KMJ
KUWAIT MEDICAL JOURNAL

The Official Journal of The Kuwait Medical Association

KUWAIT MEDICAL JOURNAL

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<div align="center">Review Article</div>

Pharmacotherapy of kidney transplant rejection: A narrative review on current therapy and future aspects

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ABSTRACT

Objectives: The current article was designed to review the main clinical and pharmacological data on the currently approved immunosuppressants used for the prevention and treatment of kidney transplant rejection.

Design: A narrative review manuscript

Settings: A comprehensive search for the available literature on renal transplant pharmacotherapy on PubMed, Google, Medline Scopus and clinicalTrials.gov.

Subjects: Key related articles published until May 2020

Intervention: Non-interventional

Main outcome measures: The induction therapy should be started with high doses of immunosuppressants immediately after transplantation, followed by gradual dosage reduction in maintenance therapy. It commonly involves thymoglobulin or alemtuzumab. Basiliximab is also approved as an induction agent, especially in recipients with low risk of rejection.

Results: Thymoglobulin is superior in patients on a steroid-free maintenance regimen. The maintenance therapy should be started early after transplantation or even before. It can be made up of different combinations of calcineurin inhibitors (CNIs) like cyclosporine A / tacrolimus, mycophenolate mofetil (MMF), mTOR inhibitors (sirolimus/everolimus) and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy and it is associated with a better allograft function. MMF is a major drug in the current maintenance therapy in combination with CNIs, and a main part in CNI-free regimens. Corticosteroids are still a main component in immunosuppressive regimens, despite the current interest in avoiding them due to their various long-term side effects. The rates of transplant loss are still unacceptably high, mainly due to dose-limiting toxicities.

Conclusion: New drugs are being developed to improve the efficacy and safety profile of maintenance therapy. Some of them showed promising results.

KEY WORDS: alemtuzumab, cyclosporine, sirolimus, tacrolimus, thymoglobulin

INTRODUCTION

Chronic kidney disease (CKD) is defined as a decrease in kidney function indicated by a glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m^2, presence of kidney damage markers, or both for more than three months, regardless of the underlying cause. The two main causes of CKD are found to be diabetes and hypertension[1].

CKD is a major health care problem. According to a recent study, 13.4% of the population worldwide are CKD patients, which is thought to be more than the prevalence of diabetes mellitus[2]. Treatment of CKD mainly aims to delay the progression towards end-stage renal disease[3], in which the only treatment becomes dialysis or renal transplantation when possible[4]. In the year 2016, renal transplants comprised around 66% of the different total transplants worldwide according to the Global Observatory on Donation and Transplantation[5]. However, this therapeutic option is challenged by the reaction of the immune system against the transplant, which necessitates a chronic control by immunosuppressants[6].

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Despite the continuous development of immunosuppressive agents over recent decades, renal graft loss as a consequence of rejection response cannot be totally prevented. The United Network for Organ Sharing reported that the probability of new graft rejection from a living donor at one-year post-transplantation is 3% and increases up to 14% at five years[7]. Generally, graft survival is affected by different factors including recipient age, the number of human leukocyte antigen mismatches, the onset of graft function after transplantation, donor type (deceased or living) and the presence of infections[8-10].

This review discusses the current protocols, the new approaches and potential drug targets that might improve the prevention of renal graft rejection.

There are three types of graft rejection; hyperacute, acute and chronic rejection. Each one has a unique onset and mechanism[11].

Hyperacute rejection

Hyperacute rejection appears only in the vascularized organs. It is a fast response that aims to eliminate foreign intruders. It occurs within the first few minutes to hours of transplantation[11,12]. In this reaction, the preexisting recipient antidonor antibodies bind to endothelial cells of the graft and activate the complement cascade, which recruits inflammatory cells such as polymorphonuclear cells[11-14]. The recruited inflammatory cells secrete enzymes that cause injury to the endothelial cells, resulting in the release of von Willebrand factor that stimulates platelet aggregation and thrombus formation, which ultimately leads to graft loss[11,14]. A high risk for hyperacute rejection occurs if the lymphocytotoxicity crossmatch was positive[15].

Acute rejection

This is the most common type of renal transplant rejection. It reduces the graft half-life by around 34% and accounts for almost 6.2% of the causes of graft loss[12,16,17]. It is a combination of humoral and cellular responses[11]. The manifestations of this type begin within a week to three months after transplantation[11,12]. It involves initially the activation of the innate response against the foreign grafts[18]. It is triggered as a consequence of a graft tissue injury caused by ischemic reperfusion or infection during transplantation[11], where specific substances called damage-associated molecular patterns are released from the site of injury, causing the activation of certain receptors expressed by innate immune cells called pattern-recognition receptors[19]. Upon activation, these receptors mediate danger signals that initiate the innate response through the activation of dendritic cells (DCs) and the secretion of chemokines and cytokines[11,19]. Activated DCs migrate to the secondary lymphoid tissues to activate T-cells and induce their migration to the graft area to initiate the adaptive response[11,17].

The adaptive response is more specific than the innate one. It plays a major role in the rejection mechanism[11,18]. This response occurs when T-cells recognize the peptide antigen presented by major histocompatibility complex, which is expressed on the surface of the donor antigen-presenting cells (APC); this process is called direct pathway[11-13,17,18]. Full activation of T-cells is not only achieved by binding with major histocompatibility complex, but also requires other interactions between molecules on the surface of T-cells and their ligands on the APC, such as CD28: CD80L or CD 86L, CD2: CD58L, CD5: CD72L. APC secret IL-12 that activates the helper T-cells (CD4+ cells) and induce their differentiation into TH1 cells, which produce interleukin-2 (IL-2) and INFs, both can stimulate the differentiation of cytotoxic T-cells. On the other hand, when CD4+ cells are activated by DCs expressing CD86, they will be differentiated into TH2 cells and thereby acquire the ability to secrete IL-4, IL-5, IL-9, IL-10, IL-13 and IL-14, which in turn stimulate B-cells to produce antibodies against the graft and to present antigens to T-cells[14,18].

Chronic rejection

It represents the most common reason for graft failure after one year of transplantation. The exact cause of developing chronic rejection is still unknown. It is thought to be a combination of different factors including infections, chronic diseases and immunosuppressant induced nephrotoxicity[15]. Forty-seven percent of graft loss cases are attributed to this type of rejection[16], compared to 6.2% of graft loss by acute rejection as mentioned earlier. This difference in the rejection rate could be due to the response of acute type to current immunosuppressive therapy in contrast with chronic type, which is not adequately controlled by any of the available immunosuppressants until now[17].

METHODS

Combinations of different related keywords were fed to PubMed, Google, Medline Scopus and clinicalTrials.gov. The keywords included terms like "kidney/renal transplant rejection", "renal transplant therapy" (RTT), "immunosuppressants", and known drug names like thymoglobulin, tacrolimus, *etc*. The suggested related articles by PubMed and Google were also investigated. The study considered only the articles published in peer-reviewed journals or websites and lay in the scope of the manuscript. The literature search was performed between October 2019 to April 2020.

LITERATURE REVIEW
Conventional immunosuppressive agents

To ensure successful renal transplantation and long-term survival of the graft, two categories of immunosuppressive agents are used sequentially for induction and maintenance[20]. Induction agents include potent immunosuppressive drugs that are given immediately after transplantation and continued over about 1-10 days to reduce the risk of early acute rejection[20,21]. Thereafter, maintenance therapy is continued life-long in lower doses to maintain long-term survival with the least possible side effects[22,23].

Induction agents
Antithymocyte globulin: Atgam and Thymoglobulin

Antithymocyte globulins (ATG) are polyclonal antibodies produced in horse or rabbit by injecting them with human lymphocytes to act as an immunogen so that anti-lymphocyte serum is produced, from which the most potent portion, immune gamma-globulins, are purified[22,24]. These antibodies mediate immunosuppression by targeting markers on T-cell surfaces that are responsible for the immune-activating signals such as CD2, CD3, CD4, CD8, CD16, CD18, CD25 and CD45[20,24]. Consequently, these antigen-antibody interactions result in complement-mediated lysis of lymphocytes[20,23].

Equine ATG (also known as Atgam, Lymphoglobulin and Thymogam) was indicated for treating and preventing acute rejection after kidney transplantation[20,24]. While rabbit ATG (also known as rATG or thymoglobulin) was initially approved only for treating acute rejection, it later on became the most commonly used agent for induction in the United States[20,21,24,25], as it is associated with lower acute rejection rates and better graft survival[20]. A randomized prospective multicenter six-month-long study assessed the efficacy and safety of using thymoglobulin as an induction agent in combination with tacrolimus or cyclosporine A (CsA), in comparison with tacrolimus-based therapy without induction with thymoglobulin. The patients (n=555) were randomly assigned to tacrolimus triple therapy, ATG induction with tacrolimus or ATG induction with CsA. All three treatment groups also received azathioprine and corticosteroid. This study demonstrated that the rate of biopsy-proven acute rejection (BPAR) in the groups of thymoglobulin-tacrolimus was significantly lower than the other two groups. However, thymoglobulin treatment was associated with a higher incidence of serum sickness, hematological toxicities (thrombocytopenia, leucopenia) and cytomegalovirus infections[26]. Besides these side effects, other studies also reported increased risks for lymphoma and anaphylactic reactions[20,22]. The symptoms of hypersensitivity reactions could be prevented by slow infusion of thymoglobulin (over 4-8 hours) and by pretreatment with antihistamines, glucocorticoids and paracetamol[24]. Recently, in 2017, thymoglobulin has gained US FDA approval for prophylaxis as an induction agent[25].

Interleukin-2 receptor antagonist: Basiliximab

Basiliximab is a chimeric monoclonal antibody developed by DNA recombinant technology. It is a fusion of the variable region of murine immunoglobulin with the constant region of human immunoglobulin to produce an antibody that binds with the alpha chain (CD25) of IL-2 receptor (IL-2R) presented on the surface of the activated helper T-cells so that it blocks its binding with IL-2 and consequently prevents helper T-cell proliferation without causing their lysis, as in the case with the use of ATG[20].

Basiliximab is approved as a prophylaxis against rejection following renal graft transplantation[20]. It is currently recommended to be used in recipients with a low risk of rejection[27]. A systematic study emphasized on the effectiveness of using Basiliximab as an induction agent with an acceptable side effect profile[28]. However, a study that targeted patients at the Organ Procurement and Transplantation Network registry between the years 2000-2012 evaluated the necessity of induction therapy with an IL-2R antagonist in patients receiving living donor renal transplantation, who were treated with tacrolimus/mycophenolic acid with and without steroids. The study demonstrated that the induction with IL-2R antagonist had no advantage over no-induction group in patients receiving a maintenance therapy with tacrolimus/mycophenolic acid/steroid. The study also demonstrated that, in the no-steroid group, thymoglobulin resulted in lower rates of acute rejection compared to IL2-R antagonist[29]. From the author's point of view, it appears that although current guidelines recommend the use of induction agents regardless of the risk of rejection, corticosteroids use or the constituents of the used maintenance regimen, it seems that future guidelines might restrict the use of induction agents for higher risk groups or when steroids are to be avoided. In such cases, thymoglobulin would be the preferred agent. However, large randomized clinical trials in this concern are needed.

Anti-CD-52 antibodies: Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody that triggers antibody-dependent cell lysis by binding to CD-52 molecule on the surface of T- and B-cells, macrophages and natural killer cells[20]. Although it has an FDA approval only for B-cell lymphocytic leukemia, its use for induction therapy in

kidney transplantation is increasing since 1998[30]. For this purpose, it is administered intravenously as a single dose of 30 mg intraoperatively[31]. Like other monoclonal antibodies, alemtuzumab might cause hematological side effects such as thrombocytopenia, neutropenia and lymphopenia. It may also cause cytokine release syndrome and infusion-related reactions[20].

Several trials were published that investigated alemtuzumab's efficacy and safety in kidney transplantation. Of these, alemtuzumab was compared with basiliximab and thymoglobulin in low and high-risk groups. All groups received tacrolimus, mycophenolate mofetil (MMF) and five-day steroid as maintenance therapy. The study showed that alemtuzumab was superior to basiliximab in reducing the acute rejection rate (10% vs. 22%), but almost similar to thymoglobulin (18% vs. 15%)[32]. However, another study showed no difference between alemtuzumab and basiliximab on graft and patient survival[30]. Recently, a systemic review and meta-analysis of randomized controlled trials reported superior beneficial effects of alemtuzumab over thymoglobulin; however, the comparison was statistically insignificant due to the limited number of the trials[33]. Therefore, more controlled comparative studies are required to determine the superiority of these drugs.

Maintenance agents
Calcineurin inhibitors: Cyclosporin A and tacrolimus

CsA and tacrolimus are two of the most widely used immunosuppressive agents. They are considered the mainstay of renal transplant maintenance therapy. They are called calcineurin inhibitors (CNI) due to their ability to inhibit the activity of a protein called calcineurin[34], which is a calcium/calmodulin-dependent serine-threonine phosphatase, consisting of two subunits, a catalytic subunit, calcineurin-A and a regulatory calcium-binding subunit, calcineurin-B[35,36]. It plays a major role in T-cell activation. When an alloantigen is recognized by T-cell receptors, the intracellular calcium concentration increases, this in turn activates calcineurin-B. Once activated, it stimulates calcineurin-A to dephosphorylate a cytoplasmic transcription factor called the cytoplasmic nuclear factor of activated T-cells, allowing its transfer along with the activated calcineurin to the nucleus, where it induces the expression of cytokines and co-stimulatory substances like IL-2, which in turn, upon interaction with their receptors, result in cell activation and proliferation[36-38].

CsA and tacrolimus have the same pharmacodynamic activity although they differ in their structures[34,38]. CNIs bind cytoplasmic proteins called immunophilins, cyclophilin in the case of CsA and FK-binding protein in case of tacrolimus[39]. This complex then binds to calcineurin, leading to inhibition of its activity[36,40].

CNIs are associated with many adverse effects due to their narrow therapeutic window and the presence of their target (calcineurin) in many cell types other than immune cells. The most frequent adverse effect is nephrotoxicity, which can be acute and reversible or chronic and irreversible. Chronic CNIs-induced nephrotoxicity may result in interstitial fibrosis, tubular atrophy or glomerulosclerosis, which contributes to the deterioration of the function of the transplanted kidney with time. Other side effects include new-onset diabetes, dyslipidemia, neurotoxicity, de novo cancers and infections[41].

Different studies showed that tacrolimus is superior to CsA in terms of the rates of graft loss, acute rejection and hypercholesterolemia in addition to lower nephrotoxic potential. While CsA is more likely to cause gingival hyperplasia, hirsutism and increased levels of low-density lipoprotein and triglycerides, tacrolimus increases the risk of developing diabetes (dose-dependent) and gastrointestinal upsets. No differences in malignancy and infections were observed[42,43].

Currently, tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation[27,44]. Extended-release tacrolimus formulation is available now as an alternative to the immediate release one with comparable efficacy[45].

Purine synthesis inhibitors: Azathioprine and mycophenolate mofetile
Azathioprine

Azathioprine inhibits purine synthesis as it is converted within cells to its metabolites, mercaptopurine and thioguanine. Mercaptopurine, which is involved in the formation of thioinosinic acid nucleotides, consequently blocks the purine biosynthesis pathway. Besides, these metabolites are incorporated into the DNA, blocking its replication[46].

Azathioprine, since its development in the 1960s, was used as a main part of the standard regimens for immunosuppression[22]. However, lately, it has been replaced by mycophenolic acid[27], as this drug was found to be more effective in the prevention of acute rejection[47]. However, this claim has been challenged by later studies, as discussed in the following section of this manuscript.

The main side effects associated with azathioprine use are bone marrow suppression, mainly in patients who take gout medications, allopurinol or febuxostat. Other side effects are nausea, joint pain and hepatotoxicity[46,48].

Mycophenolate mofetil

MMF is a prodrug of the active agent mycophenolic acid. It is directed against the process of guanosine nucleotides production, mostly through the inhibition of the enzyme inosine monophosphate dehydrogenase that is expressed in T and B lymphocytes, thus negatively affecting their proliferation rate[49]. However, MMF's ability to inhibit the recruitment of immune cells at the site of rejection and to decrease the proliferation of arterial smooth muscle cells are also considered main mechanisms by which MMF may improve graft survival[50]. MMF is considered a major drug in the current maintenance therapy of renal transplant along with CNIs, and a main part in CNI-free, toxicity sparing regimens that may take a place in the future[51]. Although many studies have shown that MMF may play a role in preventing tubular chronic graft rejection and prolong patient survival[47,52,53], there is a need for additional clinical trials on larger scales to confirm this effect.

In many systematic reviews and randomized controlled clinical trials, MMF was found to be associated with a superior benefit in preventing acute renal graft rejection compared with azathioprine, and with comparable adverse effects profile[47,54]. Therefore, MMF has largely replaced azathioprine in immunosuppression protocols, and it is considered now the first-line agent in RTT[27]. However, this approach was opposed by a prospective, multicenter, randomized parallel group trial published in 2004 by Remuzzi and colleagues, which demonstrated that MMF had no advantages over azathioprine in preventing acute rejection in kidney transplant patients who were on CsA microemulsion and steroid over six months, followed by CsA alone over another 15 months. Interestingly, treatment with MMF was fifteen times more expensive than azathioprine[55]. A follow-up study by the same group followed the patients over five years demonstrated that the clinical outcomes were similar between MMF and azathioprine. In addition, based on the relatively high cost of MMF, they recommended the replacement of MMF with azathioprine in the standard immunosuppression treatment for kidney transplant recipients[56]. In agreement with these recommendations, another review of the respective clinical data in 2013 did not reveal any long-term benefits of MMF over azathioprine[57]. Therefore, we think that caregivers should balance the potential benefits and harms of MMF and azathioprine according to individual patient's risks and preferences.

Inhibitors of mammalian target of Rapamycin: Sirolimus and everolimus
Sirolimus (also known as rapamycin)

It is a potent antiproliferative immunosuppressive agent. It acts by blocking the action of a protein kinase called the mammalian target of Rapamycin (mTOR), which plays a major role in multiple signal transduction pathways. Sirolimus inhibits the proliferation of T- and B-cells. It also decreases the production of antibodies. This unique mechanism of action, as well as its adverse effect profile, make this drug one of the major agents used in the prevention of acute renal transplant rejection[58].

A synergistic effect was observed when sirolimus was combined with CsA, that is, the risk of developing acute renal rejection was reduced[58,59]. Furthermore, in different studies, when CNI was withdrawn and a CNIs-free maintenance regimen consisting of sirolimus and MMF was introduced, a comparable efficacy (in terms of graft and patient survival) with more safety was observed and maintained for four years post transplantation[60,61].

The advantages of Sirolimus use include the lack of causing severe nephrotoxicity or major organ damage. However, gastrointestinal toxicity, hypertension, hyperglycemia, hyperlipidemias, bone marrow suppression, infections, impaired wound healing, arthralgia, edema and increased risks for certain malignancies remain major side effects by sirolimus that require attention[62,63].

Everolimus

It is an oral sirolimus analog, with improved solubility and oral bioavailability, but similar immunosuppressive efficacy and safety profile[64,65]. As an advantage, everolimus may have an ability to lower the toxicity of CNI on the kidneys, in contrast to sirolimus which potentiates CNI-associated nephrotoxicity. However, this ability is claimed only by expert opinions and clinical trials on limited scales[65,66], and therefore further studies and randomized controlled clinical trials are needed to confirm this observation.

Corticosteroids

For decades, corticosteroids have been used as a major part of immunosuppressive regimens due to their various inhibitory effects on inflammatory and lymphocytic activation processes[67]. Their anti-inflammatory effects include suppression of immune cell migration to inflamed tissues as well as inhibition of the production of inflammatory mediators such as prostaglandins. Steroids also decrease cytokines and interleukins production, thus affecting T- lymphocyte proliferation[68].

Methylprednisolone and prednisolone are the main steroids used for immunosuppression preoperatively and as a part of the triple maintenance therapy consisting of CNI and mycophenolic acid along with corticosteroids[27]. Intravenous methylprednisolone dose is used commonly at induction and tapered postoperatively. At 3-5 days post-transplantation, methylprednisolone is switched to a fixed oral dose of prednisolone[67].

Unfortunately, long term use of corticosteroids is associated with unfavorable side effects that underlay several major health problems, such as dyslipidemia, hypertension, hyperglycemia and post-transplant diabetes mellitus[69]. To obtain a better side effect profile, current protocols suggest that steroids could be withdrawn in patients with low risk of rejection during the first week after transplantation. However, if steroids are not avoided in the first month after transplantation, prednisolone should be continued with the lowest possible effective dose (5 mg daily)[27].

A recent retrospective cohort study included two groups of patients. All patients received a perioperative dose of basiliximab and a second dose on day 4. They also received a single perioperative dose of methylprednisolone as induction agents. The first group included low-risk patients in whom prednisolone was administered from day 1-7 and then maintained with tacrolimus and MMF. The other group included high-risk patients, who were maintained with tacrolimus, MMF and prednisolone for the first three months, and afterward, they were maintained with tacrolimus and prednisolone. The data demonstrated a close incidence of BPAR between the high and low-risk groups. Moreover, one-year post-transplantation, the rates of graft and patient survival, and the graft function were also similar between the two groups[70]. Interestingly, a systemic review and meta-analysis study advocated the withdrawal or avoidance of steroids, even in high-risk patients, as it was demonstrated in this study as being safe, in terms of graft survival and rejection, and associated with a reduced risk of death and post-transplantation diabetes mellitus. However, the authors recommended future prospective studies to further validate these results[71].

Guidelines in renal transplant therapy

Traditionally, the goal of therapy during the first six months after transplantation is mainly to prevent acute graft rejection and to prolong its survival. However, lately, the aim has expanded to also include the prevention/treatment of the major complications associated with the use of immunosuppressants such as malignancies and infections, and to improve patient's adherence and to ultimately preserve a good

transplant function[27,72]. The current guidelines recommend the use of multi-drug regimens in a way that produces a synergistic effect against acute rejection, starting with high doses immediately after transplantation followed by gradual dosage reduction in maintenance therapy[44].

To prevent hyperacute transplant rejection, immunosuppressive therapy should be started at the time of transplantation. Antibody agents including ATG and IL-2R antagonists represent the cornerstone of the induction therapy in all kidney transplant recipients[27,44]. The use of ATG is preferred when there is a high risk for rejection or when corticosteroids are avoided early at induction[72,73].

Treatment with CNIs like CsA or tacrolimus should be started early after transplantation or even before[27,72]. Tacrolimus is preferred as it is associated with a lower rate of rejection. The plasma trough level of tacrolimus is not recommended to exceed 4-8 ng/ml[27]. The anti-proliferative agent mycophenolic acid is the drug of choice to be used in combination with CNIs[27,44].

The mTOR inhibitors, sirolimus or everolimus, are considered second line drugs to be used when CNIs are causing serious adverse effects including chronic graft disease, tubular atrophy or interstitial fibrosis. CNI induced nephrotoxicity should be confirmed by biopsy[27].

Despite the recent great interest in corticosteroids avoidance and withdrawal from RTT as better side effect profile could be obtained, corticosteroids, so far, remain a main part in immunosuppressive regimens. Studies showed that avoidance of steroids early after transplantation is associated with an increased risk for acute rejection. However, graft survival and mortality seem not to be affected by the current antibody induction and maintenance regimens used[74,75]. A recently updated review article revealed that there is a significant increase in acute rejection associated with early steroids avoidance or discontinuation, but graft survival seems to be similar in a time frame of five years. However, further clinical studies are required to assess longer corticosteroid withdrawal effects[76].

New approaches in renal transplant therapy
Targeting specific cytoplasmic signaling proteins

Tofacitinib (also known as tositinib, CP-690,550) has a unique mechanism of action in immune suppression. It is directed against a family of cytoplasmic signaling proteins called Janus kinases (JAK), mainly JAK2 and JAK3, which mediate the signal from cytokine receptors, especially in this context, the IL-2Rγ. The inhibition of this signaling pathway in B-cells, T- cells and natural killer cells results in their apoptosis[77]. In the year 2012, this drug gained FDA approval to treat rheumatoid arthritis and

recently, in 2018, it has also been approved for the treatment of moderate to severe ulcerative colitis[77,78].

Clinical trials are being conducted to evaluate the efficacy and safety of its use in renal transplantation, especially as a part of CNIs-free regimens. Tofacitinib has successfully passed animal studies as well as phase 1 clinical trials regarding transplantation[77]. In a phase I study, 28 stable kidney transplant patients were included and randomized to receive tofacitinib 5 mg b.i.d, 15 mg b.i.d, 30 mg b.i.d or placebo (n=6, 6, 10 and 6 respectively). The study reported a dose-dependent decrease in hemoglobin, natural killer cell count and mild to moderate viral and bacterial infections, especially with the highest dose of tofacitinib. The study did not report any clinical rejection or renal toxicity[79].

Another larger pilot study was carried out in the year after. It was a prospective, randomized study over 12 months and involved 61 kidney transplant patients who received induction therapy with basiliximab and concomitant treatment with MMF and corticosteroids. The patients were distributed in three treatment groups; 15 mg tofacitinib twice daily, 30 mg tofacitinib twice daily and tacrolimus (control group). The results showed that the combination therapy of 30 mg tofacitinib twice daily with MMF was associated with over-immunosuppression, while 15 mg tofacitinib twice daily showed efficacy and safety profile that was comparable to the control group (tacrolimus), except that it showed a higher risk for viral infections. A limitation of this study was that the treatment groups did not have equivalent demographics[80].

Based on the outcome of this study, a larger prospective randomized multicenter phase-IIb trial was carried out on tofacitinib. The study included 331 renal transplant recipient patients with low to moderate risk of rejection. The patients were randomly distributed into three groups. All groups received basiliximab for induction and mycophenolic acid and corticosteroids for maintenance therapy. One group received CsA microemulsion, while the other two groups received tofacitinib with two different intensities; more intensive tofacitinib (15 mg twice daily (b.i.d) in months 1-6, then 10 mg b.i.d in months 7-12), and less intensive tofacitinib group (15 mg b.i.d in months 1-3, then 10 mg b.i.d in months 4-12). After 12 months of post-transplantation therapy, patient and graft survival was similar among the different treatment groups. Despite the advantageous lower rates of chronic nephropathy associated with tofacitinib use as compared to CsA, patients treated with this drug developed more severe side effects in the context of anemia, leucopenia, viral infections and lymphoproliferative disorders, which was believed to be a result of excessive immunosuppression[81]. Similar results were obtained by a more recent study, which assessed the long term effect of tofacitinib in comparison with CsA over three years post-transplantation. The study also concluded an association between the increased risk of serious infections and the use of mycophenolic acid with tofacitinib[82].

Possible future approaches need to be investigated for the aim of reducing the side effects profile of tofacitinib, which might include serum drug level monitoring to individualize the therapeutic levels of tofacitinib to achieve the lowest possible effective doses or testing tofacitinib-containing regimens that include lower therapeutic doses of MMF. Additionally, tofacitinib could be tested as a part of new CNI-free protocols.

Targeting co-stimulatory pathways

The interaction between the T-cell receptors and the antigen presented on APCs is not sufficient for full T-cell activation, but other co-stimulatory signals are also needed. A new insight for targeting these signals is currently being conducted to treat solid organ transplantation rejection and autoimmune diseases[83]. The inhibition of co-stimulatory pathways such as CD40-CD154 and CD80/CD86-CD28 negatively affects APCs activation, antibody-production and cytokine releasing processes[84].

Anti-CD40 monoclonal antibodies

The interaction between CD40, presented by APCs such as B-cells and DCs, and CD40L (or CD154), presented by T-lymphocytes, plays a major role in T-cell activation[17]. Once CD40 interacts with its ligand, the activated APCs upregulate co-stimulatory proteins leading to T-cell proliferation and differentiation. Likewise, the activation of B-cells by the CD40-CD154 pathway causes their differentiation into memory or plasma cells, which play a major role in chronic renal graft rejection as mentioned earlier in this article[85].

Recently, different agents directed against the CD40-CD154 pathway were introduced to treat chronic graft rejection and auto-immune diseases, besides some kinds of cancers. Anti-CD154 antibodies were studied and they were considered to have a partial efficacy in renal transplant. They are suggested to be combined with B7/CD28 pathway inhibition for full efficacy in preventing chronic rejection[86]. Moreover, anti-CD154 antibodies are associated with thromboembolic events that are thought to be mediated by anti-CD154 Fc domain-platelets interaction[87].

Bleselumab

Bleselumab (ASKP1240) is a fully-humanized IgG4 monoclonal antibody that binds with CD40 on B-cells

and APCs to block its interaction with CD154 ligand on T-cells, resulting in co-stimulatory pathway inhibition[88]. The mean maximal CD40 receptor occupancy is reached at a dose greater than 0.01 mg/kg and the receptor occupancy did not exceed 87%. After that, any increase in the dose will only prolong the duration of occupancy[89,90].

Regarding the safety of bleselumab, the most commonly reported side effects were procedure pain, hypophosphatemia, hypomagnesemia, nausea, tremor, edema and BK viral infection[88,91]. Unlike some investigational chimeric anti CD40 monoclonal antibodies, it does not cause cytotoxicity and immunogenicity. Also, there were no thromboembolic events caused by this antibody[90].

A phase 2 randomized open-label multicenter study was designed to assess the efficacy and safety of bleselumab combined with immediate-release tacrolimus (IR-TAC) or MMF in comparison with the standard of care treatment, which consisted of IR-TAC and MMF. Basiliximab as an induction treatment and corticosteroids were used in all recipients. The dose of IR-TAC was decreased gradually after 28 days of treatment in the bleselumab + IR-TAC group until a trough level of 2-5ng/ml was reached by four months. The BPAR after 6 and 36 months was 6.3% and 14.6% respectively in standard of care treatment, while it was 37% and 41% in bleselumb + MMF and 9.1% and 9.1% in bleselumab + IR–TAC. These results suggest the noninferiority of bleselumb plus IR-TAC in comparison to SoC in the prevention of BPAR. However, the incidence of new-onset diabetes mellitus was lower in bleselumab + MMF than other regimens. Finally, all regimens had no significant differences in eGFR and graft and patient survival rates[88].

Iscalimab

Iscalimab, CFZ533A, is a fully-humanized Fc-silent anti-CD40 immunoglobulin that was recently invented. This drug can inhibit humoral immunity that is stimulated by helper T-cells without depleting peripheral blood B-cells[92,93]. With its suspected efficacy in prolonging graft survival and no evidence for developing thromboembolic complication, this drug is now being studied to be considered in the main part of CNI-free renal transplant protocols.

In phase-IIa randomized clinical trials conducted in 2018 (ClinicalTrials.gov Identifier: NCT02217410), 51 patients were studied to compare the therapeutic outcome between iscalimab and tacrolimus. All patients received basiliximab and corticosteroids as induction therapy and MMF and steroids as maintenance therapy. Results showed that iscalimab has a better side effect profile and improved renal graft outcomes with no risk for nephrotoxicity[94]. Allograft

biopsies were reviewed by blind pathologist and results were presented by American Transplant Congress, 2019. Chronic allograft damage index was lower in patients treated with iscalimab[95], indicating that this novel drug could be a valuable add-on therapy to the current standard tacrolimus containing regimens. The results need to be confirmed by phase-IIb clinical trials (NCT03663335) that may be completed by 2021[96].

Anti CD80/86 monoclonal antibodies
Belatacept

Belatacept is a CTLA4-Ig fusion protein developed from abatacept by substituting leu104 with glutamic acid and ala29 with tyrosine. It was approved in 2011 for acute rejection prophylaxis in kidney transplant recipients as an alternative for CNIs to reduce their renal and cardiometabolic side effects. Belatacept interacts with CD80/86 on APCs to block its binding to CD28 on T-cells, resulting in the prevention of T-cell activation[97,98].

Belatacept has a volume of distribution of 0.11L/kg, clearance of average 0.49013± mL/kg/h and a terminal half-life of almost 11 days. It is administrated intravenously over 30 minutes with 10 mg/kg on days 1 and 5 and at the end of weeks 2, 4 and 8. After that, 5mg/kg is given on week 16 of transplantation and it is repeated every four weeks. It does not require therapeutic monitoring as its plasma concentration is not affected by renal or hepatic functions, therefore, the doses are adjusted only according to the factor of bodyweight[99,100].

The most common adverse effects related to belatacept are infusion-related reactions, anemia, headache, peripheral edema and infections[97]. Also, the FDA puts a black box warning about the post-transplant lymphoproliferative diseases, especially in Epstein-Barr virus seronegative recipients, which contraindicates its use in this group of patients[99,101].

The two major clinical trials that evaluated belatacept efficacy and safety are Belatacept Evaluation of Nephroprotection and Efficacy as a First-line Immunosuppression Trial (BENEFIT) and the BENEFIT–Extended Criteria Donors Trial that followed the patients with extended criteria donor kidney transplant. In these studies, patients were randomized into three groups, more intensive belatacept, low intensive belatacept and CsA. All recipients were given induction with basiliximab and maintenance with MMF and corticosteroids. In BENEFIT, after 12 months, both the graft and patient survival rates were not significantly different between the groups (95% more intensive vs. 97% low intensive vs. 93% CsA respectively)[97]. Concerning safety, belatacept groups had a higher renal function than

CsA[100] and lower chronic renal allograft nephropathy. Finally, belatacept approved its safety in reducing the cardiometabolic side effects related to CsA use, as it showed a lower incidence of hypertension, hyperlipidemia and new-onset diabetes mellitus after transplantation. However, in contrast to all of these advantages for belatacept over CsA, it remains associated with higher acute rejection rates. After the end of seven years, the belatacept groups also remain non-inferior to CsA groups in the survival rates for patients and graft. It showed better results for kidney function and cardiometabolic effects[97].

To compare between belatacept and tacrolimus, two studies were conducted. In the first one, basiliximab was used as an induction agent while MMF and steroids were used as maintenance agents. Both belatacept and tacrolimus resulted in the same renal function but the acute rejection rate was higher with belatacept (55% vs. 10%)[102]. In the second study, the patients were divided into three groups, group 1 received tacrolimus and MMF as maintenance agents, both groups 2 and 3 received belatacept and MMF for maintenance, however, for induction, ATG was used in groups 1 and 2 and tacrolimus with basiliximab in group 3. In this study, the belatacept groups had better eGFR and patient and graft survival. Nevertheless, the acute rejection rate was still the lowest in the tacrolimus group (3.4%, 34.5% and 36.9%, respectively). This study has not been published yet[103].

As discovered later, the CTLA4 pathway could play a role in the function of regulatory T-cells promoting their co-inhibitory signal. Therefore, the blockade of this pathway by belatacept might explain why belatacept is associated with increased rates of acute rejection when compared with CNI, as well as a high incidence of lymphoproliferative disorders[104,105].

Selective CD28 blockade

In contrast to belatacept, selective CD28 blockers inhibit CD28 co-stimulatory effects, while preserving the co-inhibitory effect of the CTLA-4 pathway. Therefore, they are considered to be more effective in inhibiting T-cell activation and proliferation[106,107].

The most advanced selective anti-CD28 agent is FR104, a novel humanized pegylated CD28 antagonist that is being developed for the treatment of graft rejection. A nonhuman primate study was conducted to evaluate its efficacy for the aim of allowing the minimization of steroids and CNI use. The results were promising as the combination of FR104 and low doses of tacrolimus with no steroid exposure significantly improved graft survival. FR104 was also found to be effective in preventing alloimmunization and in decreasing the development of donor-specific alloantibodies. CNI-free regimens showed different results as (FR104/MMF/Steroids) was associated with increased risk for acute rejection, but the combination between FR104 and sirolimus prevented acute rejection and showed improvement in graft survival[108]. Later evidence for synergistic effect between FR104 and mTOR inhibitors was demonstrated in a recent study that showed a significant inhibition for T-cell activation, resulting in more immunosuppression and more infectious complications[109]. The first-in-human phase 1 clinical study in 64 healthy volunteers to assess the efficacy and safety of FR104 was conducted in 2016. With all tested doses, FR104 was found to be safe and clinically efficacious, supporting further studies to determine ideal dosage regimens[110].

Summary

The prevention of kidney transplant rejection requires a short-term induction therapy, as prophylaxis against early acute rejection, and a life-long maintenance therapy against chronic rejection.

The induction therapy should be started with high doses immediately after transplantation, followed by gradual dosage reduction in maintenance therapy. It commonly involves thymoglobulin (ATG), which targets several surface antigens on T-lymphocytes, resulting in complement-mediated lysis of these cells. It is also commonly used for the treatment of acute rejection. Alemtuzumab is another induction agent that causes antibody-dependent immune cell lysis. It demonstrated some statistically insignificant beneficial effects over thymoglobulin, therefore, more controlled comparative studies are required to determine the superiority of these two drugs. Both drugs are associated mainly with hematological toxicities. On the other hand, basiliximab is an IL-2R antagonist that targets activated helper T-cells and prevents their proliferation without causing their lysis. It is approved as an induction agent, especially in recipients with low risk of rejection. However, thymoglobulin might be superior in patients on a steroid-free maintenance regimen.

The maintenance therapy should be started early after transplantation or even before. It can be made up of different combinations of CNIs (CsA/tacrolimus), MMF, mTOR inhibitors (sirolimus/everolimus) and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation. Studies showed that tacrolimus has lower nephrotoxic potential than CsA and it is associated with a better allograft function. CNIs are generally associated with cardiovascular, metabolic and immunosuppression-related side effects, as well as nephrotoxicity, which is the most frequent. MMF is also considered a major drug in the current maintenance therapy of renal transplant along with CNIs, and a main part in CNI-

free regimens that may take a place in the future, although some studies recommended its replacement with azathioprine based on cost-effectiveness data. Sirolimus (rapamycin) and everolimus inhibit the proliferation of T- and B-cells and decrease the production of antibodies. They can produce a synergistic effect in combination with CNI and in CNI-free maintenance regimen along with MMF. They are considered second-line drugs to be used when CNIs are causing serious adverse effects. The inhibitors of mTOR are associated with cardiovascular, metabolic and immunosuppression related side effects. Until now, corticosteroids are considered a main part in immunosuppressive regimens, despite the current interest in avoiding them due to their various side effects.

New drugs are being developed to improve the efficacy and safety profile of maintenance therapy. A promising one is tofacitinib that inhibits JAK signaling pathways resulting in lymphocytes apoptosis. It showed comparable anti-rejection activity to CsA with a lower risk of nephropathy, but more hematological toxicity and increased risk for infections and lymphoproliferative disorders. Other drug classes are being developed, some of them target co-stimulatory pathways like bleselumab and iscalimab that targets CD40 antigens on B-cells, another one targets CD/8086 on APC like belatacept, and finally the recent selective CD28 blockers like FR104. These classes are still in clinical trials to assess their efficacy and safety profile in comparison to the currently approved drugs. Some of them showed some promising results.

CONCLUSION

Induction therapy is most commonly achieved by ATG and Alemtuzumab. The maintenance therapy can be made up of different combinations of CNIs, MMF, mTOR inhibitors and corticosteroids. Tacrolimus is considered a first-line agent in maintenance therapy in renal transplantation. MMF is another major drug that is used in combination with CNIs and is being investigated as a main part in future CNI-free regimens. New promising drugs are being developed to improve the efficacy and safety profile of the maintenance therapy.

ACKNOWLEDGMENT

REFERENCES

1. Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet 2017; 389(10075):1238-1252.
2. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS, et al. Global prevalence of Chronic Kidney Disease - A systematic review and meta-analysis. PLoS One 2016; 11(7):e0158765.
3. Turner JM, Bauer C, Abramowitz MK, Melamed ML, Hostetter TH. Treatment of chronic kidney disease. Kidney Int 2012; 81(4):351-362.
4. Breyer MD, Susztak K. The next generation of therapeutics for chronic kidney disease. Nat Rev Drug Discov 2016; 15(8):568-588.
5. Global Observatory on Donation and Transplantation. Kidney Transplants (%). 2016 [cited 2019, 8/7]; Available from: www.transplant-observatory.org/countkidney/.
6. de Brito DC, de Paula AM, Grincenkov FR, Lucchetti G, Sanders-Pinheiro H. Analysis of the changes and difficulties arising from kidney transplantation: a qualitative study. Rev Lat Am Enfermagem 2015; 23(3):419-426.
7. United Network of Organ Sharing. Transplant from a living donor. [cited 2019 8/7]; Available from: https://transplantliving.org/kidney/what-is-life-like-after-living-donor-transplant/.
8. Cippa PE, Schiesser M, Ekberg H, van Gelder T, Mueller NJ, Cao CA, et al. Risk stratification for rejection and infection after kidney transplantation. Clin J Am Soc Nephrol 2015; 10(12):2213-2220.
9. Almond PS, Matas A, Gillingham K, Dunn DL, Payne WD, Gores P, et al. Risk factors for chronic rejection in renal allograft recipients. Transplantation 1993; 55(4):752-756; discussion 756-757.
10. Ravindra KV, Sanoff S, Vikraman D, Zaaroura A, Nanavati A, Sudan D, et al. Lymphocyte depletion and risk of acute rejection in renal transplant recipients at increased risk for delayed graft function. Am J Transplant 2019; 19(3):781-789.
11. Moreau A, Varey E, Anegon I, Cuturi MC. Effector mechanisms of rejection. Cold Spring Harb Perspect Med 2013; 3(11):a015461.
12. Nasr M, Sigdel T, Sarwal M. Advances in diagnostics for transplant rejection. Expert Rev Mol Diagn 2016; 16(10):1121-1132.

13. Martinu T, Pavlisko EN, Chen DF, Palmer SM. Acute allograft rejection: cellular and humoral processes. Clin Chest Med 2011; 32(2):295-310.

14. Chinen J, Buckley RH. Transplantation immunology: solid organ and bone marrow. J Allergy Clin Immunol 2010; 125(2 Suppl 2):S324-S335.

15. Dipiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM. Pharmacotherapy: A pathophysiologic approach. 10th ed. McGraw Hill companies; 2017.

16. Katsuma A, Yamakawa T, Nakada Y, Yamamoto I, Yokoo T. Histopathological findings in transplanted kidneys. Renal Replacement Therapy 2017; 3(1):6.

17. Ingulli E. Mechanism of cellular rejection in transplantation. Pediatr Nephrol 2010; 25(1):61-74.

18. Chaplin DD. 1. Overview of the human immune response. J Allergy Clin Immunol 2006; 117(2):S430-S435.

19. Cucchiari D, Podesta MA, Ponticelli C. The critical role of innate immunity in kidney transplantation. Nephron 2016; 132(3):227-237.

20. Chouhan KK, Zhang R. Antibody induction therapy in adult kidney transplantation: A controversy continues. World J Transplant 2012; 2(2):19-26.

21. Hardinger KL, Brennan DC, Klein CL. Selection of induction therapy in kidney transplantation. Transpl Int 2013; 26(7):662-672.

22. Muntean A, Lucan M. Immunosuppression in kidney transplantation. Clujul Med 2013; 86(3):177-180.

23. Zdanowicz MM. The pharmacology of immunosuppression. Am J Pharm Educ 2009; 73(8):144.

24. Hardinger KL. Rabbit antithymocyte globulin induction therapy in adult renal transplantation. Pharmacotherapy 2006; 26(12):1771-1783.

25. Alloway RR, Woodle ES, Abramowicz D, Segev DL, Castan R, Ilsley JN, et al. Rabbit anti-thymocyte globulin for the prevention of acute rejection in kidney transplantation. Am J Transplant 2019; 19(8):2252-2261.

26. Charpentier B, Rostaing L, Berthoux F, Lang P, Civati G, Touraine JL, et al. A three-arm study comparing immediate tacrolimus therapy with antithymocyte globulin induction therapy followed by tacrolimus or cyclosporine A in adult renal transplant recipients. Transplantation 2003; 75(6):844-851.

27. Baker RJ, Mark PB, Patel RK, Stevens KK, Palmer N. Renal association clinical practice guideline in post-operative care in the kidney transplant recipient. BMC Nephrol 2017; 18(1):174.

28. Webster AC, Ruster LP, McGee R, Matheson SL, Higgins GY, Willis NS, et al. Interleukin 2 receptor antagonists for kidney transplant recipients. Cochrane Database Syst Rev 2010; 2010(1):CD003897.

29. Tanriover B, Zhang S, MacConmara M, Gao A, Sandikci B, Ayvaci MU, et al. Induction therapies in live donor kidney transplantation on tacrolimus and mycophenolate with or without steroid maintenance. Clin J Am Soc Nephrol 2015; 10(6):1041-1049.

30. Morgan RD, O'Callaghan JM, Knight SR, Morris PJ. Alemtuzumab induction therapy in kidney transplantation: a systematic review and meta-analysis. Transplantation 2012; 93(12):1179-1188.

31. Kalluri HV, Hardinger KL. Current state of renal transplant immunosuppression: Present and future. World J Transplant 2012; 2(4):51-68.

32. Hanaway MJ, Woodle ES, Mulgaonkar S, Peddi VR, Kaufman DB, First MR, et al. Alemtuzumab induction in renal transplantation. N Engl J Med 2011; 364(20):1909-1919.

33. Zheng J, W Song. Alemtuzumab versus antithymocyte globulin induction therapies in kidney transplantation patients: A systematic review and meta-analysis of randomized controlled trials. Medicine (Baltimore) 2017; 96(28):e7151.

34. Kapturczak MH, Meier-Kriesche HU, Kaplan B. Pharmacology of calcineurin antagonists. Transplant Proc 2004; 36(2 Suppl):25S-32S.

35. Bram RJ, Hung DT, Martin PK, Schreiber SL, Crabtree GR. Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcineurin binding and cellular location. Mol Cell Biol 1993; 13(8):4760-4769.

36. Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later, can't live without. J Immunol 2013; 191(12):5785-5791.

37. Shibasaki F, Price ER, Milan D, McKeon F. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. Nature 1996; 382(6589):370-373.

38. Schreiber SL, Crabtree GR. The mechanism of action of cyclosporin A and FK506. Immunol Today 1992; 13(4):136-142.

39. Fruman DA, Klee CB, Bierer BE, Burakoff SJ. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proc Natl Acad Sci U S A 1992; 89(9):3686-3690.

40. Martinez-Martinez S, Redondo JM. Inhibitors of the calcineurin/NFAT pathway. Curr Med Chem 2004; 11(8):997-1007.

41. Malvezzi P, Rostaing L. The safety of calcineurin inhibitors for kidney-transplant patients. Expert Opin Drug Saf 2015; 14(10):1531-1546.

42. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. BMJ 2005; 331(7520):810.

43. Azarfar A, Ravanshad Y, Mehrad-Majd H, Esmaeeli M, Aval SB, Emadzadeh M, et al. Comparison of tacrolimus and cyclosporine for immunosuppression after renal transplantation: An updated systematic review and meta-analysis. Saudi J Kidney Dis Transpl 2018; 29(6):1376-1385.

44. Faba OR, Boissier R, Budde K, Figueiredo A, Taylor CF, Hevia V, et al. European Association of Urology Guidelines on Renal Transplantation: Update 2018. Eur Urol Focus 2018; 4(2):208-215.

45. Saengram W, Vadcharavivad S, Poolsup N, Chancharoenthana W. Extended release versus immediate release tacrolimus in kidney transplant recipients: a systematic review and meta-analysis. Eur J Clin Pharmacol 2018; 74(10):1249-1260.

46. Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: Mechanisms of action and therapeutic efficacy. Crit Rev Oncol Hematol 2005; 56(1):23-46.

47. Wang K, Zhang H, Li Y, Wei Q, Li H, Yang Y, et al. Efficacy of mycophenolate mofetil versus azathioprine after renal transplantation: a systematic review. Transplant Proc 2004; 36(7):2068-2070.

48. Mohammadi O, TA Kassim. Azathioprine, in StatPearls. 2020: Treasure Island (FL).

49. Allison AC, EM Eugui. Mycophenolate mofetil and its mechanisms of action. Immunopharmacology 2000; 47(2-3):85-118.

50. Allison AC. Mechanisms of action of mycophenolate mofetil in preventing chronic rejection. Transplant Proc 2002; 34(7):2863-2866.

51. Dalal P, Grafals M, Chhabra D, Gallon L. Mycophenolate mofetil: safety and efficacy in the prophylaxis of acute kidney transplantation rejection. Ther Clin Risk Manag 2009; 5(1):139-149.

52. Mihovilovic K, Maksimovic B, Kocman B, Gustin D, Vidas Z, Bulimbasic S, et al. Effect of mycophenolate mofetil on progression of interstitial fibrosis and tubular atrophy after kidney transplantation: a retrospective study. BMJ Open 2014; 4(7):e005005.

53. Meier-Kriesche HU, Steffen BJ, Hochberg AM, Gordon RD, Liebman MN, Morris JA, et al. Long-term use of mycophenolate mofetil is associated with a reduction in the incidence and risk of late rejection. Am J Transplant 2003; 3(1):68-73.

54. Knight SR, Russell NK, Barcena L, Morris PJ. Mycophenolate mofetil decreases acute rejection and may improve graft survival in renal transplant recipients when compared with azathioprine: a systematic review. Transplantation 2009; 87(6):785-794.

55. Remuzzi G, Lesti M, Gotti E, Ganeva M, Dimitrov BD, Ene-Iordache B, et al. Mycophenolate mofetil versus azathioprine for prevention of acute rejection in renal transplantation (MYSS): a randomised trial. Lancet 2004; 364(9433):503-512.

56. Remuzzi G, Cravedi P, Costantini M, Lesti M, Ganeva M, Gherardi G, et al. Mycophenolate mofetil versus azathioprine for prevention of chronic allograft dysfunction in renal transplantation: the MYSS follow-up randomized, controlled clinical trial. J Am Soc Nephrol 2007; 18(6):1973-1985.

57. Maripuri S, Kasiske BL. The role of mycophenolate mofetil in kidney transplantation revisited. Transplant Rev (Orlando) 2014; 28(1):26-31.

58. Sehgal SN. Sirolimus: its discovery, biological properties, and mechanism of action. Transplant Proc 2003; 35(3 Suppl):7S-14S.

59. Kuypers DRJ. Benefit-risk assessment of sirolimus in renal transplantation. Drug Saf 2005; 28(2):153-181.

60. Lebranchu Y, Thierry A, Thervet E, Buchler M, Etienne I, Westeel PF, et al. Efficacy and safety of early cyclosporine conversion to sirolimus with continued MMF-four-year results of the postconcept study. Am J Transplant 2011; 11(8):1665-1675.

61. Weir MR, Mulgaonkar S, Chan L, Shidban H, Waid TH, Preston D, et al. Mycophenolate mofetil-based immunosuppression with sirolimus in renal transplantation: a randomized, controlled Spare-the-Nephron trial. Kidney Int 2011; 79(8):897-907.

62. Kahan BD. Sirolimus: a comprehensive review. Expert Opin Pharmacother 2001; 2(11):1903-1917.

63. Medscape. Sirolimus (Rx). [March, 2020]; Available from: https://reference.medscape.com/drug/rapamune-sirolimus-343206#4.

64. MacKeigan JP, Krueger DA. Differentiating the mTOR inhibitors everolimus and sirolimus in the treatment of tuberous sclerosis complex. Neuro Oncol 2015; 17(12):1550-1559.

65. Klawitter J, Nashan B, Christians U. Everolimus and sirolimus in transplantation-related but different. Expert Opin Drug Saf 2015; 14(7):1055-1070.

66. Kamar N, Allard J, Ribes D, Durand D, Ader JL, Rostaing L. Assessment of glomerular and tubular functions in renal transplant patients receiving cyclosporine A in combination with either sirolimus or everolimus. Clin Nephrol 2005; 63(2):80-86.

67. Steiner RW, Awdishu L. Steroids in kidney transplant patients. Semin Immunopathol 2011; 33(2):157-167.

68. Baxter JD. The effects of glucocorticoid therapy. Hosp Pract (Off Ed) 1992; 27(9):111-114, 115-118, 123 passim.

69. Miller LW. Cardiovascular toxicities of immunosuppressive agents. Am J Transplant 2002; 2(9):807-818.

70. Phanish MK, Hull RP, Andrews PA, Popoola J, Kingdon EJ, MacPhee IAM, et al. Immunological risk stratification and tailored minimisation of immunosuppression in renal transplant recipients. BMC Nephrol 2020; 21(1):92.

71. Song TR, Jiang YM, Liu JP, Wang ZL, Zeng J, Huang ZL, et al. Steroid withdrawal or avoidance is safe in high-risk kidney transplants: A systematic review and meta-analysis. Kaohsiung J Med Sci 2019; 35(6):350-357.

72. Kidney Disease: Improving Global Outcomes Transplant Work Group. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009; 9 Suppl 3:S1-155.

73. Tanriover B, Jaikaransingh V, MacConmara MP, Parekh JR, Levea SL, Ariyamuthu VK, et al. Acute rejection rates and graft outcomes according to induction regimen among recipients of kidneys from deceased donors treated with tacrolimus and mycophenolate. Clin J Am Soc Nephrol 2016; 11(9):1650-1661.

74. Woodle ES, First MR, Pirsch J, Shihab F, Gaber AO, Van Veldhuisen P, et al. A prospective, randomized, double-blind, placebo-controlled multicenter trial comparing early (7 day) corticosteroid cessation versus long-term, low-dose corticosteroid therapy. Ann Surg 2008; 248(4):564-577.

75. Matas AJ, Gillingham K, Kandaswamy R, Dunn TB, Payne WD, Sutherland DE, et al. Kidney transplant half-life (t[1/2]) after rapid discontinuation of prednisone. Transplantation 2009; 87(1):100-102.

76. Haller MC, Royuela A, Nagler EV, Pascual J, Webster AC. Steroid avoidance or withdrawal for kidney transplant recipients. Cochrane Database Syst Rev 2016; (8):CD005632.

77. Zand MS. Tofacitinab in renal transplantation. Transplant Rev (Orlando) 2013; 27(3):85-89.

78. Tran V, Shammas RM, Sauk JS, Padua D. Evaluating tofacitinib citrate in the treatment of moderate-to-severe active ulcerative colitis: design, development and positioning of therapy. Clin Exp Gastroenterol 2019; 12:179-191.

79. van Gurp E, Weimar W, Gaston R, Brennan D, Mendez R, Pirsch J, et al. Phase 1 dose-escalation study of CP-690 550 in stable renal allograft recipients: preliminary findings of safety, tolerability, effects on lymphocyte subsets and pharmacokinetics. Am J Transplant 2008; 8(8):1711-1718.

80. Busque S, Leventhal J, Brennan DC, Steinberg S, Klintmalm G, Shah T, et al. Calcineurin-inhibitor-free immunosuppression based on the JAK inhibitor CP-690,550: a pilot study in de novo kidney allograft recipients. Am J Transplant 2009; 9(8):1936-1945.

81. Vincenti F, Tedesco Silva H, Busque S, O'Connell P, Friedewald J, Cibrik D, et al. Randomized phase 2b trial of tofacitinib (CP-690,550) in de novo kidney transplant patients: efficacy, renal function and safety at 1 year. Am J Transplant 2012; 12(9):2446-2456.

82. Busque S, Vincenti FG, Tedesco Silva H, O'Connell PJ, Yoshida A, Friedewald JJ, et al. Efficacy and safety of a tofacitinib-based immunosuppressive regimen after kidney transplantation: Results From a long-term extension trial. Transplant Direct 2018; 4(9):e380.

83. van der Zwan M, Hesselink DA, van den Hoogen MWF, Baan CC. Costimulation blockade in kidney transplant recipients. Drugs 2020; 80(1):33-46.

84. Malvezzi P, Jouve T, Rostaing L. Costimulation blockade in kidney transplantation: An update. Transplantation 2016; 100(11):2315-2323.

85. Xu Y, Song G. The role of CD40-CD154 interaction in cell immunoregulation. J Biomed Sci 2004; 11(4):426-438.

86. Yamada A, Sayegh MH. The CD154-CD40 costimulatory pathway in transplantation. Transplantation 2002; 73(1 Suppl):S36-S39.

87. Law CL, Grewal IS. Therapeutic interventions targeting CD40L (CD154) and CD40: the opportunities and challenges. Adv Exp Med Biol 2009; 647:8-36.

88. Harland RC, Klintmalm G, Jensik S, Yang H, Bromberg J, Holman J, et al. Efficacy and safety of bleselumab in kidney transplant recipients: A phase 2, randomized, open-label, noninferiority study. Am J Transplant 2020; 20(1):159-171.

89. Goldwater R, Keirns J, Blahunka P, First R, Sawamoto T, Zhang W, et al. A phase 1, randomized ascending single-dose study of antagonist anti-human CD40 ASKP1240 in healthy subjects. Am J Transplant 2013; 13(4):1040-1046.

90. Okimura K, Maeta K, Kobayashi N, Goto M, Kano N, Ishihara T, et al. Characterization of ASKP1240, a fully human antibody targeting human CD40 with potent immunosuppressive effects. Am J Transplant 2014; 14(6):1290-1299.

91. Vincenti F, Klintmalm G, Yang H, Ram Peddi V, Blahunka P, Conkle A, et al. A randomized, phase 1b study of the pharmacokinetics, pharmacodynamics, safety, and tolerability of bleselumab, a fully human, anti-CD40 monoclonal antibody, in kidney transplantation. Am J Transplant 2020; 20(1):172-180.

92. Ristov J, Espie P, Ulrich P, Sickert D, Flandre T, Dimitrova M, et al. Characterization of the in vitro and in vivo properties of CFZ533, a blocking and non-depleting anti-CD40 monoclonal antibody. Am J Transplant 2018; 18(12):2895-2904.

93. Pinelli DF, Ford ML. Novel insights into anti-CD40/CD154 immunotherapy in transplant tolerance. Immunotherapy 2015; 7(4):399-410.

94. Nashan B, Tedesco H, van den Hoogen M, Berger S, Cibrik D, Mulgaonkar S, et al. CD40 inhibition with CFZ533 - A new, fully human, non-depleting, Fc silent mAB - improves renal allograft function while demonstrating comparable efficacy vs. tacrolimus in de-novo CNI-free kidney transplant recipients. Transplantation 2018; 102:S366.

95. Farkash E, Naik A, Tedesco-Silva H, Nashan B, Witzke O, van den Hoogen M, et al. CNI-free therapy with iscalimab (anti-CD40 mAb) preserves allograft histology compared to standard of care after kidney transplantation. Am J Transplant 2019; 19(suppl 3).

96. Novartis (Novartis Pharmaceuticals). Study of efficacy, safety, tolerability, pharmacokinetic (PK) and pharmacodynamic (PD) of an anti-CD40 monoclonal antibody, CFZ533, in kidney transplant recipients (CIRRUS I). 2018 January 14, 2020 [cited 2020 February 1]; Available from: https://clinicaltrials.gov/ct2/show/NCT03663335#wrapper.

97. Kumar D, LeCorchick S, Gupta G. Belatacept as an alternative to calcineurin inhibitors in patients with solid organ transplants. Front Med (Lausanne) 2017; 4:60.

98. Wojciechowski D, Vincenti F. Belatacept in kidney transplantation. Curr Opin Organ Transplant 2012; 17(6):640-647.

99. Su VCH, Harrison J, Rogers C, Ensom MH. Belatacept: a new biologic and its role in kidney transplantation. Ann Pharmacother 2012; 46(1):57-67.

100. Hardinger KL, Sunderland D, Wiederrich JA. Belatacept for the prophylaxis of organ rejection in kidney transplant patients: an evidence-based review of its place in therapy. Int J Nephrol Renovasc Dis 2016; 9:139-150.

101. Vincenti F, Rostaing L, Grinyo J, Rice K, Steinberg S, Gaite L, et al. Belatacept and long-term outcomes in kidney transplantation. N Engl J Med 2016; 374(4):333-343.

102. de Graav GN, Baan CC, Clahsen-van Groningen MC, Kraaijeveld R, Dieterich M, Verschoor W, et al. A randomized controlled clinical trial comparing belatacept with tacrolimus after de novo kidney transplantation. Transplantation 2017; 101(10):2571-2581.

103. Hehenberger K, Heilborn JD, Brismar K, Hansson A. Inhibited proliferation of fibroblasts derived from chronic diabetic wounds and normal dermal fibroblasts treated with high glucose is associated with increased formation of l-lactate. Wound Repair Regen 1998; 6(2):135-141.

104. Noble J, Jouve T, Janbon B, Rostaing L, Malvezzi P. Belatacept in kidney transplantation and its limitations. Expert Rev Clin Immunol 2019; 15(4):359-367.

105. Adams AB, Ford ML, Larsen CP. Costimulation blockade in autoimmunity and transplantation: The CD28 pathway. J Immunol 2016; 197(6):2045-2050.

106. Ville S, Poirier N, Branchereau J, Charpy V, Pengam S, Nerriere-Daguin V, *et al*. Anti-CD28 antibody and Belatacept exert differential effects on mechanisms of renal allograft rejection. J Am Soc Nephrol 2016; 27(12):3577-3588.

107. Poirier N, Azimzadeh AM, Zhang T, Dilek N, Mary C, Nguyen B, *et al*. Inducing CTLA-4-dependent immune regulation by selective CD28 blockade promotes regulatory T cells in organ transplantation. Sci Transl Med 2010; 2(17):17ra10.

108. Poirier N, Dilek N, Mary C, Ville S, Coulon F, Branchereau J, *et al*. FR104, an antagonist anti-CD28 monovalent fab' antibody, prevents alloimmunization and allows calcineurin inhibitor minimization in nonhuman primate renal allograft. Am J Transplant 2015; 15(1):88-100.

109. Watkins BK, Tkachev V, Furlan SN, Hunt DJ, Betz K, Yu A, *et al*. CD28 blockade controls T cell activation to prevent graft-versus-host disease in primates. J Clin Invest 2018; 128(9):3991-4007.

110. Poirier N, Blancho G, Hiance M, Mary C, Van Assche T, Lempoels J, *et al*. First-in-human study in healthy subjects with FR104, a pegylated monoclonal antibody fragment antagonist of CD28. J Immunol 2016; 197(12):4593-4602.

Original Article

The frequency of celiac disease in children with chronic constipation

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ABSTRACT

Objective: To investigate the frequency of celiac disease (CD) in children with chronic constipation unresponsive to conventional treatment

Design: A retrospective study

Setting: Clinics of Pediatric Gastroenterology, Mersin City Training and Research Hospital, Mersin, Turkey

Subjects: Six hundred and sixty-two pediatric patients between the ages of 2 and 18 who were diagnosed with chronic constipation according to Rome III criteria and did not respond to conservative treatments were included in the study.

Interventions: All patients underwent tissue transglutaminase antibody (tTG) IgA and total IgA tests. The cut-off value of tTG IgA is 20 U/ml. Esophago-gastroduodenoscopy was performed on all patients with tTG positivity.

Main outcome measure: All patients were evaluated in regards to celiac disease.

Results: Of the 662 patients (341 girls), the mean age was 6.48±4.32 years. The mean tTG IgA value was 7.07±17.33 U/ml and the mean total IgA value was 107.37±64.74 mg/dl. tTG positivity was detected in 22 patients (3.32%) and six of them refused endoscopy. Gastroduodenoscopy was performed to the other patients. The pathological biopsy results of nine patients (1.36%) were consistent with Marsh 3 classification score and those were diagnosed with CD. The pathological biopsy results of the other seven patients were consistent with Marsh 0 classification score.

Conclusion: We found that the prevalence of CD was three times higher in children with functional constipation than in the general population. Therefore, we suggest that serologic tests for CD should be considered in children with functional constipation unresponsive to conventional treatment.

KEY WORDS: celiac disease, chronic constipation, intestinal biopsy

INTRODUCTION

Chronic constipation is one of the most common problems of childhood and mostly functional. Its prevalence is estimated to be 3% worldwide. In addition, it may be found as a sign of other organic diseases such as celiac disease (CD). Acording to the Rome III criteria, chronic constipation is defined as a decrease in the frequency of defecation or painful defecation for at least two months[1-4].

CD is an immune-mediated systemic disease triggered by gluten intake in genetically susceptible individuals. It is one of the most common life-long diseases and its prevalence is estimated to be approximately 1% worldwide[5]. With the development of sensitive and specific serologic tests and increased awareness of CD, its prevalence has increased during the recent years.

The clinical manifestations of CD can vary extensively in clinical findings ranging from classical malabsorption symptoms including chronic diarrhea, weight loss, vitamin deficiencies and growth delay to extra-intestinal findings such as osteopenia, isolated short stature, iron deficiency anemia, neurological diseases and elevated liver enzymes[6-8].

The clinical findings of CD have changed over recent years. Instead of classical malabsorption symptoms, atypical symptoms such as abdominal pain and chronic constipation may be the only sign of CD[8].

Chronic constipation is one of the most common presenting causes of pediatric gastroenterology and

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Table 1: The demographic and laboratory characteristics of patients with chronic constipation and celiac disease

Variable	Children diagnosed with chronic constipation (n=653)	Children diagnosed with celiac disease (n=9)	P
Age (years)[&]	5.00 (IQR 6.00)	6.00 (IQR 7.00)	.321
Hemoglobine (g/dl)*	12.36±1.13	12.27±1.14	.815
tTG IgA (U/ml)[&]	3.90 (IQR 2.00)	95.00 (IQR 124.00)	<.001
Total IgA[&]	92.00 (IQR 84.00)	110.00 (IQR 73.00)	.316
Duration of constipation (months)[&]	7.00 (IQR 21.00)	24.00 (IQR 24.00)	.006

tTG: tissue transglutaminase; IQR: interquartile range; *data are presented as mean ± standard deviation; [&]data are presented as median (interquartile range)

general pediatric outpatient clinics[9]. It is mostly functional; most cases have no identifiable organic lesions or disorders. A small number of children with chronic constipation do not respond to medical treatment and may create an inescapable situation for physicians and parents. According to the latest guidelines of ESPGHAN and NASPGHAN, although the routine screening test for organic causes such as CD is not recommended in the absence of alarming symptoms, most physicians apply these tests to exclude organic causes at the first application[4].

There are a few studies about the prevalence of CD in children with chronic constipation[3,10-14]. In the current study, we aimed to investigate the frequency of CD in children with chronic constipation unresponsive to conventional treatment.

SUBJECTS AND METHODS

The present cross-sectional study was carried out between March 2017 and October 2018. Six hundred and sixty-two pediatric patients between the ages of 2 and 18 who were diagnosed with chronic constipation according to Rome III criteria and did not respond to

conservative treatments were included in the study[15,16].

Patients with any organic disease such as CD, neurologic disease, thyroid dysfunction and hypercalcemia were excluded from the study. Gluten-free patients were also excluded from the study. The current study was approved by the Ethics Committee of Mersin University.

All patients underwent tissue transglutaminase antibody (tTG) IgA and total IgA tests. The cut-off value of tTG IgA is 20 U/ml. tTG IgG test was analysed in patients with selective IgA deficiency. Esophago-gastroduodenoscopy was offered to all patients with tTG positivity, and then endoscopy was performed to those patients who accepted. At least four biopsies from duodenum and two biopsies from bulbus were obtained. The endoscopic mucosal biopsies were evaluated according to the modified Marsh classification[17].

Statistical evaluation

Statistical analysis was performed using SPSS software version 13.0 (SPAA Inc, Chicago IL, USA). Categorical data were reported as percentages and

Table 2: The laboratory data of patients with tTG IgA positivity

Patient No.	Age (year)/sex	Duration of constipation (months)	tTG IgA (U/ml)	Total IgA (mg/dl)	Pathology
1	4/f	36	174.0	107	Marsh 3a
2	5.5/f	12	181.0	72	Marsh 3c
3	7/f	48	166.7	155	Marsh 3b
4	11.5/f	12	95.0	153	Marsh 3a
5	4/f	12	33.5	110	Marsh 3b
6	6/f	12	72.6	91	Marsh 3c
7	2/f	12	200.0	72	Marsh 3a
8	11/f	12	78.4	160	Marsh 3a
9	17/f	72	30.0	115	Marsh 3a
10	4/f	42	27.3	83	Marsh 0
11	3/f	9	20.0	70	Marsh 0
12	2/f	12	27.0	55	Marsh 0
13	9/f	48	20.0	135	Marsh 0
14	5/m	48	20.0	192	Marsh 0
15	3/m	24	20.2	143	Marsh 0
16	11/m	36	37.1	201	Marsh 0

tTG: tissue transglutaminase antibody

continuous data as mean standard deviation or median (range). Mann Whitney U test was used for the parameters which did not show a normal distribution.

RESULTS

Of the 662 patients (341 girls), the mean age was 6.48±4.32 years. The mean value of tTG IgA was 7.07±17.33 U/ml and the mean value of total IgA was 107.37±64.74 mg/dl. The median duration of constipation in children with chronic constipation and CD were 7.00 (IQR 21.00) and 24.00 (IQR 24.00) months respectively (P <.05; Table 1). Two patients had selective IgA deficiency. tTG IgA positivity was detected in 22 patients (3.32%) and six of them refused endoscopy. Esophago-gastroduodenoscopy was performed for the other patients. The pathological biopsy results of nine patients (1.36%) were consistent with Marsh 3 classification score and those were diagnosed with CD. The duration of constipation ranged from 12-72 months in all patients with CD. The age of onset of constipation ranged from 1-11 years. There was no difference in regard to the age of presentation and the severity of chronic constipation between children with CD and functional chronic constipation. The pathological biopsy results of the other seven patients were consistent with Marsh 0 classification score (Table 2).

Seven of nine celiac patients had anemia and one of them also had folate deficiency. Six patients had low vitamin D levels. B12 deficiency was detected in two patients. In addition to that, two patients had growth retardation.

DISCUSSION

Chronic constipation is a very common and extremely troublesome disorder and has a negative impact on quality of life[18-20]. The relationship between chronic constipation and CD was first reported in 1972[21]. In this study, 12 patients (10.7%) were diagnosed with constipation before the diagnosis of CD.

The clinical findings of CD change over time. Atypical symptoms such as abdominal pain and chronic constipation instead of classical malabsorption symptoms may be the only sign of CD. Abdominal pain and constipation are one of the most common presenting complaints in pediatric gastroenterology outpatient clinics, and are often considered to be functional abdominal pain in the absence of alarming symptoms. Those symptoms are common with CD findings[7,22]. CD is not routinely excluded in patients with chronic constipation because of the identification of functional diseases, and routine testing for CD is also not recommended[1,12,22-25].

The rate of patients admitted only with constipation and then diagnosed with CD to all patients diagnosed with CD was reported between 6.8% to 38.9%[7,26-28].

There are only limited studies investigating the prevalence of CD in children with chronic constipation. The prevalence of CD in children with chronic constipation is reported to be 0.99-7.2%[3,10-14,23,29].

In a study including 370 patients, the prevalence of biopsy-proven CD was found to be 1.9%[12]. The authors recommend that serological tests for CD should be performed to patients with constipation unresponsive to medical treatment.

In another study involving 550 pediatric patients with chronic functional constipation, tTG positivity was found in 42 patients (7.6%), and 40 (7.2%) of them were diagnosed with CD by an intestinal biopsy[29].

In a recent study including 1046 children in Turkey, the prevalence of biopsy-proven CD was found to be 1/28 in children with constipation. Authors recommended that screening tests for CD should be considered in children with conventional treatment-resistant constipation[3].

In contrast, there are studies reporting that the prevalence of CD is not much higher in patients with constipation than in the general population[10,13,23,27]. For this reason, the authors do not recommend screening tests for CD in children with chronic constipation.

In a study conducted in Turkey including 313 children with chronic constipation, tTG positivity was found to be 2.5% in children with chronic constipation[10]. Eight patients with tTG positivity underwent gastroduodenoscopy. The pathological results of five patients were Marsh 1 and the other was Marsh 0, and those cases were evaluated as potential CD. In that study, serological evaluation of CD is not recommended in children with chronic constipation because a majority of the patients had negative serologic tests for CD.

In the present study, nine of the patients (1.36%) with chronic constipation were diagnosed with CD. All the patients with CD were closely monitored. Two patients were incompatible with gluten-free diet; they were still complaining of constipation. We have recommended psychiatric support for these patients. After adherence to gluten-free diet, constipation symptoms dissappeared in the other seven patients in six months (2-6 months).

The prevalence of CD in adults and children were found to be 0.39% and 0.47% in our country respectively[30,31]. In the current study, the prevalence of biopsy-proven CD was found to be 1.36%. In accordance with some literature, we found that the prevalence of CD was approximately three times higher in children with functional constipation than in the general population.

Limitations

The major limitations of our study was that six patients with tTG positivity refused the process of gastroduodenoscopy. If they had accepted the endoscopy, the biopsy-proven prevalence of CD would be higher. Hence, the effectiveness of the current study may be weak. Second, HLA-DQ2/8 analysis could not be performed to patients due to the high costs of HLA analysis. Third, because CD can be diagnosed at all stages in life, the follow-up period may be short. Therefore, we may have detected a lesser prevalence of CD than expected.

CONCLUSION

We found that the prevalence of CD was three times higher in children with chronic constipation than in the general population. Therefore, we suggest that serological screening tests for CD be considered in children with chronic constipation unresponsive to conventional treatment.

REFERENCES

1. Gfroerer S, Rolle U. Pediatric intestinal motility disorders. World J Gastroenterol 2015; 21(33):9683-9687.
2. Drossman DA. The functional gastrointestinal disorders and the Rome III process. Gastroenterology 2006; 130(5):1377-1390.
3. Akman S, Sahaloglu O, Dalkan C, Bahçeciler NN, Arikan C. Is celiac disease misdiagnosed in children with functional constipation. Turk J Gastroenterol 2018; 29(2):210-214.
4. Tabbers MM, DiLorenzo C, Berger MY, Faure C, Langendam MW, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. J Pediatr Gastroenterol Nutr 2014; 58(2):258-274.
5. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. J Pediatr Gastroenterol Nutr 2012; 54(1):136-160.
6. Catassi C, Anderson RP, Hill ID, Koletzko S, Lionetti E, Mouane N, et al. World perspective on celiac disease. J Pediatr Gastroenterol Nutr 2012; 55:494-499.
7. Shalaby SA, Sayed MM, Ibrahim WA, Abdelhakam SM, Rushdy M. The prevalence of celiac disease in patients fulfilling Rome III criteria for irritable bowel syndrome. Arab J Gastroenterol 2016; 17(2):73-77.
8. Khatib M, Baker RD, Ly EK, Kozielski R, Baker SS. Presenting pattern of pediatric celiac disease. J Pediatr Gastroenterol Nutr 2016; 62(1):60-63.
9. Aydoğdu S, Cakir M, Yüksekkaya HA, Arikan C, Tümgör G, Baran M, et al. Chronic constipation in Turkish children: clinical findings and applicability of classification criteria. Turkish J Pediatr 2009; 51:146-153.
10. Cakir M, Cezaroglu S, Cobanoglu U. Celiac disease in children with chronic constipation. Turk J Med Sci 2016; 46(3):651-656.
11. Khanna V, Poddar U, Yachha SK. Etiology and clinical spectrum of constipation in Indian children. Indian Pediatr 2010; 47(12):1025-1030.
12. Pelleboer RA, Janssen RL, Deckers-Kocken JM, Wouters E, Nissen AC, Bolz WE, et al. Celiac disease is overrepresented in patients with constipation. J Pediatr (Rio J) 2012; 88(2):173-176.
13. Chogle A, Saps M. Yield and cost of performing screening tests for constipation in children. Can J Gastroenterol 2013; 27(1):e35-e38.
14. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med 2003; 163(3):286-292.
15. Hyman PE, Milla PJ, Benninga MA, Davidson GP, Fleisher DF, Taminiau J. Childhood functional gastrointestinal disorders: neonate/toddler. Gastroenterology 2006; 130(5):1519-1526.
16. Rasquin A, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology 2006; 130(5):1527-1537.
17. Marsh MN. Gluten, major histocompatibility complex, and the small intestine: a molecular and immunobiologic approach to the spectrum of gluten sensitivity (celiac sprue). Gastroenterology 1992; 102(1):330-354.
18. Bellini M, Usai-Satta P, Bove A, Bocchini R, Galeazzi F, Battaglia E, et al. Chronic constipation diagnosis and treatment evaluation: the ''CHRO.CO.DI.T.E.'' study. BMC Gastroenterol 2017; 17:11.
19. Bharucha AE, Pemberton JH, Locke GR 3rd. American Gastroenterological Association technical review on constipation. Gastroenterology 2013; 144(1):218-238.
20. Suares NC, Ford AC. Prevalence of, and risk factors for, chronic idiopathic constipation in the community: systematic review and meta-analysis. Am J Gastroenterol 2011; 106(9):1582-1591.
21. Egan-Mitchell B, McNicholl B. Constipation in childhood coeliac disease. Arch Dis Child 1972; 47(252):238-240.
22. Shannahan S, Leffler DA. Diagnosis and updates in celiac disease. Gastrointest Endosc Clin N Am 2017; 27(1):79-92.
23. Dehghani SM, Ehsaei Z, Honar N, Javaherizadeh H. Frequency of celiac disease in children with chronic functional constipation in Shiraz-Iran. Middle East J Dig Dis 2015; 7:166-169.

24. Choung RS, Rubio-Tapia A, Lahr BD, Kyle RA, Camilleri MJ, Locke GR 3rd, *et al*. Evidence against routine testing of patients with functional gastrointestinal disorders for celiac disease: a population-based study. Clin Gastroenterol Hepatol 2015; 13(11):1937-1943.

25. Squires JE, Fei L, Cohen MB. Role of celiac disease screening for children with functional gastrointestinal disorders. JAMA Pediatr 2014; 168(6):514-515.

26. Pare P, Ferrazzi S, Thompson WG, Irvine EJ, Rance L. An epidemiological survey of constipation in Canada: Definitions, rates, demographics, and predictors of health care seeking. Am J Gastroenterol 2001; 96(11):3130-3137.

27. Catassi C, Kryszak D, Louis-Jacques O, Duerksen DR, Hill I, Crowe SE, *et al*. Detection of celiac disease in primary care: A multicenter case-finding study in North America. Am J Gastroenterol 2007; 102(7):1454-1460.

28. Balamtekin N, Uslu N, Baysoy G, Usta Y, Demir H, Saltik-Temizel IN, *et al*. The presentation of celiac disease in 220 Turkish children. Turkish J Pediatr 2010; 52(3):239-244.

29. Sadjadei N, Hosseinmardy S, Hakimzadeh M, Ziaei Kajbaf T, Javaherizadeh H. AntiTTG among children with chronic functional constipation unresponsive to 6 weeks of treatment of constipation. Arq Gastroenterol 2017; 54:197-200.

30. Dalgic B, Sari S, Basturk B, Ensari A, Egritas O, Bukulmez A, *et al*. Turkish Celiac Study Group. Prevalence of celiac disease in healthy Turkish school children. Am J Gastroenterol 2011; 106(8):1512-1517.

31. Sezgin O, Sarıtaş B, Aydın İ, Şaşmaz T, Lınke ES. Celiac disease prevalence in Turkey: A Population based cross-sectional study. Acta Medica Mediterranea 2016; 32:719-727.

<div align="center">Original Article</div>

Testing the depth of anesthesia in total intravenous and balanced anesthesia

<div align="center">
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ABSTRACT

Objective(s): Monitoring the depth of anesthesia during general balanced and total intravenous anesthesia using bispectral index (BIS) values and exploring potential occurrence of intraoperative awareness episodes as related to the applied anesthesia modality

Design: Randomized prospective study

Setting: Clinical Center of Vojvodina, Novi Sad, Serbia

Subjects: The study included 40 patients, 20 who received propofol anesthesia, while the other 20 received sevoflurane.

Intervention(s): Patient monitoring involved BIS values and hemodynamic parameters. After surgical intervention, the subjects were interviewed applying modified Brice questionnaire.

Main Outcome Measure(s): Modified Brice questionnaire was used to identify the episodes of intraoperative awareness. The questionnaire consisted of six questions to which multiple answers were offered.

Result(s): There was no difference in the depth of anesthesia between the two groups. There is a relationship between body weight and BIS value during the induction to anesthesia. Anesthetic dosage to maintain BIS values between 40 and 60 achieved an adequate depth of anesthesia in all patients, none of the patients reported that he/she has been awake during surgery. The patients who received propofol stated anxiety as the worst experience of surgery, while pain was reported as the worst experience by those who received sevoflurane.

Conclusion(s): Regardless of the type of anesthetic used (inhalation or intravenous), compliance with the principles of good anesthesiology practice results in no differences in the anesthetic depth and awareness during anesthesia is rare.

<div align="center">**KEY WORDS:** awareness, propofol, sevoflurane</div>

INTRODUCTION

The fear of being awake under anesthesia is one of the greatest anxieties among patients facing a surgical procedure. The incidence of anesthesia awareness ranges from 0.1 to 0.3% of anesthetized patients[1,2]. Although such situations occur only occasionally, anesthesiologists take this matter very seriously because of the correlation of shallow anesthesia with the occurrence of post-traumatic stress disorder, which can be subject to a medico legal responsibility of the anesthesiologist[3,4].

The assessment of anesthetic depth is routinely performed by monitoring and analyzing arterial blood pressure, heart rate, frequency and rhythm of breathing, muscle tone, eye tearing and perspiration. These signs are indirect and non-specific. Considering that awareness, *i.e,* patient's subjective sensation of being awake, needed to be objectively interpreted, the technology of Electroencephalogram Bispectral Analysis was introduced about 20 years ago as a direct measurement of intraoperative consciousness.

Bispectral index (BIS) is a Continuous Electroencephalogram (EEG)-derived technology used to monitor the depth of anesthesia[5]. The method relies on continuous EEG analysis of the frontal area of the brain reflecting the level of hypnosis. BIS represents a direct measure of the effects of anesthetics and sedatives on the brain, thereby enabling

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Table 1: Descriptive statistics of the investigated anthropometric and hemodynamic parameters

Variable	Average value	Standard deviation	Minimum value	Maximum value
Age (years)	58.10	15.00	22	87
Body weight (kg)	76.73	12.08	53	100
Body height (cm)	171.85	8.04	152	189
Systolic arterial pressure (mmHg)	142.60	22.70	98	189
Diastolic arterial pressure (mmHg)	79.63	10.49	54	101
Mean arterial pressure (mmHg)	105.40	16.71	69	144
Heart rate (beats per minute)	75.47	13.18	48	100

Table 2: Gender distribution according to the type of anesthesia

χ^2 test	Group S		Group P		χ^2 test	
	n	%	n	%	χ^2	P
Gender					0.107	.744
Females	8	40	7	35		
Males	12	60	13	65		
Total	20	100	20	100		

anesthesiologists to accurately manage anesthesia and monitor the status of the patient during surgical procedure and react adequately to any change of his clinical condition. Calculation of BIS value integrates the four diverse EEG parameters. Each of these parameters correlates with the relevant level of anesthetic depth. The data for each parameter are processed, analyzed and combined using appropriate software to obtain the final figure – BIS value. BIS values can range between 0 and 100. BIS values greater than 80 indicate that the patient is awake[6], whereas values between 40 and 60 are considered to provide optimal anesthetic depth during surgery[7].

BIS monitor is portable and easy to interpret, and prevents unpleasant episodes of intraopertive awareness. Optimal dosage of anesthetics allows less adverse effects and faster recovery after anesthesia[8].

The basic objective of this study was monitoring the depth of anesthesia during general balanced inhalational and total intravenous anesthesia using BIS values and exploring potential occurrence of intraoperative awareness episodes as related to the applied anesthesia modality.

SUBJECTS AND METHODS

According to the approval of the Ethics Committee of the Clinical Center of Vojvodina, the research was conducted at the Clinic for Anesthesia and Intensive Therapy of the Clinical Center of Vojvodina as a randomized prospective clinical study. The research included 40 patients of both sexes, older than 18, and categorized as American Society of Anesthesiologists group I and II according to its physical status classification system. The patients were informed about the objective of the study and written informed consent was obtained from all participants. The patients were subjected to different surgical procedures.

In line with the aims of the research, the patients were randomly distributed into two groups of 20

Table 3: The ratio between age, anthropometric and hemodynamic parameters

Variable	Group	Average value	F	P
Age (years)	P	56.9	1.324	.619
	S	59.3		
Body weight (kg)	P	79.4	0.542	.156
	S	74.0		
Body height (cm)	P	173.2	0.004	.295
	S	170.5		
Systolic arterial pressure (mmHg)	P	142.2	6.289	.902
	S	143.0		
Diastolic arterial pressure (mmHg)	P	80.4	2.274	.668
	S	78.9		
Mean arterial pressure (mmHg)	P	105.9	3.953	.853
	S	104.9		
Heart rate (beats per minute)	P	76.6	2.513	.580
	S	74.3		

Table 4: Average BIS-values according to the phase of anesthesia

Variable	Average value	Standard deviation	Minimum value	Maximum value
BIS - phase before beginning of anesthesia	96.65	3.79	79	100
BIS – induction phase	54.47	20.56	22	96
BIS – intubation phase	36.05	12.32	20	67
BIS – extubation phase	83.37	7.78	62	95

individuals. All patients received premedication with midazolam i.v. (0.03 mg/kg). Group S: Induction of anesthesia was performed using intravenous anesthetic propofol (2 mg/kg) and depolarizing neuro-muscular blocker succinylcholine (1 mg/kg). Inhalation anesthetic sevoflurane and opioid analgesic fentanyl were used at the maintenance phase of anesthesia, whereas muscle relaxation during surgery was provided using rocuronium bromide. Group P: Induction of anesthesia was performed using intravenous anesthetic propofol (2 mg/kg) and depolarizing neuro-muscular blocker succinylcholine (1 mg/kg). Maintenance of anesthesia and analgesia was accomplished using propofol and fentanyl, respectively. Rocuronium bromide was administered to provide muscle relaxation during surgery. In both groups, the anesthesia was administered according to standard protocols of routine clinical practice. The dosage of inhalation and intravenous anesthetics used to maintain anesthesia was adjusted guided by the BIS-values to maintain the range between 40 and 60. Anesthetic depth was monitored using BIS technology (BIS modul - Aspect®), whereas vital parameters were monitored using the monitor Infinity Delta® – Dräger. Visualization of BIS-values was possible immediately upon attaching the electrodes on the forehead of the patient. The values were recorded during induction of anesthesia, maintenance phase as well as during termination of anesthesia.

Modified Brice questionnaire (interview method) was used to identify the episodes of intraoperative awareness. The questionnaire consisted of six questions to which multiple answers were offered. The questions pertained to the quality and content of memories before, during and after anesthesia. Moreover, one of the questions applied to patient's subjective experience of the entire course of surgical procedure. Standard non-invasive clinical monitoring was performed during surgery, including monitoring of systolic, mean and diastolic arterial blood pressure and heart rate at five-minute intervals.

Numerical data description was performed using arithmetic mean, standard deviation and minimum/maximum values, whereas attributive properties were described using percentage and frequency distribution. During preliminary statistical analysis of numerical properties and to the purpose of assessing Gauss distribution, Kolmogorov-Smirnov and the Shapiro-Wilk tests were applied. In cases where the variable deviated from normal distribution range (Kolmogorov-Smirnov and Shapiro-Wilk test P <.05), the most adequate transformation was applied to the purpose of using parametric tests. Assessment of the interrelations between independent variables (hemodynamic and anthropometric parameters) and dependent variable (BIS-values) standard parametric (Pearson's correlation coefficient, Student's t-test for independent samples, Student's t-test for paired samples, analysis of variance, multiple linear regression) and non-parametric methods (chi-squared test, Spearman correlation coefficient) were used, depending on the nature of the data. The level of statistical significance was P <.05.

Table 5: BIS-values according to gender distribution

Variable	Average value	95% Confidence Interval for Mean		F	P
		Lower Bound	Upper Bound		
BIS - phase before beginning of anesthesia				0.127	.723
Male	96.08	0.3920	0.6952		
Female	97.60	0.4281	0.5881		
BIS – induction phase				0.025	.875
Male	54.88	45.3791	64.3809		
Female	53.80	44.7144	62.8856		
BIS – intubation phase				0.658	.422
Male	37.28	31.8084	42.7516		
Female	34.00	28.0614	39.9386		
BIS – extubation phase				1.458	.235
Male	84.52	81.4492	87.5908		
Female	81.46	76.9055	86.0278		

Table 6: Correlation relationship between hemodynamic and anthropometric parameters with BIS values

Parameters	Bispectral index values	
	r	P
Systolic arterial pressure	0.04	.85
Diastolic arterial pressure	0.03	.90
Mean arterial pressure	0.11	.62
Heart rate	0.11	.62
Age	0.04	.83
Body weight	0.55	.01
Body height	0.36	.11

r - correlation coefficient; *P* - P value

Statistical analysis of the data was performed using statistical software package SPSS (Statistical Package for Social Sciences).

RESULTS

Sample population in this research included 62.5% males and 37.5% females with an average age of 58.10±15.00 years, weight of 76.73±12.08 kg and height of 171.85±8.04 cm. Average values of hemodynamic parameters such as systolic, diastolic, mean arterial blood pressure and heart rate were 142.60±22.70 mmHg, 79.63±10.49 mmHg, 105.40±16.71 mmHg and 75.47±13.18 beats per minute, respectively (Table 1).

Distribution according to gender analyzed using χ^2 test did not reveal any statistical significance between groups receiving maintenance anesthesia with sevoflurane (group S) and propofol (group P) (χ^2 = 0.107; *P* >.05) (Table 2).

Analysis of variance did not establish any statistically significant differences between the groups with respect to age and investigated anthropometric and hemodynamic parameters (Table 3).

In the investigated sample population, the average BIS-values were as following: before anesthesia 96.65±3.79, during induction phase 54.47±20.56, during intubation phase 36.05±12.32, and during extubation phase 83.37±7.78 (Table 4).

The analysis of BIS-values at different phases of anesthesia did not reveal any statistically significant difference with respect to gender distribution (*P* >.05) (Table 5).

The assessment of correlation between anthropometric and hemodynamic parameters and BIS-values revealed statistically significant positive correlation between body weight and BIS-values at the phase of induction to anesthesia (r = 0.55; *P* =.01) (Table 6).

Statistical significance between investigated parameters at other phases of anesthesia was not established by Pearson's correlation test, irrespective of the applied anesthetic drug (propofol or sevoflurane). The analysis of BIS-values at different phases of anesthesia did not show any statistically significant difference between the group S and group P (*P* >.05) (Table 7).

Table 7: Average BIS-values at different phases of anesthesia

Variable	Group S	Group P	t	P
BIS - phase before beginning of anesthesia	96.8	96.5	0.28	.81
BIS – induction phase	58.2	50.8	1.02	.26
BIS – intubation phase	37.6	34.4	2.79	.42
BIS – extubation phase	84.5	82.2	0.88	.37

Regarding the question about the last thing the patient remembered happening before they went to sleep, the analysis of modified Brice questionnaire did not show statistically significant differences between the groups S and P (*P* >.05) (Table 8).

There was no statistically significant difference between the groups regarding the answer to the question about the first thing the patient remembered happening on waking (*P* >.05) (Table 9).

All participants answered negatively when asked if they remembered anything during anesthesia. When asked if they were dreaming during anesthesia and if the dreams were disturbing, the answers did not

Table 8: Last memories of the patient before anesthesia

Questions	Group P		Group S	
	n	%	n	%
Question 1. What was the last thing you remembered happening before you went to sleep?				
1. Time spent in the pre-op area	3	15	1	5
2. Seeing the operating room	2	10	3	15
3. Time spent with family	0	0	1	5
4. Hearing voices	8	40	7	35
5. Feeling mask on face	3	15	8	40
6. Smell of gas	0	0	0	0
7. Burning or stinging in the IV line	2	10	0	0
8. Other	2	10	0	0
Total	20	100	20	100

Table 9: First memories of the patient upon waking up after anesthesia

Questions	Group P		Group S	
	n	%	n	%
Question 2. What was the first thing you remembered happening on waking?				
1. Hearing voices	13	65	12	60
2. Feeling breathing tube	1	5	2	10
3. Feeling mask on face	1	5	0	0
4. Feeling pain	3	15	3	15
5. Seeing the operating room	1	5	0	0
6. Being in the recovery room	0	0	0	0
7. Being with family	0	0	0	0
8. Being in ICU	0	0	0	0
9. Nothing	1	5	2	10
10. Other	0	0	1	5
Total	20	100	20	100

reveal any statistically significant differences between groups S and P (Table 10).

The answer to the question regarding the patient's worst experience about the operation did not establish any statistically significance between the groups (P >.05). However, if absolute numbers are taken into consideration, larger number of patients from group P reported anxiety as the worst experience, as compared to the patients from group S, where pain was most frequently reported as the worst experience (Table 11).

Table 10: Answers to the question if the patients were dreaming during anesthesia

Group	Question 4. Did you dream during your procedure?		Total
	No	Yes	
Propofol			
n	17	3	20
%	85	15	100
Sevoflurane			
n	17	3	20
%	85	15	100

Statistically significant difference was established with respect to heart rate during extubation phase. The heart rate values established in group S were significantly higher than those in group P (P <.05) (Table 12).

DISCUSSION

Awareness during anesthesia is one of the biggest fears and distress among patients scheduled for surgical procedure. The occurrence of awareness is determined by a range of factors, mainly the type and dose of the anesthetic, metabolic characteristics of the patient and competence of the anesthesiologist to recognize the signs of awareness. Possible awareness episodes can be associated with subsequent psychical complications such as anxiety, nightmares or insomnia.

Table 11: Answers to the question regarding the patient's worst experience

Questions	Group P		Group S	
	n	%	n	%
Question 6. What was the worst thing about your operation?				
1. Anxiety	4	20	1	5
2. Pain	6	30	11	55
3. Recovery process	2	10	1	5
4. Unable to carry out usual activities	1	5	2	10
5. Awareness	0	0	0	0
6. Other	7	35	5	25
Total	20	100	20	100

Our research, which encompassed 40 participants of both sexes (62.5% males and 37.5% females) aged around 58 years, did not reveal any statistically significant differences for BIS-values in regard to the gender. Also, there were no statistically significant differences in view of anthropometric and hemodynamic parameters between the investigated groups. Similar results were reported by Ching-Kuo et al[9].

The analysis of correlation between anthropometric and hemodynamic parameters and BIS-values revealed statistically significant positive correlation of

Table 12: Hemodynamic parameters recorded during extubation phase

Variable	Group		F	P
Systolic arterial pressure (mm Hg)	S	143.95	0.10	.96
	P	144.40		
Diastolic arterial pressure (mm Hg)	S	83.95	0.88	.64
	P	86.60		
Mean arterial pressure (mm Hg)	S	109.30	0.09	.95
	P	108.90		
Heart rate (beats per minute)	S	84.45	0.57	.02
	P	73.70		

S: sevoflurane; P: propofol

body weight and BIS-values, *i.e*, BIS-values were significantly higher in patients with higher body weight. Consequently, the induction and maintenance of anesthesia in persons with higher body weight require higher doses of anesthetics[10].

Differences in anesthetic depth accomplished with inhalation and total intravenous anesthesia have been reported by Somvanshi *et al*[11]. The study included 50 participants aged 18-60, who were randomly distributed into two groups receiving maintenance anesthesia with propofol and sevoflurane. The authors established faster emergence from anesthesia in sevoflurane group. Our research did not establish any differences in BIS-values during the emergence from anesthesia. Similar results were obtained in a study of Siampalioti *et al*[12], which encompassed 100 participants allocated into four groups (sevoflurane, sevoflurane with BIS monitoring, propofol and propofol with BIS monitoring).

Considering the relatively small number of participants in our research, Brice questionnaire did not reveal any statistically significant differences between the answers obtained in the two groups. Similar results were reported in a study conducted at the Clinic for Otorhinolaryngology and Maxillofacial Surgery of the Clinical Center of Serbia[13].

The analysis of modified Brice questionnaire regarding the question about the last thing the patient remembered happening before they went to sleep did not show statistically significant differences between the groups. However, if absolute numbers are taken into consideration, the majority of patients from groups P and S reported remembering voices and having a mask on their face, respectively. Anxiety (group P) and pain (group S) were reported by majority of patients as the worst experiences about the operation.

In all participants of our study, BIS-values were maintained within a range between 40 and 60. Considering that awareness episodes did not occur in any of the patients, we can confirm that the recommended BIS range is the appropriate level to accomplish adequate anesthetic depth[7].

Do the patients dream during anesthesia? This still remains one of the mysteries behind anesthesia. Our research revealed that the same number of patients from both groups recalled some kind of dreams. Similar research[14] showed that the incidence of dreaming was significantly higher in the group receiving sevoflurane. In a study conducted by Errando *et al*[15], which encompassed 4001 individuals, dreaming was observed in 52.6% of participants, unlike only 15% of patients who reported dreaming during anesthesia and surgery in our study.

Comparison of hemodynamic parameters during extubation phase revealed statistically significant differences in heart rate values, being higher in participants receiving sevoflurane maintenance anesthesia. Fredman *et al*[16] reported correlation between propofol and consistently higher heart rate values.

CONCLUSION

Hypnosis is the essential component of general anesthesia; however, episodes of awareness may occur in clinical practice. This is a frequently raised concern, which has been receiving growing attention among professionals. Besides the indirect signs of awareness, technologies for direct monitoring of anesthetic depth such as BIS-monitoring system are available to the anesthesiologists. Based on the results of our study, we may conclude that, with strict compliance with the principles of good anesthesiology practice, there are no differences in anesthetic depth irrespective of the type of the applied anesthetic (inhalation or intravenous), and the episodes of anesthetic awareness are extremely rare.

ACKNOWLEDGMENT

Author's contributions in the manuscript: Vickovic Sanja participated in the design of the study, data collection, statystical analysis, interpretation of data and results, preparation of manuscript and literature search. Popovic Radmila participated in data systematization, design of manuscript, critical review and final revision of manuscript. Bozic Teodora participated in coordination and assisted to draft the manuscript, data interpretation, statystical analysis and literature search. Uvelin Arsen participated in preparation of manuscript, literature search, critical review and final revision of manuscript. Lukic-Sarkanovic Mirka participated in data collection, data systematization, statistical analysis, literature search and preparation of manuscript. Zdravkovic Ranko participated in data systematization, data interpretation, statistical analysis, preparation of manuscript and literature search.

REFERENCES

1.   Ranta SO, Laurila R, Saario J, Ali-Melkkila T, Hynynen M. Awareness with recall during general anesthesia: incidence and risk factors. Anesth Analg 1998; 86(5):1084-1089.
2.   Hellwagner K, Holzer A, Gustorff B, Schroegendorfer K, Greher M, Weindlmayr-Goettel M, *et al*. Recollection of dreams after short general anaesthesia: influence on

patient anxiety and satisfaction. Eur J Anaesthesiol 2003; 20(4):282-288.

3. Ghoneim M. The trauma of awareness: history, clinical features, risk factors, and cost. Anaesth Analg 2010; 110(3):666-667.

4. Mashour JA. Posttraumatic stress disorder after intraoperative awerness and high-risk surgery. Anaesth Analg 2010; 110(3):668-670.

5. Schneider G, Gelb AW, Schmeller B, Tschakert R, Kochs E. Detection of awareness in surgical patients with EEG based indices—bispectral index and patient state index. Br J Anaesth 2003; 91(3):329-335.

6. Schuller PJ, Newell S, Strickland PA, Barry JJ. Response of bispectral index to neuromuscular block in awake volunteers. Br J Anaesth 2015; 115(Suppl 1):i95-i103.

7. Recart A, Gasanova I, White PF, Thomas T, Ogunnaike B, Hamza M, *et al*. The effect of cerebral monitoring on recovery after general anesthesia: a comparison of the auditory evoked potential and bispectral index devices with standard clinical practice. Anesth Analg 2003; 97(6):1667-1674.

8. Duygu K, Asli D, Alev S, Umit K, Tahsin D, Busra T. Do we use too much propofol for sedation in colonoscopy? An Observational Study with Sedline Monitor. Kuwait Med J 2018; 50(4):432-436.

9. Ching-Kuo L, Yu-Tung F, Shiuh-Lin H, Chih-Lung L, King-The L, Kuang-I C. A comparison of propofol target controlled infusion-based and sevoflurane-based anesthesia in adults undergoing elective anterior cervical discectomy and fusion. Kaohsiung Journal of Medical Sciences 2015; 31:150-155.

10. Varagić VM, Milošević MP. Farmakologija. ELIT MEDICA. Beograd 2012.

11. Somvanshi M, Agarwal D, Tripathi A. Comparison of recovery profiles of propofol and sevoflurane anesthesia with bispectral index monitoring (BIS) in general anesthesia. National Journal of Medical Research 2015; 5(4):52-56.

12. Siampalioti A, Karavias D, Zotou A, Kalfarentzos F, Filos K. Anesthesia management for the super obese: is sevoflurane superior to propofol as a sole anesthetic agent? A double-blind randomized controlled trial. Eur Rev Med Pharmacol Sci 2015; 19(13):2493-2500.

13. Radošić NN, Kastratović DA, Tomić SD, Terzić MK, Marković SZ, Milaković BD. Detekcija budnosti tokom anestezije u otorino-maksilofacijalnoj hirurgiji primenom bispektralne analize elektroencefalograma. Med Pregl 2012; LXV(3-4):111-114.

14. Xu GH, Liu XS, Yu FQ, Gu EW, Zhang J, Wang K, *et al*. Dreaming during sevoflurane or propofol short-term sedation: a randomised controlled trial. Anaesth Intensive Care 2012; 40(3):505-510.

15. Errando CL, Sigl JC, Robles M, Calabuig E, García J, Arocas F, *et al*. Awareness with recall during general anaesthesia: a prospective observational evaluation of 4001 patients. Br J Anaesth 2008; 101(2):178-185.

16. Fredman B, Nathanson MH, Smith I, Wang J, Klein K, White PF. Sevoflurane for outpatient anesthesia: a comparison with propofol. Anesth Analg 1995; 81(4):823-828.

Original Article

Comparison of diagnostic tests performed in patients with suspected acute osteomyelitis

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ABSTRACT

Objective: Diagnosis of acute osteomyelitis (OM) is a multidisciplinary challenge, and the aim of this study was to assess the effectiveness of diagnostic tests of acute OM performed in our hospital.
Design: Retrospective study
Setting: Gulhane Training and Research Hospital, Turkey
Subjects: The charts of 119 patients with suspected acute OM were retrospectively reviewed.
Intervention: The sensitivity and specificity of Three Phase Bone Scintigraphy (TPBS), Single Photon Emission Computed Tomography/Computed Tomography (SPECT/CT), Labeled Leukocyte Scintigraphy (LLS), and Magnetic Resonance Imaging (MRI), microbiological culture results, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were determined. The clinicians confirmed final diagnosis of OM and non-OM by clinical examinations, consultations and results of all performed tests.

Main outcome measures: Diagnostic value of imaging techniques and laboratory tests regarding their cost effectiveness for diagnosis of OM were evaluated.
Results: The clinicians diagnosed 64 patients as acute OM and 55 as non-OM. The sensitivity and specificity of TPBS, SPECT/CT, LLS, MRI, CRP and ESR were between 80.8-100% and 67.3-100%, respectively. The presence of diabetes mellitus was higher in the OM than in the non-OM group (P=.01), while the level of HbA1c was not significantly different (P=.66). The most grown microorganism was *Staphylococcus aureus*. The mean cost of diagnosis was 52.07±36.59 USD for the OM group and 44.98±37.19 USD for the non-OM group (P=.016).
Conclusion: Our results support the reliability of TPBS and LLS, particularly when combined with SPECT/CT, as diagnostic tools when MRI is contraindicated or unavailable for evaluating patients with suspected acute OM.

KEY WORDS: acute osteomyelitis, diagnosis, imaging, nuclear medicine

INTRODUCTION

Osteomyelitis (OM) is a progressive infection of bone that can lead to bone devastation, necrosis and deformation due to inflammatory reactions. The disease can be classified as acute or chronic based on the duration of the illness, and as hematogenous or contiguous based on the mechanism[1]. Acute OM is diagnosed when the disease is discovered within two weeks after the initial onset, whereas chronic OM refers to a disease that has existed for several months at the time of diagnosis[2]. Contiguous OM is divided into two categories based on the presence or absence of associated vascular insufficiency[1,3].

Hematogenous OM occurs more frequently in children, with the metaphysis of long bones, particularly the tibia and femur, usually affected. In adults, the most common site is the vertebral bodies[1,4]. Contiguous OM usually spreads from adjacent tissues or following direct inoculation during trauma or surgery in young adults, whereas older adults typically show infection in relation to decubitus ulcers and infected joint arthroplasties. OM due to vascular insufficiency is frequently associated with the presence of underlying diabetes mellitus[2,3].

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Hematogenous OM is generally monomicrobial, whereas contiguous OM may be either monomicrobial or polymicrobial. The most commonly isolated organism is *Staphylococcus aureus* in all types of OM in adults and children. Other common pathogens are coagulase-negative staphylococci, beta-hemolytic streptococci, enterococci, aerobic gram-negative bacilli (including *Pseudomonas* species), *Enterobacter* species and *Escherichia coli*. The identification of the causative microorganism is crucial for both diagnosis and treatment[1,5].

Signs and symptoms may vary depending on the category of infection, the infective organism, the anatomic location and other factors related to the patient. A suspicion of OM arises from the initial history and physical examination findings. Confirmation usually entails a combination of radiologic, biochemical, microbiologic and pathologic tests[2,6].

The most useful imaging techniques are Three Phase Bone Scintigraphy (TPBS), Single Photon Emission Computed Tomography/Computed Tomography (SPECT/CT), Labeled Leukocyte Scintigraphy (LLS) and Magnetic Resonance Imaging (MRI). Blood cultures should be taken in suspected OM cases, even though they are often negative, except in hematogenous OM. The diagnostic gold standard of OM is doing bone biopsy for histopathologic examination and tissue culture[2-4,7]. The erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels are often elevated. The white blood cell (WBC) count can be normal or elevated[8-10].

Many diagnostic tools are available; however, failure to diagnose and promptly treat OM can lead to chronic OM, whereas early intervention, followed by a proper antibiotic regimen, prevents chronic OM[11].

The aims of the present study were to evaluate the diagnostic tests for OM performed in our hospital, to analyze their performance in diagnosis and to determine their cost effectiveness.

SUBJECTS AND METHODS

We retrospectively reviewed the medical charts of 119 patients admitted to the Gulhane Training and Research Hospital with clinically suspected acute OM between January 2017 and December 2018. Patients who had a previous history of both acute and chronic OM were excluded from the study. The hospital records, including laboratory test results and radiodiagnostic and radionuclide-imaging tools, of the remaining patients were used in the study. The tools used for the diagnosis of acute OM, including microbiology (wound and blood culture), biochemistry (WBC, CRP, ESR) tests, radionuclide scanning (TBPS, LLS, SPECT/CT), and radiodiagnostics (MRI), were

determined and their diagnostic compatibilities, up to the final clinical diagnosis, were statistically evaluated.

TPBS was performed with technetium-99m-methylene diphosphonate, and the in vitro leukocyte labeling for LLS was done with technetium-99m-hexamethylpropyleneamine oxime.

The clinicians' definitive diagnoses were made based on their clinical examinations, consultations and the results of all performed tests. Patient history of diabetes mellitus, orthopedic prosthesis, bone surgery and trauma were also recorded. This study was approved by the decisions of the Ethics Committee of the University of Health Sciences (April 26, 2018, numbered 18/110) and The Medical Board of the Gulhane Training and Research Hospital (24 May 2018/ 799).

Statistical tests

The data were statistically analyzed using SPSS 15.0 software (SPSS Inc., Chicago, IL, USA). Categorical data were presented as frequencies and percentages, while continuous data that had a normal distribution were presented as means and standard deviations. The diagnostic tests were analyzed for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). The distribution of variables was controlled using the Kolmogorov-Smirnov test. Continuous values, in cases of normal distribution, were analyzed with Student t-test for two independent groups and with one-way analysis of variance for three or more independent groups. The Mann-Whitney U test was used for the continuous variables that did not show a normal distribution. The Chi-square test was used to compare categorical data. A *P*-value <.05 between groups was considered statistically significantly.

RESULTS

In total, 119 adults (42 women [35.3%], 77 men [64.7%]) who presented with suspected acute OM between January 2017 and December 2018 were included in the study. The cases enrolled in the study were under follow up at different clinics, mainly orthopedics (n=72), infectious disease (n=32), plastic surgery (n=4), internal medicine (n=3) and miscellaneous others (n=8). The age range was 18 to 85 years (52.68±17.63). No statistical difference was noted between the patients with and without a diagnosis of acute osteomyelitis (OM and non-OM, respectively) in terms of age or gender (*P*=.17 and *P*=.72, respectively).

The distributions of the involved regions in patients diagnosed with acute OM were as follows: 52 cases (81.3%) with OM of the lower extremities, nine cases with OM of the upper extremities (14%) and three cases with OM of the cranium. The affected bones in

Table 1: The distribution of radionuclide and radiodiagnostic tools and their diagnostic value in the study groups

Final diagnosis	Test results	TPBS		SPECT/CT		LLS		MRI	
		n	%	n	%	n	%	n	%
OM	Positive	62	96.9	52	92.9	16	100	21	80.8
(n=64)	Negative	2	3.1	4	7.1	0	0	5	19.2
	Sum	64	100	56	87.5	16	25.0	26	40.6
Non-OM	Positive	18	32.7	9	27.3	0	0	1	9.1
(n=55)	Negative	37	67.3	24	72.7	14	100	10	90.9
	Sum	55	100	33	60.0	14	25.5	11	20.0
P-value		<.001		<.001		<.001		<.001	
Sensitivity		96.9%		92.9%		100%		80.8%	
Specificity		67.3%		72.7%		100%		90.9%	
Positive Predictive Value		77.5%		85.2%		100%		95.5%	
Negative Predictive Value		94.9%		85.7%		100%		66.7%	

TPBS: Three Phase Bone Scintigraphy; SPECT/CT: Single Photon Emission Computed Tomography/Computed Tomography; LLS: Labeled Leukocyte Scintigraphy; MRI: Magnetic Resonance Imaging; OM: Osteomyelitis; non-OM: Diagnosed as other than osteomyelitis.

the lower extremity were the femur (n=5), tibia (n=10) and feet bones (n=32). The 27 patients with acute OM of the feet also had diabetes mellitus.

TPBS was performed in all patients who were followed up with a preliminary diagnosis of acute OM. SPECT/CT imaging was conducted in 89 (74.8%), LLS in 30 (25.2%) and MRI in 37 (31.1%) patients. The results were summarized in Table 1. Statistical significance was calculated for each of four tests performed in the diagnosis of OM (P <.001). Seven patients underwent all four imaging tests and six of them were diagnosed with acute OM. The CRP and ESR values were significantly higher for the OM than for the non-OM group (P <.001; Fig 1 and 2), whereas

the WBC counts were not significantly different (P=.56; Table 2).

The statistical analysis revealed no significant differences between OM and non-OM groups in terms of previous orthopedic surgery, prosthesis and trauma (P=.78). However, the number of patients with diabetes mellitus was significantly higher in the OM than in the non-OM group (P=.01). The glycated hemoglobin (HbA1c) levels did not differ significantly between the two groups (P=.66; Table 3).

Microbiological cultures (50 wound cultures and 15 of which had a synchronous blood culture) were performed in 50 of 119 patients, and 39 wound cultures showed microbial growth (29 monomicrobial, 9

Table 2: The CRP, ESR and WBC values of cases in study groups

Final diagnosis	Test results	CRP		ESR		WBC	
		Frequency (n)	Mean±SD	Frequency (n)	Mean±SD	Frequency (n)	Mean±SD
OM	High positive	50	61.62±40.02	47	78.26±18.70	-	-
(n=64)	Positive	14	11.73±4.04	14	36.50±10.52	4	15.31±5.51
	Normal	0	0	3	15.67±5.51	60	7.53±1.75
	Mean±SD		50.71±41.01		66.19±26.56		8.02±2.81
Non-OM	High positive	7	35.21±12.70	10	62.70±15.70	-	-
(n=55)	Positive	19	11.94±3.57	17	37.82±9.74	5	12.23±1.62
	Normal	29	3.28±1.51	28	11.81±6.54	50	7.47±1.84
	Mean±SD		10.34±11.45		29.10±21.88		7.90±2.26
P-value		<.001		<.001		.56	
Sensitivity of high positive		100%		94.0%			
Sensitivity of positive		100%		95.3%		6.3%	
Specificity of high positive		80.6%		73.7%			
Specificity of positive		52.7%		50.9%		90.9%	
PPV of high positive		87.7%		82.5%			
PPV of positive		71.1%		69.3%		44.4%	
NPV of high positive		100%		90.3%			
NPV of positive		100%		90.3%		45.5%	

OM: Osteomyelitis; non-OM: Diagnosed as other than osteomyelitis; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; WBC: white blood cells; PPV: positive predictive value; NPV: negative predictive value. High positive describes >20 mg/l for CRP and >50 mm/h for ESR. Positive describes between 5-20 mg/l for CRP, 25-50 mm/h for ESR and >10x103 cells/μl for WBC.
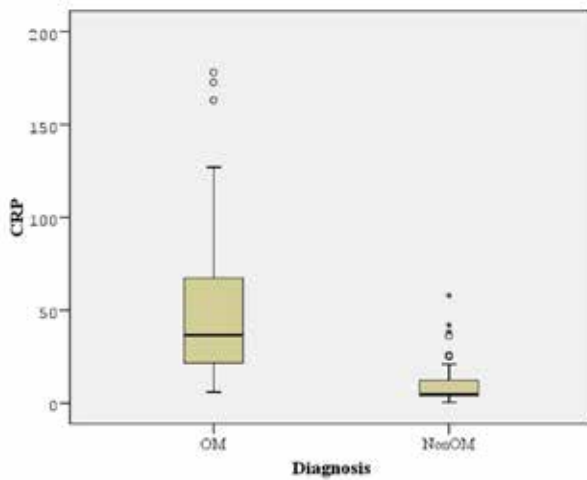
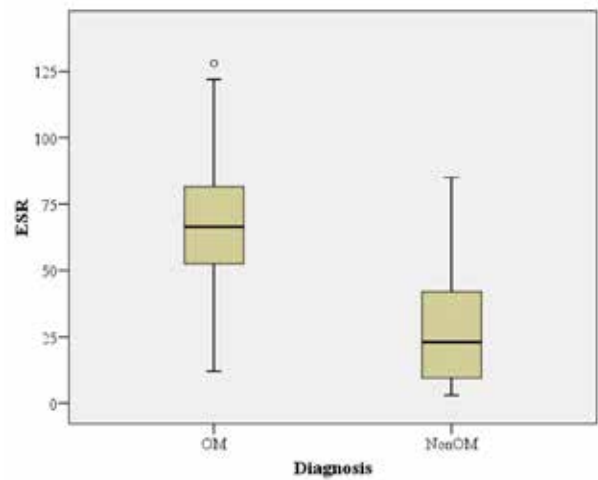**Fig 1:** C-reactive protein (CRP) values of the groups (*P* <.001).

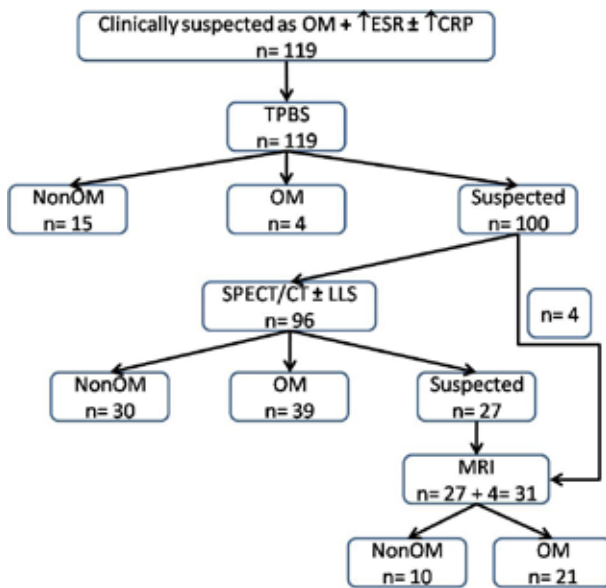**Fig 2:** The erythrocyte sedimentation rate (ESR) values of the study groups (*P* <.001).

Fig 3: Diagnostic flow chart used in our hospital for patients with suspected osteomyelitis.
ESR: Erythrocyte Sedimentation Rate, CRP: C-Reactive Protein, TPBS: Three Phase Bone Scintigraphy, SPECT/CT: Single Photon Emission Computed Tomography/Computed Tomography, LLS: Labeled Leukocyte Scintigraphy, MRI: Magnetic Resonance Imaging, OM: Osteomyelitis, NonOM: Diagnosed as other than osteomyelitis.

bimicrobial and one trimicrobial), however bacterial growth were observed only in two blood cultures. In the OM group, wound cultures and blood cultures were available for 35 (27 positive) and 13 (1 positive; *Prevotella bivia*) patients, respectively. In the non-OM group, wound cultures and blood cultures were available for 15 (12 positive) and two (one positive; *Pseudomonas aeruginosa*) patients, respectively. The most common Gram-positive organism was *Staphylococcus aureus*, followed in frequency by *Streptococcus* subspecies, and the most common Gram-negative organism was *Pseudomonas aeruginosa* in the wound cultures. Descriptive details of wound culture results are given briefly in Table 4.

We also performed a cost analysis for the patients with suspected acute OM. The mean cost per person was 48.79±36.89 USD. The mean cost of patients diagnosed with OM was 52.07±36.59 USD and this cost was statistically higher than the mean cost of 44.98 ± 37.19 USD for patients whose findings ruled out OM (*P*=.016).

DISCUSSION

The many classifications of OM are based on different criteria, such as the location of infection, duration of illness, infection source and the condition

Table 3: The concomitant findings and diabetes mellitus in patients suspected with osteomyelitis

Final diagnosis	Results	Prosthesis		Tr/Op		DM		HbA1c	
		n	%	n	%	n	%	n	Mean±SD
OM (n=64)	Yes	16	25.0	16	25.0	36	56.3	22	9.53±2.84
	No	48	75.0	48	75.0	28	43.8		
Non-OM (n=55)	Yes	15	27.3	16	29.1	18	32.7	11	9.08±2.30
	No	40	72.7	39	70.9	37	67.3		
P-value		.78		.78		.01		.66	

Tr/Op: Trauma or non-prosthetic orthopedic surgery; DM: diabetes mellitus; HbA1c: glycated hemoglobin; OM: osteomyelitis; non-OM: diagnosed as other than osteomyelitis.

Table 4: Results of bacterial isolates in wound cultures

Variables	OM (n)	Non-OM (n)	Total (n)
Number of samples	35	15	50
Positive cultures	27	12	39
Monomicrobial	19	10	29
Polymicrobial	8	2	10
Gram (+)	21	7	28
Gram (-)	15	7	22
S. aureus	7	6	13
Coagulase-negative staphylococci	4	1	5
P. aeruginosa	3	2	5
Streptococcus spp.	4[1]	0	4
Enterococcus spp.	3[2]	0	3
Acinetobacter spp.	3[3]	1[4]	4
Actinomycetes urogenitalis	2	0	2
Escherichia coli	2	2	4
Morganella morganii	1	1	2
Serratia marcescens	1	1	2
Arcanobacterium haemolyticum	1	0	1
Providencia rettgeri	1	0	1
Citrobacter freundii	1	0	1
Neisseria subflava	1	0	1
Klebsiella pneumoniae	1	0	1
Stenotrophomonas maltophilia	1	0	1

OM: osteomyelitis; non-OM: diagnosed as other than osteomyelitis.
[1]S.pyogenes 2, S.anginosus 1, S.oralis 1
[2]E.faecalis 2, E.cloacae 1
[3]A.baumannii 3
[4]A.lwoffii

of the host. The diagnosis of OM is not always apparent, so radiologic, microbiologic, biochemical and pathologic tests are frequently necessary for the diagnostic work-up. The current guidelines suggest that a diagnosis of OM should be based both on clinical examination, microbiological tests and imaging studies[1].

In our study, the 119 adult patients who were pre-diagnosed with acute OM underwent TPBS and SPECT/CT (n=89, 74.8%), LLS (n=30, 25.2%) and MRI (n=37, 31.1%). No association was noted between the departments involved in the follow-up and the examinations performed (P=.138). A diagnostic flowchart is shown in Fig 3. Nineteen patients had only undergone a TPBS and 41 patients had undergone both TPBS and SPECT/CT, without other radio-diagnostic or radionuclide scans. In 15 of the 19 patients who had no other tests, acute OM diagnosis was initially ruled out; however, the rest of these patients were diagnosed with acute OM based on clinical findings and laboratory tests. Of the 41 patients who had undergone both TPBS and SPECT/CT, 25 were diagnosed with acute OM, whereas the diagnosis was excluded in 16. The mean cost of diagnosis in 59 patients who underwent either TPBS or TPBS and SPECT/CT was 23.35±8.15 USD, whereas the diagnostic cost for the other 60 patients was 73.81±37.04 USD

(P <.001). Consequently, almost half of the patients with a pre-diagnosis of OM would require additional examinations and the associated costs for an accurate clinical decision.

The sensitivity and specificity of TPBS are generally reported as 90% and 25%, respectively. If a TPBS is negative, then OM is very unlikely. However, the findings of TPBS are not specific for OM. The reason for the low specificity is that TPBS has low discrimination when the bony structures are complicated by trauma or surgery because localized uptake in TPBS is observed both in cases of active infection and in all other pathologies that result in increased osteoblastic activity. TBPS also fails to differentiate between soft tissue infection and OM[4,12,13]. An abnormal TPBS result therefore requires further investigation, especially in cases of trauma or prosthesis. Adding SPECT/CT to TPBS enhances the diagnostic performance by providing an exact localization of the increased radionuclide uptake[11,13]. The sensitivity, specificity, PPV and NPV values of TPBS alone were 96.9%, 67.3%, 77.5% and 94.9%, respectively, whereas these values for TPBS with SPECT/CT were 96.9%, 75.8%, 87.1% and 92.6%, indicating an increase in specificity and PPV when compared to TBPS alone.

LLS is related to the accumulation of radiolabeled WBC in areas suspected of infection; therefore, it differs from TPBS, which reflects the status of perfusion and osteoblastic activity[11]. The ranges in the sensitivity and specificity of LLS have been reported as 75-100% and 67-100%, respectively[14]. Our groups of cases showed LLS sensitivity, specificity, PPV and NPV values of 100%. In our study, 30/80 patients who had undergone a TPBS were also scanned with LLS. The LLS findings supported OM in 16 of these patients, and OM was excluded in 14. LLS improved the diagnostic evaluation of OM by excluding false positives obtained with TPBS, and our results were consistent with previous studies[15,16].

MRI is considered to be the most useful imaging technique for evaluating suspected acute OM, as it provides anatomic detail, including cortical destruction and soft tissue extension, within three to five days of the disease onset[4,14]. The ranges of sensitivity and specificity of MRI were reported as 78-90% and 60-90%, respectively[4]. In the present study, MRI had a modest sensitivity (80.8%), a high specificity (90.9%) and PPV (95.5%), and a low NPV (66.7%).

The serum inflammatory markers CRP and ESR can be utilized for both the diagnosis and the follow-up of patients with OM. Previous studies suggested that elevated CRP and ESR levels may be used for the diagnosis of OM with a sensitivity and specificity of >70%. However, the sensitivity and specificity of CRP

and ESR for the diagnosis of OM vary depending on the cutoff value, as higher cutoff values decrease the sensitivity and increase the specificity[10,17,18]. In our study, when the cutoff value of ESR was accepted as 25 mm/h, the sensitivity and specificity were 95.3% and 50.9%, respectively. When the cutoff value of ESR was increased to 50 mm/h, the sensitivity decreased to 94% and the specificity increased to 73.7%. Similarly, when the cutoff value of CRP was chosen as 5 mg/dl or 20 mg/dl, the specificity values were 52.7% and 80.6%, respectively. The mean CRP and ESR values were 50.71±41.01 mg/dl and 66.19±26.56 mm/h respectively in the acute OM group and 10.34±11.45 mg/dl and 29.10±21.88 mm/h respectively in the non-OM group (Fig 1 and 2). Our results suggest that the mean ESR and CRP levels were significantly higher in the patients who had OM than in those who did not (both $P < .001$).

Healthy bone is highly resistant to infection. Most hematogenous OM is monomicrobial, although OM due to trauma or implanted prosthetic material is often polymicrobial (with organisms such as *S. aureus*, coagulase-negative staphylococci and gram-negative enteric bacteria). *S. aureus* is typically the most common pathogen responsible for hematogenous, trauma-related or prosthetic-material-related, and diabetic foot OM. The gold standard technique for obtaining a specimen for microbiological culture is a bone biopsy, but purulent material from drainage wounds can be cultured, although it often does not reflect the OM agent and may be confused with a wound infection or contamination by skin flora[3,4].

In the present study, microbiological wound cultures were obtained from 50 of the 119 patients with suspected OM. Bacterial growth was observed in 39 patients, and 27 of those patients were diagnosed with OM. In those cases, the microbiological cultures were performed to determine the treatment rather than to confirm the diagnosis of OM. The most frequently isolated bacterium was *S. aureus* in our study, and this result was consistent with previous studies.

Acute hematogenous OM generally originates as a bloodstream infection and occurs predominantly in children, whereas a reason, such as diabetes mellitus, trauma or orthopedic prosthesis, usually underlies OM in adults[1]. Our analysis of 64 patients with acute OM revealed 27 (42.2%) diagnoses associated with diabetic feet, 16 (25%) with orthopedic prostheses, and 16 (25%) with extra-prosthetic operations or traumas. A total of 59 (92.2%) patients with OM had an underlying etiology. However, no statistically significant difference was noted between prosthesis, trauma or the presence of any previous operation involving the suspect bone among the groups (P=.78). Diabetes mellitus, as an important underlying cause of

OM, was present in 54 of 119 patients and 36 of them were diagnosed with OM (27 diabetic feet). A statistically significant difference was noted between the two groups regarding the presence of diabetes mellitus (P=.01), but no significant difference was evident in HbA1c values between groups (P=.66).

The diagnosis of OM remains a challenge, and all imaging techniques have advantages and disadvantages. Both TPBS and LLS, when used alone, are relatively poor at distinguishing between soft tissue infection and OM because of their reduced spatial resolutions[19]. Previous surgical treatment or trauma also reduces the specificity of TPBS. Negative results for most of the imaging techniques virtually ruled out OM, but positives were generally inconclusive for diagnosis[20]. SPECT/CT, combined with TPBS or LLS, is helpful for the diagnosis of OM[16,21,22]. Our study has also shown that excluding OM is more cost effective and less expensive than diagnosing OM. The mean cost of the patients diagnosed with OM was 52.07±36.59 USD, and the mean cost of diagnostic tests in the cases without OM was 44.98±37.19 USD, and this difference was statistically significant (P=.016).

CONCLUSION

MRI is the imaging modality of choice for the investigation of suspected OM in the current evidence-based guidelines. Previous studies and our results support that, in cases where MRI is contraindicated or unavailable, TPBS and LLS, particularly when combined with SPECT/CT, are useful diagnostic tools in evaluating patients with suspected OM.

REFERENCES

1. Schmitt SK. Osteomyelitis. Infect Dis Clin North Am 2017; 31(2):325-338.
2. Carek PJ, Dickerson LM, Sack JL. Diagnosis and management of osteomyelitis. Am Fam Physician 2001; 63(12):2413-2420.
3. Fritz JM, McDonald JR. Osteomyelitis: approach to diagnosis and treatment. Phys Sportsmed 2008; 36(1):nihpa116823.
4. Hatzenbuehler J, Pulling TJ. Diagnosis and management of osteomyelitis. Am Fam Physician 2011; 84(9):1027-1033.
5. Jorge LS, Chueire AG, Rossit AR. Osteomyelitis: a

current challenge. Braz J Infect Dis 2010; 14(3):310-315.

6. Berbari EF, Steckelberg JM, Osmon DR. Osteomyelitis. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. 7th ed. PA: Philadelphia, Churchill Livingstone/Elsevier; 2010. p. 1457-1467.

7. Love C, Palestro CJ. Nuclear medicine imaging of bone infections. Clin Radiol 2016; 71(7):632-646.

8. Chiappini E, Mastrangelo G, Lazzeri S. A case of acute osteomyelitis: An update on diagnosis and treatment. Int J Environ Res Public Health 2016; 13(6):539.

9. Lin Z, Vasudevan A, Tambyah PA. Use of erythrocyte sedimentation rate and C-reactive protein to predict osteomyelitis recurrence. J Orthop Surg (Hong Kong) 2016; 24(1):77-83.

10. Michail M, Jude E, Liaskos C, Karamagiolis S, Makrilakis K, Dimitroulis D, et al. The performance of serum inflammatory markers for the diagnosis and follow-up of patients with osteomyelitis. Int J Low Extrem Wounds 2013; 12(2):94-99.

11. Kim C, Lee SJ, Kim JY, Hwang KT, Choi YY. Comparative analysis of (99m)Tc-MDP Three-Phase Bone Scan with SPECT/CT and (99m)Tc-HMPAO-Labeled WBC SPECT/CT in the differential diagnosis of clinically suspicious post-traumatic osteomyelitis. Nucl Med Mol Imaging 2017; 51(1):40-48.

12. Horger M, Eschmann SM, Pfannenberg C, Storek D, Vonthein R, Claussen CD, et al. Added value of SPECT/CT in patients suspected of having bone infection: preliminary results. Arch Orthop Trauma Surg 2007; 127(3):211-221.

13. Palestro CJ. Radionuclide imaging of osteomyelitis. Semin Nucl Med 2015; 45(1):32-46.

14. Lauri C, Tamminga M, Glaudemans A, Juarez Orozco LE, Erba PA, Jutte PC, et al. Detection of osteomyelitis in the diabetic foot by imaging techniques: A systematic review and meta-analysis comparing MRI, White Blood Cell Scintigraphy, and FDG-PET. Diabetes Care 2017; 40(8):1111-1120.

15. Ballani NS, Al-Huda FA, Khan HA, Al-Mohannadi S, Mahmood H, Al-Enezi F. The value of quantitative uptake of (99m)Tc-MDP and (99m)Tc-HMPAO white blood cells in detecting osteomyelitis in violated peripheral bones. J Nucl Med Technol 2007; 35:91-95.

16. Poirier JY, Garin E, Derrien C, Devillers A, Moisan A, Bourguet P, et al. Diagnosis of osteomyelitis in the diabetic foot with a 99mTc-HMPAO leucocyte scintigraphy combined with a 99mTc-MDP bone scintigraphy. Diabetes Metab 2002; 28(6 Pt 1):485-490.

17. Ertugrul BM, Savk O, Ozturk B, Cobanoglu M, Oncu S, Sakarya S. The diagnosis of diabetic foot osteomyelitis: examination findings and laboratory values. Med Sci Monit 2009; 15(6):CR307-12.

18. Rabjohn L, Roberts K, Troiano M, Schoenhaus H. Diagnostic and prognostic value of erythrocyte sedimentation rate in contiguous osteomyelitis of the foot and ankle. J Foot Ankle Surg 2007; 46(4):230-237.

19. Simpfendorfer CS. Radiologic approach to musculoskeletal infections. Infect Dis Clin North Am 2017; 31(2):299-324.

20. Trevail C, Reddy PR, Sulkin T, Bartlett G. An evaluation of the role of nuclear medicine imaging in the diagnosis of periprosthetic infections of the hip. Clin Radiol 2016; 71(3):211-219.

21. Lee YJ, Sadigh S, Mankad K, Kapse N, Rajeswaran G. The imaging of osteomyelitis. Quant Imaging Med Surg 2016; 6(2):184-198.

22. Govaert GA, IJpma FF, McNally M, McNally E, Reininga IH, Glaudemans AW. Accuracy of diagnostic imaging modalities for peripheral post-traumatic osteomyelitis - a systematic review of the recent literature. Eur J Nucl Med Mol Imaging 2017; 44(8): 1393-1407.

Original Article

Comparison of psoriasis area and severity index and physician's global assessment in determining psoriasis severity

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ABSTRACT

Objective: In clinical studies, it is crucial to assess psoriasis severity accurately and with no substantial variation between different raters. The Psoriasis Area and Severity Index (PASI) and Physician Global Assessment (PGA) are the two most commonly used tools for the assessment of psoriasis severity. The aim of this study was to evaluate the intra-rater and inter-rater reliability of these methods and to determine whether inter-rater reliability is affected by rater experience.
Design: An open uncontrolled study
Setting: Dermatology Department of Ege University, Medical Faculty
Subjects: Fifty-five patients with plaque psoriasis who were examined between 15 August 2012 and 15 November 2012 in the dermatology department of Ege University
Interventions: Three dermatology residents with varying

experience evaluated the patients individually using both the PASI and PGA for each patient (in that order).
Main Outcome Measure: PASI and PGA
Results: PASI and PGA scores showed high intra-rater correlation for all three residents. Inter-rater reliability for PASI was high between the most experienced and second most experienced resident and between the most experienced and least experienced resident. However, inter-rater reliability for PGA was high between the most experienced and second most experienced residents, but only moderate between the most experienced and least experienced resident.
Conclusions: There were no significant interrater differences between PGA and PASI scores in our study. However, because PGA is more subjective and may be affected by rater experience, PASI is considered to be a more reliable method for assessing severity of psoriasis.

KEY WORDS: physician global assessment, psoriasis area and severity index, psoriasis severity score

INTRODUCTION

Psoriasis is a chronic skin disease which affects people for many years. Many different methods have been used to attempt to treat psoriasis; current treatment options include topical treatments, phototherapy, systemic and biological agents. Treatment is selected based on disease severity[1].

The development of new treatment modalities has enhanced the importance of accurate psoriasis severity evaluation in daily clinical assessment and in clinical trials comparing disease severity before and after

treatment to evaluate drug efficacy[2,3]. Psoriasis severity is multidimensional, and there is currently no method that assesses all of these dimensions[4]. Despite the fact that psoriasis severity is the most important determiner of treatment, there is no ideal method which is fully verified or globally accepted[5-7]. Naldi *et al* reviewed 249 randomized clinical studies on psoriasis treatment conducted from 1977 to 2000 and determined that 44 different scoring systems were used in 171 studies, with the most commonly used being the Psoriasis Area and Severity Index (PASI) (83

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Table 1: The Psoriasis Area and Severity Index (PASI)

PASI	Area coefficient	Erythema	Induration	Scaling	Area
Scalp	0.1 x	(+	+	)	x
Body	0.3 x	(+	+	)	x
Upper extremity	0.2 x	(+	+	)	x
Lower extremity	0.4 x	(+	+	)	x
	Area%		Severity		
	0= none		0= none		
	1=0-9		1=mild		
	2=10-29		2=moderate		
	3=30-49		3=severe		
	4=50-69		4=very severe		
	5=70-89				
	6=90-100				

studies, 33.3%)[8]. Spuls *et al* reviewed psoriasis studies conducted between 2000 and 2007 and discovered that 53 different psoriasis severity assessment methods were used, with PASI again being the most common[7].

Although PASI is the most validated and comprehensive scoring scale[6], it does have certain limitations. The PASI can be difficult to interpret, and does not evaluate the hands, nails, feet, face and genital area, impact on life quality or comorbidities. Furthermore, the sensitivity of the upper part of the scale is low and the upper half of the scale is unnecessary, and it has low sensitivity for mild and moderate psoriasis with low body surface involvement[3,6].

After PASI, the Physician's Global Assessment (PGA) is the second most commonly used method in clinical trials[2]. Although the PGA is less objective compared to the PASI, the PGA is easier to apply and understand[9].

Due to the continuing uncertainty regarding the best method of assessing psoriasis severity, in the present study, we aimed to investigate the intra-rater correlation between PASI and PGA scores in patients with chronic plaque psoriasis and determine whether inter-rater reliability is affected by rater experience.

SUBJECTS AND METHODS

Fifty-five patients with chronic plaque psoriasis who presented to our Dermatology department between August 2012 and November 2012 were included in the study. Permission from local ethics committee was taken. Inclusion criteria for the study were having chronic plaque psoriasis and being over the age of 18. Patients with other forms of psoriasis and those who did not agree to be examined separately by three different dermatologists were excluded from the study. All participants provided written informed consent after a full explanation of the nature and purpose of the study.

The patients were evaluated by three dermatology residents with different levels of experience: Physician A was most experienced with three years of residency; Physician B had two years of residency; and Physician C was the least experienced with one year of residency. Each physician used the PASI and PGA to evaluate the 55 psoriasis patients. The patients' demographic data and treatments received for psoriasis were recorded by Physician A in their case report forms.

All three residents examined the patients individually. They completed the erythema, induration, scale and area sections of the PASI table (Table 1) and indicated a number for the patient in the PGA table (Table 2) in the patients' case report forms. Physician A later did the necessary calculations for the PASI scores.

In our study, PGA 0-2 and PASI ≤4 were accepted as mild, PGA 3-4 and PASI 4.1-10 as moderate, and PGA 5-6 and PASI > 10 as severe psoriasis.

Statistical analysis of the study data was done using SPSS 15.0 software. Descriptive continuous variables

Table 2: The Physician's Global Assessment (PGA)

Definition	Description	Score
Severe	Very marked plaque elevation, scale and/or erythema	6
Moderate to severe	Marked plaque elevation, scale and/or erythema	5
Moderate	Moderate plaque elevation, scale and/or erythema	4
Mild to moderate	Mild to moderate plaque elevation, scale and/or erythema	3
Mild	Mild marked plaque elevation, scale and/or erythema	2
Almost clear	İntermediate between mild features to clear	1
Clear	No feature of psoriasis (Post-inflammatory hyperpigmentation can be seen)	0

were expressed as means and standard deviations, and categorical variables were expressed in frequency and percent distribution. Means were compared between two groups using t-test and Spearman correlation analysis.

RESULTS

Fifty-five psoriasis patients who presented to our dermatology clinic between August and November 2012 volunteered to participate in the study. The gender and age distribution of the study participants are given in Table 1. The patient group included 45.5% males and 54.5% females (Table 3). Their mean age was 45.4±14.1 years. In terms of age distribution, 34.5% of the participants were 51-65 years old, and only 5.5% of the patients were over 65.

Table 3: Age and gender distribution of the study participants

Epidemiological characteristics	Variables	n	%
Gender	Male	25	45.5
	Female	30	54.5
Age (years)	18-35	16	29.1
	36-50	17	30.9
	51-65	19	34.5
	>65	3	5.5

The distribution of the 55 psoriasis patients in our study according to treatment regimen is evaluated. Systemic therapies were separated into two categories: Group 1 therapies included methotrexate, acitretin and cyclosporine, while group 2 included biological therapies such as adalimumab, infliximab and etanercept. Topical agents were being used by 47.35% of the patients, phototherapy by 12.7%; group 1 systemic therapy by 25.5% and group 2 systemic therapies by 7.3%. Four (7.3%) of the participants were not receiving any treatment for psoriatic lesions.

In our study, patients were evaluated separately by three dermatology residents with different levels of experience. The three physicians assessed each patient using both the PASI and PGA. Physician A was the most experienced, Physician B had an intermediate level of experience and Physician C was the least experienced. Table 4 shows the three residents'

Table 4: PASI and PGA scores from three physicians performing with different levels of experience

Severity Scale	Observer	Mean	Min.	Max.	SD
PASI	Physician A	12.1	2.2	33.4	±8.9
	Physician B	10.0	1.6	29.2	±8.0
	Physician C	12.8	2.1	30.6	±8.6
PGA	Physician A	2.7	1.0	6.0	±1.3
	Physician B	2.7	1.0	5.0	±1.5
	Physician C	3.4	1.0	6.0	±1.4

Table 5: Evaluation of the correlation between independent PASI and PGA scores given the same patient by three different residents with different levels of experience (A-C ranked most to least experienced).

Observer	Correlation coefficient (r)	P
Physician A	0.933	0.00
Physician B	0.941	0.00
Physician C	0.889	0.00

independent PASI and PGA values for psoriasis severity. Physicians A, B and C assigned mean PASI scores of 12.1±8.9, 10.0±8.0 and 12.8±8.6 and mean PGA scores of 2.7±1.3, 2.7±1.5 and 3.4±1.4, respectively (Table 4).

Table 5 presents Spearman correlation analysis of the PASI and PGA scores for the same patient obtained independently by residents with different levels of experience. Correlation coefficient (r) values between 0-0.25 are accepted as no to very weak correlation, 0.25-0.50 as moderate correlation, 0.50-0.75 as strong correlation and 0.75-1.00 as very strong correlation. Correlations with $P <0.05$ were considered statistically significant. The correlation coefficients for Physicians A, B and C were 0.933, 0.941 and 0.889, indicating very strong correlation between PASI and PGA scores for all three residents ($P <.001$ for all; Table 5).

Table 6: Evaluation of correlation between PASI and PGA scores given independently by three different residents (A-C ranked most to least experienced) to patients grouped according to psoriasis severity.

Observer	Severity classification	N	Correlation coefficient (r)	P
Physician A	Mild	10	0.503	0.138
	Moderate	20	0.747	0.000
	Severe	25	0.850	0.000
Physician B	Mild	10	0.611	0.061
	Moderate	20	0.769	0.000
	Severe	25	0.902	0.000
Physician C	Mild	10	0.562	0.091
	Moderate	20	0.789	0.000
	Severe	25	0.602	0.001

The patients were grouped by Physician A into three levels of severity using the PASI. Table 6 shows the results of the Spearman correlation analysis between PASI and PGA scores of each resident at different levels of severity. Correlation between PASI and PGA for Physician A was good for mild (r=0.503) and moderate cases (r=0.747) and very good for severe cases (r=0.850). For Physician B, correlation between PASI and PGA scores were good for mild cases (r=0.611) and very good for moderate (r=0.769) and severe cases (r=0.902). For Physician C, correlation was good for mild cases (r=0.562), very good for moderate cases (r=0.789) and good for severe cases (r=0.602) (Table 6).

Table 7 shows the results of Spearman correlation analysis of the relationships between PASI and PGA scores given by the most experienced resident and the patients' treatment for psoriasis lesions. Treatment regimen was not significantly correlated with PASI score (r=0.227) or PGA score (r=0.205).

Table 7: Evaluation of correlation between psoriasis treatment and PASI and PGA scores

Severity score	Medical Treatment	
	r	P
PASI	0.227	0.095
PGA	0.205	0.134

mild cases, 80% for severe cases and 65% for moderately severe psoriatic lesions (Table 8).

Kappa values were used to evaluate the inter-rater agreement between PGA scores given by Physicians A and B for the psoriasis patients at the three different severity levels (Table 9). There was good agreement between Physicians A and B in severity discrimination using the PGA (kappa=0.637). Agreement was 100% for severe cases and 92.3% for mild cases, but was lower (59.3%) for moderate cases.

Table 10 shows kappa values used to evaluate inter-rater agreement between PASI scores assigned by Physicians A and C for the psoriasis patients at three different severity levels. There was good

Table 8: Assessment of interrater agreement between PASI scores of the most experienced resident (Physician A) and second most experienced resident (Physician B) in patients with different levels of psoriasis severity.

Observer	Severity classification	Physician B			kappa	P
		Mild	Moderate	Severe		
Physician A	Mild	10 (100.0%)	0 (0.0%)	0 (0.0%)	0.667	0.000
	Moderate	6 (30.0%)	13 (65.0%)	1 (5.0%)		
	Severe	0 (0.0%)	5 (20.0%)	20 (80.0%)		

Cohen's kappa coefficient was calculated to evaluate the inter-rater agreement between PASI scores given by Physicians A and B for the psoriasis patients at the three different severity levels. Kappa values of 0-0.20 were accepted as poor agreement, 0.20-0.40 as fair, 0.40-0.60 as moderate, 0.60-0.80 as good and >0.80 as very good agreement. There was good agreement between Physicians A and B in severity discrimination using PASI (kappa=0.667). The inter-rater agreement in PASI scoring was much higher in patients with mild disease compared to those with moderate disease severity. Agreement was 100% for

agreement between Physicians A and C in severity discrimination using PASI (kappa=0.737). Agreement was 96% for severe cases, 80% for mild cases and 70% for moderately severe psoriatic lesions.

Table 11 shows the kappa values for inter-rater agreement in PGA scores given by Physicians A and C for the psoriasis patients at three different severity levels. There was moderate agreement between the PGA scores of Physicians A and C in the discrimination of psoriasis severity (kappa=0.442). Agreement was 100% for severe cases and 69.2% for mild cases, but only 50.0% for moderate cases.

Table 9: Assessment of interrater agreement between PGA scores of the most experienced resident (Physician A) and the second most experienced resident (Physician B) in patients with different levels of psoriasis severity.

Observer	Severity classification	Physician B			kappa	P
		Mild	Moderate	Severe		
Physician A	Mild	24 (92.3%)	2 (7.7%)	0 (0.0%)	0.637	0.000
	Moderate	6 (25.0%)	14 (59.3%)	4 (16.7%)		
	Severe	0 (0.0%)	0 (0.0%)	5 (100.0%)		

Table 10: Assessment of interrater agreement between PASI scores of the most experienced resident (Physician A) and least experienced resident (Physician C) in patients with different levels of psoriasis severity.

Observer	Severity classification	Physician B			kappa	P
		Mild	Moderate	Severe		
Physician A	Mild	8 (80.0%)	2 (20.0%)	0 (0.0%)	0.737	0.000
	Moderate	2 (10.0%)	14 (70.0%)	4 (20.0%)		
	Severe	0 (0.0%)	1 (4.0%)	24 (96.0%)		

Table 11: Assessment of interrater agreement between PGA scores of the most experienced resident (Physician A) and least experienced resident (Physician C) in patients with different levels of psoriasis severity.

Observer	Severity classification	Physician B			kappa	P
		Mild	Moderate	Severe		
Physician A	Mild	18 (69.2%)	8 (30.8%)	0 (0.0%)	0.442	0.000
	Moderate	1 (4.2%)	12 (50.0%)	11 (45.8%)		
	Severe	0 (0.0%)	0 (0.0%)	5 (100.0%)		

DISCUSSION

The PASI, developed in 1978 by Fredericksonn and Petersson[10], rates the severity of psoriasis on a scale from 0 to 72, and is difficult to interpret[6]. Interpretations of the severity indicators, particularly area of involvement, may vary between raters, especially if they are not experienced[5].

The PGA includes static, dynamic, 5-point and 7-point variations. Static PGA classifies severity between 'clear' and 'very severe'. The 7-point PGA is the most commonly used form in clinical studies[5]. It provides an average assessment of all psoriatic lesions based on erythema, scaling and induration[3]. Although it is subjective and provides no information about what proportion of the body is affected, it is easy to apply and understand[5]. PGA allows raters to make an estimate of the severity of psoriasis[2]. Robinson *et al* stated that while PGA is an appropriate and practical method for community-based studies, but due to its variability, it offers no benefit over the use of PASI alone for assessing the severity of psoriasis in large-scale clinical studies, including patients with medium to severe psoriasis[3]. They also observed in their literature review that there is weaker correlation between PASI and PGA when evaluating early treatment response or less effective treatments[3].

Compared to the r values in our study, PASI and PGA correlation was reported as high (r=0.87) in a study by Langley *et al*[2], and as moderate (r=0.75) and high (r=0.79) by Berth-Jones *et al* in 2008[5] and 2006[9] respectively. In our study, PASI and PGA correlation was very high for all three physicians.

Langley *et al* compared severity measurements made by 17 experienced and inexperienced physicians using PASI, PGA and Lattice System Physician's General Assessment (LSPGA) with 35 psoriasis patients and reported that inter-rater variation was highest in the PASI. They noted that PASI was affected by experience, whereas the PGA and LSPGA were not. The authors proposed that the high level of variability in the PASI may be related to the fact that area of involvement is difficult to determine accurately, even for experienced physicians[2]. In a study by Berth-Jones *et al* evaluating the reliability of Copenhagen Psoriasis Severity Index and comparing the PASI and PGA, inter-rater reliability was high for the PASI and

Copenhagen Psoriasis Severity Index and moderate for the PGA[5]. In another study by Berth-Jones *et al* investigating the reliability of PASI, PGA and LSPGA, inter-rater reliability was high for the PASI and LSPGA and moderate for the PGA. They suggested that the contradiction between their results and those of Langley *et al* may be due to the fact that they familiarized the raters with the scales beforehand, and improved the statistical methods used[9]. In our study, we observed a good level of agreement between both rater pairs for PASI, whereas the PGA showed good agreement between Physicians A and B but only moderate agreement between Physicians A and C. Despite the fact that these three residents had not been involved in any previous psoriasis research, the PASI is used in our clinic to measure psoriasis severity, and all of the residents were trained in its use before they began working, which may explain the high inter-rater agreement.

It has been stated in previous studies that differences in PASI scores may result from body area calculations[2]. Moreover, other severity indicators may be interpreted differently by the raters[5]. The variations among the PASI scores assigned by the residents in our study may be the result of differences in the calculation of body surface area and/or the other PASI components.

Limitations of this study are low total number and dissimilar number of participants in the groups separated by the psoriasis severity.

CONCLUSION

In summary, our results showed higher inter-rater agreement when using the PASI than the PGA. Agreement among both PASI and PGA scores was lower in patients with moderately severe psoriasis. In our study, inter-rater agreement for the PGA was affected by the rater's experience level. Despite being quick and easy to use and understand, the PGA is subjective and may be interpreted differently by different raters. This may explain the lower inter-rater reliability we observed for PGA in this study.

Author Contribution

Ayda Acar: concept, literature search, clinical study, data acquisition, statistical analysis, manuscript preperation, manuscript editing. Ada Bozkurt and Gizem Kocabas Yenipazar: clinical study, data acquisition. Sibel Alper and Can Ceylan: concept, design, manuscript review

REFERENCES

1. Başkan EB. Hastalık Şiddetinin tanımlanması in Turkish Guideline for the Treatment of Psoriasis-2016. Turkderm - Arch Turk Dermatol Venerology 2016; 50(Suppl 1):4-6.
2. Langley RG, Ellis CN. Evaluating psoriasis with Psoriasis Area and Severity Index, Psoriasis Global Assessment, and Lattice System Physician's Global Assessment. J Am Acad Dermatol 2004; 51(4):563-569.
3. Robinson A, Kardos M, Kimball AB. Physician Global Assessment (PGA) and Psoriasis Area and Severity Index (PASI): why do both? A systematic analysis of randomized controlled trials of biologic agents for moderate to severe plaque psoriasis. J Am Acad Dermatol 2012; 66(3):369-375.
4. Paul C, Gourraud PA, Bronsard V, Prey S, Puzenat E, Aractingi S, et al. Evidence-based recommendations to assess psoriasis severity: systematic literature review and expert opinion of a panel of dermatologists. J Eur Acad Dermatol Venereol 2010; 24 Suppl 2:2-9.
5. Berth-Jones J, Thompson J, Papp K; Copenhagen Psoriasis Working Group. A study examining inter-rater and intrarater reliability of a novel instrument for assessment of psoriasis: the Copenhagen Psoriasis Severity Index. Br J Dermatol 2008; 159(2):407-412.
6. Puzenat E, Bronsard V, Prey S, Gourraud PA, Aractingi S, Bagot M, et al. What are the best outcome measures for assessing plaque psoriasis severity? A systematic review of the literature. J Eur Acad Dermatol Venereol 2010; 24 Suppl 2:10-16.
7. Spuls PI, Lecluse LL, Poulsen ML, Bos JD, Stern RS, Nijsten T. How good are clinical severity and outcome measures for psoriasis?: quantitative evaluation in a systematic review. J Invest Dermatol 2010; 130(4):933-943.
8. Naldi L, Svensson A, Diepgen T, Elsner P, Grob JJ, Coenraads PJ, et al. European Dermato-Epidemiology Network. Randomized clinical trials for psoriasis 1977-2000: the EDEN survey. J Invest Dermatol 2003; 120(5):738-741.
9. Berth-Jones J, Grotzinger K, Rainville C, Pham B, Huang J, Daly S, et al. A study examining inter- and intrarater reliability of three scales for measuring severity of psoriasis: Psoriasis Area and Severity Index, Physician's Global Assessment and Lattice System Physician's Global Assessment. Br J Dermatol 2006; 155(4):707-713.
10. Fredriksson T, Pettersson U. Severe psoriasis--oral therapy with a new retinoid. Dermatologica 1978; 157(4):238-244.

<div align="center">

Original Article

Case series of pancreas transplant in Kingdom of Saudi Arabia

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ABSTRACT

Objectives: To report our experience in a rare complex procedure and graft survival data

Design: Retrospective analysis of a prospectively collected data of patients undergoing pancreas transplant in two centers in Saudi Arabia

Setting: Tertiary care centers in Saudi Arabia

Subject: Patients who underwent pancreas transplant

Intervention: Pancreas transplant

Main Outcome Measure(s): Report our experience in a complex surgical procedure and graft survival

Results: We collected a total of 21 cases of pancreas transplant for type 1 diabetes mellitus (DM) patients (9 females and 12 males). Mean age at transplant was 34.9±7.47 years and mean DM duration was 148±119 months. Out of the 21 cases, four (19%) were pancreas after kidney transplants while the remaining 17 (81%) were simultaneous pancreas-kidney. Regarding the surgical technique, all procedures were standardized to be implanted in right iliac fossa with enteric drainage. The mean cold ischemia time was 591.7±185.82 minutes and mean graft survival was 624±396 months. The mean length of admission was 17.8±15.14 days. Mean graft survival (defined by return to insulin use) was 624±395 months. Unfortunately, data regarding C-peptide level was lacking in our sample size. One patient underwent graft excision after 28 days of acute life-threatening graft rejection. In our series, we had zero 90-day mortality.

Conclusion: Despite our initial small numbers, they prove that the procedure is feasible and doable in our centers with good initial graft survival and acceptable morbidity and mortality.

KEY WORDS: diabetes mellitus, insulin, pancreas

INTRODUCTION

Pancreas transplant was first introduced in humans in 1966 aiming to treat insulin dependent diabetes mellitus (DM). The number of transplants were scarce until 2004, when the surgical, medical and immunological control aided in a better outcome. The Kingdom of Saudi Arabia is a 2.1×10^6 km^2 spaced country with a population of around 33 million with an estimated median age of 30.6 years for both genders and with a 73-year life expectancy. DM is a major crisis involving about 30% of the population with a mean age of 55.3±13.1 years as of 2011[1,2]. Having a young population with a huge burden of diabetes makes pancreas transplant a good option to improve the quality of the patient's life and outcomes. In this study, our aim is to report our experience in pancreas transplant in Saudi Arabia.

SUBJECTS AND METHODS

A retrospective analysis of prospectively collected data of patients undergoing pancreas transplant in two centers in Saudi Arabia operated by Saudi surgeons

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Table 1: Table showing recipient and graft data

Recipient Data	Graft			
Type of procedure	PAK (n=4)		SPK (n=17)	
ABO				
A	0		2	
A+	1		4	
AB	1		0	
AB+	1		0	
B	0		2	
B+	0		2	
O	1		2	
O+	0		5	
Sex				
F	2		7	
M	2		10	
Recipient Data	**mean**	**SD**	**mean**	**SD**
Body mass index (kg/m^2)	30.2	15.46	40.28	24.39
Age at transplant (years)	36.25	6.7	34.59	7.8
CIT (minutes)	576.75	161.86	595.24	195.4
Duration of admission (days)	25.25	26.74	16.06	11.64
DM duration (months)	183.75	122.61	137.62	120.82
Graft survival (months)	142	161.22	784.67	299.11

PAK: pancreas after kidney; SPK: simultaneous pancreas-kidney; CIT: cold ischemia time; DM: diabetes mellitus

over the period of 2004-2016 was carried out. Written ethical approval was part of the general consent to admission, as this is a retrospective study on clinical data. Ethical approval was acquired from King Khalid University Hospital IRB Committee.

Pre-operative and surgical procedure

All patients underwent the same regimen of immunosuppression using thymoglobulin for induction, tacrolimus, mycophenolic acid and prednisone. The patients underwent standardized surgical procedure. All pancreas organs were transplanted in the right iliac fossa with systemic vascular anastomosis and enteric duct drainage.

RESULTS

We collected a total of 21 cases of pancreas transplant for type 1 DM patients (9 females and 12 males). Mean age at transplant was 34.9±7.47 years and mean DM duration was 148±119 months. Out of the 21 cases, four (19%) were pancreas after kidney transplants while the remaining 17 (81%) were simultaneous pancreas-kidney. Regarding the surgical technique, all procedures were standardized to be implanted in right iliac fossa with enteric drainage. The mean cold ischemia time was 591.7±185.82 minutes and mean graft survival was 624±396 months. The mean length of admission was 17.8±15.14 days. Mean graft survival (defined by return to insulin use) was 624±395 months (Table 1, Fig 1). Unfortunately, data regarding C-peptide level was lacking in our sample size. One patient underwent graft excision after 28
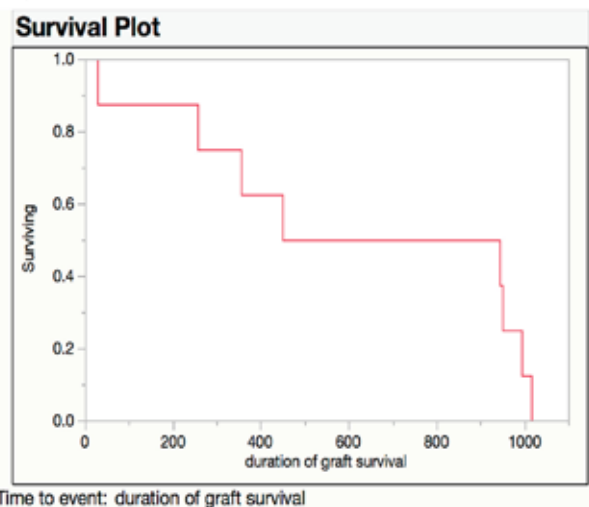
Fig 1: Survival plot

days of acute life-threatening graft rejection. In our series, we had zero 90-day mortality.

DISCUSSION

DM is classically described as two types; type I resulting from loss of pancreas insulin resulting from autoimmune disease and type II, which is a result of increased insulin resistance and demand. The treatment modalities differ according to the type. The whole mark of type I DM is insulin deficiency. Hyperglycemia causes defects in the blood cells, vessels, kidney and nerves. This causes micro and macro vascular disease, nephropathy and peripheral

neuropathy. The role of pancreas transplant has been presented in the literature since 1966[3,4]. Pancreas transplant has showed a survival benefit in type I DM. In our patient series, all the patients underwent a standardized diagnostic, pre-operative, surgical technique and post-operative pathway. In our cohort, the role of pancreas transplant is promising, with acceptable complications and graft survival rates reaching the published standards[3-5]. However, these results are limited by the small sample size and need further studying. Given our national DM rate among young patients that increase the burden on our health care system, further efforts should be directed toward this modality of treatment as it would improve patient survival and lifestyle.

CONCLUSION

Despite our initial small numbers, they prove that the procedure is feasible and doable in our centers with good initial graft survival and acceptable morbidity and mortality.

REFERENCES

1. Alotaibi A, Perry L, Gholizadeh L, Al-Ganmi A. Incidence and prevalence rates of diabetes mellitus in Saudi Arabia: An overview. J Epidemiol Glob Health 2017; 7(4):211-218.
2. Cherian MP, Al-Kanani KA, Al Qahtani SS, Yesurathinam H, Mathew AA, Thomas VS, *et al*. The rising incidence of type 1 diabetes mellitus and the role of environmental factors--three decade experience in a primary care health center in Saudi Arabia. J Pediatr Endocrinol Metab 2010; 23(7):685-695.
3. Gruessner AC, Sutherland DER, Gruessner RWG. Pancreas transplantation in the United States: A review. Curr Opin Organ Transplant 2010; 15(1):93-101.
4. Jiang AT, Rowe N, Sener A, Luke P. Simultaneous pancreas-kidney transplantation: The role in the treatment of type 1 diabetes and end-stage renal disease. Can Urol Assoc J 2014; 8(3-4):135-138.
5. van Dellen D, Worthington J, Mitu-Pretorian OM, Ghazanfar A, Forgacs B, Pararajasingam R, *et al*. Mortality in diabetes: Pancreas transplantation is associated with significant survival benefit. Nephrol Dial Transplant 2013; 28(5):1315-1322.

Original Article

The effect of neutrophil lymphocyte ratio and monocyte HDL ratio in indicating additional arterial disease in patients scheduled to undergo elective coronary bypass operation

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ABSTRACT

Objective: We aimed to investigate the role of neutrophil / lymphocyte ratio (NLR) and monocyte / high density lipoprotein ratio (MHR) in routine blood tests before surgery in predicting the presence of carotid artery and peripheral artery disease in patients scheduled for coronary artery bypass graft (CABG).
Design: Retrospective study
Setting: Department of Cardiovascular Surgery, University of Health Sciences, Mehmet Akif İnan Training and Research Hospital
Subjects: A total of 354 consecutive patients who were scheduled to undergo elective CABG operation
Intervention: Predictive values of the variables were measured for atherosclerosis extensity.
Main outcome measure(s): The relationship between preoperative NLR-MHR and concomitant arterial disease in patients scheduled to undergo CABG

Results: A total number of 310 patients in Group 1 (without additional arterial disease; 64.5% male, mean age: 59.1±8.7 years) and 44 patients in Group 2 (with additional arterial disease; 72.7% male, mean age: 65.5±12.3 years) were recorded in the study. There was a statistical difference between the two groups in terms of age (*P*=.003) and presence of diabetes mellitus (*P*=.032). In multivariate analysis, advanced age, NLR and MHR were identified as an independent predictor of concomitant arterial disease. In ROC curve analysis, a cut-off level of 2.85 for predicting concomitant arterial disease was determined for NLR (96% sensitivity and 46% specificity), and for MHR, a cut-off level of 8.65 was determined (90% sensitivity and 42% specificity).
Conclusion: In this study, we found that simple blood tests may shed light on the detection of additional arterial diseases in patients planned to undergo CABG.

KEY WORDS: carotid artery disease, coronary artery disease, monocyte to HDL ratio, neutrophil to lymphocyte ratio, peripheral artery disease

INTRODUCTION

Nowadays, the diagnosis of atherosclerotic cardiovascular diseases is more frequent with prolonged survival and progressive technology. Coronary artery disease (CAD) is one of the most frequent disease of atherosclerotic cardiovascular diseases. The gold standard treatment method is coronary artery bypass graft (CABG) when coronary artery disease presents as mutiple vessel disease[1]. CAD may be accompanied by other system arteriopathies such as peripheral arterial disease (PAD) and carotid artery stenosis (CAS). In patients scheduled for coronary artery bypass surgery, CAS with 50% and more stenosis can be seen in 30% of the patients[2]. Only carotid artery disease is even important because of possible cerebrovascular risks[3]. This situation is not a cause of cerebrovascular disease alone, but it is an important risk factor. Therefore, it is important to make a diagnosis before CABG operation. Preoperative recognition of PAD in patients undergoing CABG is also important. Possible iliac artery stenoses can lead to serious complications during and after surgery[4].

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Studies have shown that various biomarkers can demonstrate the presence and severity of atherosclerosis. In these studies, some parameters that increased in blood were related to atherosclerotic cardiovascular diseases[5,6].

In our study, we aimed to investigate the predictive value of neutrophil / lymphocyte ratio (NLR) and monocyte / high density lipoprotein ratio (MHR) that can be obtained from routine blood tests before surgery on the presence of carotid artery disease and/ or PAD in patients who scheduled to undergo CABG.

MATERIAL AND METHODS

The patients who were hospitalized with CABG between December 2014 and December 2018 were included in the study. The study was approved by the Ethical Committee of Sanlıurfa Harran University Faculty of Medicine and was conducted in accordance with the principles of the Declaration of Helsinki. The data of the patients were retrospectively obtained from the hospital data system and archive records. Patients undergoing emergency operations, patients with known systemic inflammatory disease, new myocardial infarction, patients with additional cardiac surgery (aneurysm, valvular heart diseases etc.) except for CAD were excluded from the study. After applying these exclusion criteria, 354 consecutive patients were included in the study.

According to the tests and physical examination data, patients who didn't have any additional artery disease except for CAD were included in Group 1. Patients having 50% or more stenosis in carotid or peripheral arteries were classified as Group 2. Demographic characteristics (age, sex, hypertension, etc.) and preoperative blood tests of all patients were recorded (hemogram, urea, creatinine, etc.).

Diagnosis of additional artery disease

Carotid doppler ultrasonography is performed in all patients routinely in our clinic in the preoperative preparation of coronary bypass operations. Thus, the patients can be diagnosed with a possible stenosis and occlusion. The diagnosis of PAD was made by doppler ultrasonography (in 30 patients), conventional angiography (in five patients) and/or computed tomographic angiography (in nine patients) in patients with pathology on physical examinations. According to these results, patients who had 50% or more stenosis in the peripheral and/or carotid artery system were evaluated as patients with additional artery disease.

Laboratory measurements

Preprandial blood samples were obtained from an antecubital vein of every patient when the patients entered our clinic. The tubes with EDTA were utilized for automatic blood count with reference to the procedures of our hospital. An automated hematological analyzer was used for measuring hematologic parameters (Coulter LH 780 Analyzer, CA, USA). Furthermore, NLR and MHR were assessed.

Statistical analysis

SPSS 21.0 (IBM Statistical Package for the Social Sciences Statistic Inc. version 21.0, Chicago, IL, USA) was utilized for analysis. Descriptive statistics such as mean and standard devations were calculated for continuous and ordinal variables. The frequencies and percentages of nominal variables were analyzed. In exploring the normality of the data, Kolmogorov-Smirnov test and Shapiro-Wilk tests were performed. For comparing two groups, Student's t test and Mann-Whitney U test were used for continuous variables with and without normal distribution respectively. Nominal variables were compared via Chi Square test. Predictors of other system arterial disease were defined via binary logistic regression analysis. P-value that was lower than 0.05 was determined as statistically significant. Receiver-operating characteristic (ROC) curve was applied for the prediction of other system arterial disease and the area under the curve (AUC) was calculated for NLR and MHR.

RESULTS

A total number of 310 patients in Group 1 (64.5% male, mean age: 59.1±8.7 years) and 44 patients in Group 2 (72.7% male, mean age: 65.5±12.3 years) were recorded in the study. There were statistical differences between the two groups in terms of age (P=.003) and presence of diabetes mellitus (P=.032). However, there was no statistical difference between the two groups in terms of gender, percutaneous coronary intervention history, hypertension, smoking and ejection fraction. The demographic and clinical features of the patients were shown in Table 1.

Table 1: Demographic data of the patients

Paremeters	Group 1 (n=310)	Group 2 (n=44)	P-value
Age (years, mean±sd)	59.1±8.7	65.5±12.3	.003
Male/female (n)	200/110	32/12	.448
Previous PCI, n(%)	88(28.3%)	18(40.9%)	.230
Target vessel (n, mean±sd)	2.9±1.01	3.41±1	.073
COPD, n(%)	64(20.6%)	8(18.1%)	.786
Hypertension, n(%)	206(66.4%)	28(63.6%)	.794
Diabetes mellitus, n(%)	74(23.8%)	20(45.4%)	.032
BMI (kg/m²)	28.7±4.2	27.6±3.9	.156
Smoking, n(%)	114(36.7%)	20(45.4%)	.432
Ejection fraction (%, mean±sd)	51.7±7.3	50.9±6.7	.236

PCI: percutaneus coronary intervention; COPD: chronic obstructive pulmonary disease; BMI: body mass index

The comparison of laboratory measurements between two groups were shown in Table 2. Both Group 1 and Group 2 were similar to each other in regard to laboratory findings. Additionally, there were statistically significant differences between the two groups in terms of monocyte count (P <.001), NLR (P=.001) and MHR (P <.001) (Table 2).

Factors related to determine concomitant arterial disease in patients scheduled for CABG were included in univariate logistic regression analysis. In unadjusted univariate logistic regression analysis, the presence of extracardiac arterial disease was significantly correlated with advanced age (odds ratio [OR]: 1.076, 95% confidence interval [CI]: 1.024-1.131, P=.004), diabetes mellitus (OR: 0.376, 95% CI: 0.150-0.941, P=.037), NLR (OR: 1.434, 95% CI: 1.074-1.914, P=.015) and MHR (OR: 3.099, 95% CI: 1.674-5.736, P <.001), but was not correlated with gender and target vessel. In multivariate analysis, advanced age, NLR and MHR were identified as an independent predictor of concomitant arterial disease (OR: 1.052, 95% CI: 1.002-1.105, P=.042; OR: 1.500, 95% CI: 1.036-2.171, P=.032; OR: 3.004, 95% CI: 1.540-5.860, P=.001, respectively) (Table 3).

In ROC curve analysis, a cut-off level of 2.85 for predicting concomitant arterial disease was determined for NLR (AUC: 0.722, 95% CI: 0.638-0.806, log rank P=.001, 96% sensitivity and 46% specificity),

and for MHR, a cut-off level of 8.65 was determined for predicting concomitant arterial disease (AUC: 0.791, 95% CI: 0.703-0.879, log rank P <.001, 90% sensitivity and 42% specificity) (Figure 1).
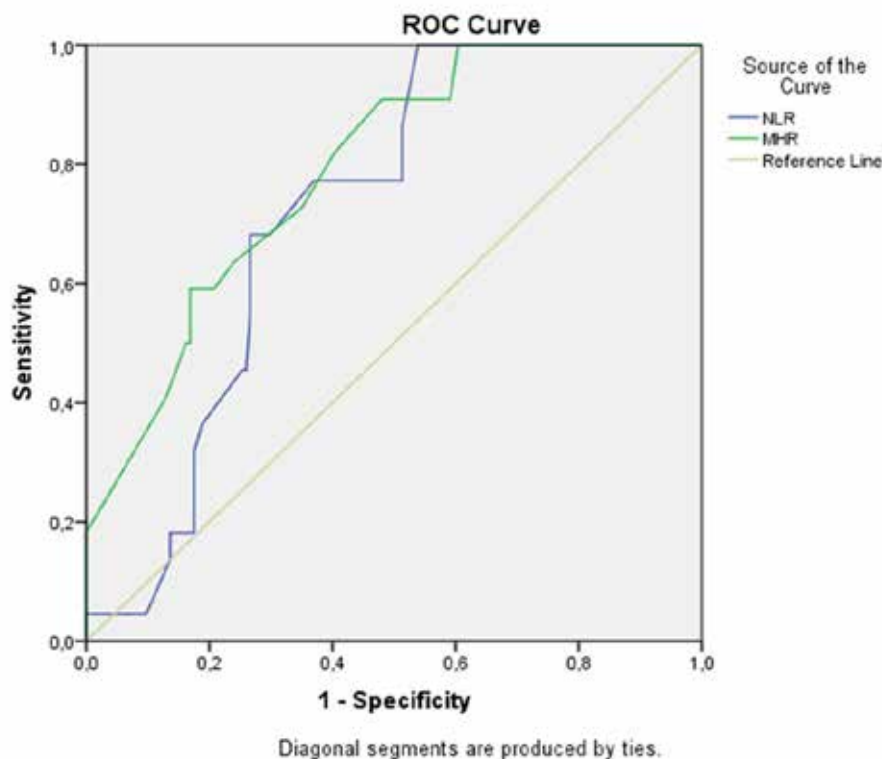
Table 2: Laboratory variables of the patients

Parameters	Group 1 (n=310; mean ± SD)	Group 2 (n=44; mean ± SD)	P-value
Hematocrit (%)	38.9±5.1	37.1±5.9	.483
White blood cell (10³/µL)	8.9±2.9	9.6±3.1	.339
Platelet (10³/µL)	279.3±73.2	288.7±69.2	.354
RDW(%)	13.7±1.4	14.4±1.3	.249
MPV(fL)	8.5±1.2	8.8±1.3	.543
Neutrophil (10³ mL)	6.4±1.2	6.9±1.1	.364
Lymphocyte (10³ mL)	2.9±1.4	2.2±1.2	.456
Monocyte (10⁹ L)	370.7±122.8	398.2±133.2	<.001
BUN (mg/dL)	17.9±6.7	19.8±9.7	.124
Creatinine (mg/dL)	1.1±0.5	0.9±0.5	.486
C-reactive protein (mg/dL)	7.8±11.3	10.1±12.6	.146
Total cholesterol (mg/dL)	191.0±68.5	199.8±53.7	.351
LDL-C (mg/dL)	126.8±43.7	129.2±38.1	.256
HDL-C (mg/dL)	44.1±6.9	41.7±9.2	.066
TG (mg/dL)	184.8±89.6	195.3±67.5	.453
NLR	3.3±1.3	4.1±1.1	.001
MHR	7.9±1.5	9.4±0.7	<.001

RDW: red cell distribution width; MPV: mean platelet volume; BUN: blood urea nitrogen; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; NLR: neutrophil to lymphocyte ratio; MHR: monocyte to HDL ratio

Fig 1: Receiver operation characteristic (ROC) curve and area under the curve (AUC) for neutrophil to lymphocyte ratio (NLR) levels and monocyte to HDL ratio (MHR) levels for predicting to identify concomitant carotid artery disease and peripheral artery disease (NLR: cut-off=2.85, 96% sensitivity, 46% specificity, MHR: cut-off= 8.65, 90% sensitivity, 42% specificity)

Table 3: Logistic regression analysis for predictors to identify other system arterial disease

Variables	Univariate analysis			Multivariate analysis		
	P	Exp(B) Odds Ratio	95% C.I. Lower Upper	P	Exp(B) Odds Ratio	95% C.I. Lower Upper
Age	.004	1.076	1.024-1.131	.042	1.052	1.002-1.105
Male gender	.450	1.467	0.543-3.964	--	--	--
Target vessel	.075	1.526	0.958-2.430	.144	1.552	0.860-2.802
DM	.037	0.376	0.150-941	.057	.731	1.111-1.284
Previous PCI	.226	1.356	0.654-2.826	--	--	--
NLR	.015	1.434	1.074-1.914	.032	1.500	1.036-2.171
MHR	<.001	3.099	1.674-5.736	.001	3.004	1.540-5.860

DM: diabetes mellitus; PCI: percutaneus coronary intervention NLR: neutrophil to lymphocyte ratio MHR: monocyte to HDL ratio

DISCUSSION

Atherosclerosis is a condition arising out of chronic vascular inflammation. As a result of this inflammation, inflamed cytokines and chemokines penetrate into the inflamed vascular endothelium[7]. The atherosclerotic process progresses within a certain relationship with the entire cardiovascular system[8]. The clinical outcomes of this condition are generally seen as CAD, PAD and CAS. These diseases can be detected separately in the same person or can be seen together. The possible PAD and/or CAS cases should be recognized in the preoperative period, especially in patients undergoing CABG.

Neutrophils in circulation lead to the progression of atherosclerosis by clinging to the damaged vascular endothelial regions. Thus, chemokines come into the region, proliferation develops and atherosclerosis progresses and the vessel wall narrows[9]. Monocyte activation also plays a role in the atherosclerotic process by modulating inflammatory cytokines. By attaching to damaged endothelial sites, monocytes aggregate the inflammatory process and play a role in thrombotic events[10]. By contrast, HDL takes a preventive role in the progression of the atherosclerotic process[11].

In recent years, the relationship between blood parameters and cardiovascular system diseases has been the subject of interest, and various studies have been carried out on this topic. In addition to the diagnostic importance, the prognostic values of these parameters after cardiovascular operations were also investigated. Aykan *et al* investigated the role of NLR in the severity of PAD. In this study, patients were divided into two groups as Transatlantic Intersociety Consensus (TASC) -A-B and TASC-C-D and NLR was significantly higher in TASC-C-D group. In the ROC analysis, the cut-off of NLR was determined as 3.05 (sensitivity=75.0%, specificity = 62.9%, AUC=0.678, 95% CI=0.688 (0.784, *P* <.001)[12].

The prognostic significance of NLR in patients undergoing CABG was investigated by Ay *et al* and the patients were divided into two groups as below 45 years of age and above. Mortality and NLR were found to be significantly higher in the elder patient group and it was stated that NLR may be a predictor of mortality in this study[13]. Arbel and his colleagues investigated the role of NLR in determining the severity of CAD by evaluating 3005 consecutive patients who had coronary angiography. They classified the patients into three groups by their NLR. The first group (30% of patients) consisted of patients whose NLR were below 2, the second one (30% of patients) comprised of patients whose NLR were between 2 and 3 and the last one (40% of patients) included patients whose NLR were more than 3. They found a significant difference in CAD severity among these three groups (*P* <.001). They also found a positive correlation between higher NLR and CAD severity (*P* <.001). At the same time, close relationship with adverse events was detected at three-year follow-up[14].

Similarly, MHR was investigated in cardiovascular system diseases. The clinical significance of MHR was evaluated by Cetin *et al* on 2661 patients with acute coronary syndrome. They determined that MHR showed the severity of CAD. In this study, the mean follow-up period was 31.6 months, and the relationship between increased MHR and major adverse cardiovascular events was determined[15]. Tekkesin *et al* investigated the effects of MHR on atrial fibrillation in patients undergoing CABG. In this study, preoperative MHR values were significantly higher in patients with atrial fibrillation in the postoperative period[16].

In our study, we aimed to investigate the predictive value of MHR and NLR in addition to the presence of CAS and PAD in patients scheduled for CABG. We divided patients into two groups as patients with CAS or PAD and patients without additional artery disease. As a result of our study, we determined that these parameters obtained from preoperative routine blood tests of the patients may guide us. Although MHR and NLR values have good sensitivity, their overall specificity is poor (42% and 46%).

Preoperative preparation of coronary bypass operations is as valuable and important as the surgical technique stage of the operation. In these patient groups, possible lung diseases, uncontrolled diabetes and other systemic diseases may overshadow our technical surgical success. Carotid artery disease and PAD should be detected in the preoperative period and essential precautions should be taken. In particular, in patients with possible iliac artery stenosis, it may be difficult to apply to intra-aortic balloon pump, a very valuable device in the weaning of cardiopulmonary bypass. In this case, it may be necessary to perform more complicated methods than the femoral approach[17]. In addition, it is very important to diagnose the potentially severe CAS at preoperative stage. We may need to make changes in our operation strategies in this clinical situation. In this case, the operation method is determined by considering the clinical evaluation of CAD and CAS[18].

CONCLUSION

In this study, we found that simple blood tests may shed light on the detection of additional arterial diseases in patients who are about to undergo CABG. Misdiagnosing possible severe PAD or CAS disease may lead to catastrophic outcomes in CABG operations, which are a major surgery. According to our study, NLR and MHR can cause us to suspect additional arterial diseases.

Limitations

This study is a retrospective study carried out in homogeneous groups of patients. A smaller number of patients is also the limiting factor of the study. In addition, Group 2 had a smaller number of patients (44 patients).

REFERENCES

1. Şahin M, Yücel C, Mert FTİ, Öztürk S. Open Heart Surgery in Haseki Training and Research Hospital: Evaluation of the First 500 Cases. Med Bull Haseki 2018; 56:1-5.
2. Venkatachalam S, Shishehbor MH. Management of carotid disease in patients undergoing coronary artery bypass surgery: is it time to change our approach? Curr Opin Cardiol 2011; 26(6):480-487.
3. Özsin KK, SanrıUS, Toktaş F, Şeker İB, Yavuz Ş. Effect of lipid parameters on carotid artery disease progression in patients undergoing carotid endarterectomy and unoperated patients with non-severe carotid artery stenosis. Eur Res J 2019; 5(1):159-165.
4. Ogoyama Y, Ogata N, Toriumi S, Kario K. Successful hybrid treatment with endovascular aorto-iliac revascularization and coronary bypass surgery in a patient with an advanced complex polyvascular disease. J Cardiol Cases 2017; 15(6):201-205.
5. Azab B, Camacho-Rivera M, Taioli E. Average values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. PLoS One 2014; 9(11):e112361.
6. Canpolat U, Çetin EH, Cetin S, Aydin S, Akboga MK, Yayla C, et al. Association of monocyte-to-HDL cholesterol ratio with slow coronary flow is linked to systemic inflammation. Clin Appl Thromb Hemost 2016; 22(5):476-482.
7. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011; 473(7347):317-325.
8. Aslan AN, Özcan AN, Ayhan H, Sivri S, Çelebi F, Ercan K, et al. Evaluation of local carotid stiffness and inflammatory biomarkers in stable angina pectoris. Adv Interv Cardiol 2017; 13(2):122-129.
9. Blake GJ, Ridker PM. Inflammatory bio-markers and cardiovascular risk prediction. J Intern Med 2002; 252(4):283-294.
10. Ancuta P, Wang J, Gabuzda D. CD16+ monocytes produce IL-6, CCL2, and matrix metalloproteinase-9 upon interaction with CX3CL1-expressing endothelial cells. J Leukoc Biol 2006; 80(5):1156-1164.
11. Akboga MK, Balci KG, Maden O, Ertem AG, Kirbas O, Yayla C, et al. Usefulness of monocyte to HDL-cholesterol ratio to predict high SYNTAX score in patients with stable coronary artery disease. Biomark Med 2016; 10(4):375-383.
12. Aykan AÇ, Hatem E, Kalaycıoğlu E, Karabay CY, Zehir R, Gökdeniz T, et al. Neutrophil-to-lymphocyte ratio may be a marker of peripheral artery disease complexity. Anatol J Cardiol 2016; 16(7):497-503.
13. Ay D, Erdolu B, Yumun G, Aydin U, Demir A, Tiryakioglu O, et al. Comparing the effectiveness of neutrophil-lymphocyte ratio as a mortality predictor on middle and advanced age coronary artery bypass graft patients. North Clin Istanb 2014; 1(2):95-100.
14. Arbel Y, Finkelstein A, Halkin A, Birati EY, Revivo M, Zuzut M, et al. Neutrophil/lymphocyte ratio is related to the severity of coronary artery disease and clinical outcome in patients undergoing angiography. Atherosclerosis 2012; 225(2):456-460.
15. Cetin MS, Cetin EHO, Kalender E, Aydin S, Topaloglu S, Kisacik HL, et al. Monocyte to HDL cholesterol ratio predicts coronary artery disease severity and future major cardiovascular adverse events in acute coronary syndrome. Heart Lung Circ 2016; 25(11):1077-1086.

16. Tekkesin AI, Hayiroglu MI, Zehir R, Turkkan C, Keskin M, Cinier G, *et al*. The use of monocyte to HDL ratio to predict postoperative atrial fibrillation after aortocoronary bypass graft surgery. North Clin Istanb 2017; 4(2):145-150.

17. Toktas F, Yavuz S, Eris C, Surer S. Transaortic intra-aortic balloon pump catheter insertion through a separate saphenous vein graft in patients with severe aortoiliac disease. Scientific World Journal 2014; 2014:247803.

18. Feldman DN, Swaminathan RV, Geleris JD, Okin P, Minutello RM, Krishnan U, *et al*. Comparison of trends and in-hospital outcomes of concurrent carotid artery revascularization and coronary artery bypass graft surgery: The United States experience 2004 to 2012. JACC Cardiovasc Interv 2017; 10(3):286-298.

Original Article

Neutrophil to lymphocyte ratio: An indicator of recurrence in primary spontaneous pneumothorax?

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ABSTRACT

Objective(s): To address the question whether neutrophil to lymphocyte ratio may be an indicator of recurrence in primary spontaneous pneumothorax
Design: Retrospective study
Setting: Bursa Yüksek Ihtisas Hospital for Education and Research, Bursa, Turkey
Subjects: A total number of 124 primary spontaneous pneumothorax patients treated in our hospital between 5th July, 2002 and 30th April, 2015. Patients with only initial episode were enrolled in Group 1 and patients presented with recurrence were enrolled in Group 2.
Intervention(s): Age, gender, cigarette smoking history, bullae presence, pneumothorax side, length of hospital stay and presence of recurrence were noted. Initial complete blood count results were used for neutrophil to lymphocyte

ratio calculation.
Main Outcome Measure(s): All data are analyzed statistically for any significant relationships between the variables and recurrence.
Result(s): Statistical analysis using chi-square test revealed a significant relationship between recurrence and neutrophil to lymphocyte ratio (X^2=62.752, P=.000) and cigarette smoking (X^2=8.116, P=.004). We did not find any significant relationship between recurrence and presence of bullae or gender.
Conclusion(s): We believe that neutrophil to lymphocyte ratio value higher than 2.48 may be an indicator of recurrence in primary spontaneous pneumothorax patients. Further multicenter studies with larger number of patients, however, are needed to verify this conclusion.

KEY WORDS: lymphocytes, neutrophils, pneumothorax, recurrence

INTRODUCTION

Pneumothorax is defined as the presence of free air between the pleural sheets[1-3]. Primary spontaneous pneumothorax (PSP) constitutes a problem for the physician because of the tendency to recur[3]. Researchers have performed studies in search for the perfect indicator of recurrence without success. Recently, a calculation depending on the physical measurements from the chest x-ray and body mass index named as the Ankara Numune Index has been reported to be of value in predicting recurrence[1,4]. Neutrophil to lymphocyte ratio (NLR) is a recent factor used in determining the severity of various disorders including seriously ill patients in the intensive care units, tuberculosis, chronic obstructive pulmonary disease (COPD), inflammatory diseases, vascular

diseases and malignancies[5-16]. Its role in PSP, however, is not yet studied.

The aim of this study is to assess the relationship between NLR and PSP recurrence within a cohort of patients treated in a single institution.

SUBJECTS AND METHODS

Following the permission granted by the Bursa Clinical Study Ethics Committee (Permission date and number: June the 30th 2015, 2015-13/17) and Hospital Management following this permission (document date and number: July the 13rd, 2015/3313), the archive files of Bursa Yüksek Ihtisas Hospital for Education and Research were screened for cases of PSP hospitalized between 2nd July, 2002 and 30th April, 2015. Patients with only an initial episode were

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Table 1: Demographic properties of the two groups of patients

Variable	Group 1		Group 2	
	Average ± SD	Range	Average ± SD	Range
Age (years)	29.23±6.53	19-42	33.04±6.84	20-56
NLR	2.13±0.27	1.58-2.85	3.16±0.81	1.83-5.33
Length of hospital stay (days)	6.01±1.30	4-8	7.52±2.64	4-18
Interval until recurrence(months)	NA	NA	5.60±3.45	1-18
	n	**%**	**n**	**%**
Gender				
Male	51	86.4	55	84.6
Female	8	13.6	10	15.4
Affected site				
Left	23	38.9	24	36.9
Right	36	61.1	41	63.1
Tobacco				
Smoker	31	52.5	50	76.9
Non-smoker	28	47.5	15	23.1
Bullae				
Absent	27	45.7	21	33.8
Present	32	54.3	44	66.2

SD: standard deviation; NLR: neutrophil to lymphocyte ratio; NA: non-applicable.

enrolled in Group 1 and patients who presented with recurrence were enrolled in Group 2. Patients who presented with pneumothorax not leading to tube thoracostomy and due to other causes, *e.g.* trauma or COPD, were excluded. Age, gender, site of pneumothorax, cigarette smoking, presence of bullae, length of hospital stay and presence of recurrence were noted. Neutrophil, lymphocyte and platelet counts from the initial complete blood count results in the emergency room at the time of admittance were recorded as well. NLR was calculated as neutrophil count divided by lymphocyte count as defined earlier[5]. For statistical analysis, MedCalc Statistical Software version 18.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018, licensed to the author) was used. Receiver operator characteristics (ROC) curve analysis and chi-square tests were used for statistical evaluation, with a *P*-value less than 0.05 accepted as significant.

RESULTS

Within the aforementioned period, a total of 124 patients were admitted to the hospital due to either an initial or a recurrent episode of PSP. Group 1 consisted of 59 patients and there were 65 patients in Group 2. There were 51 male (86.4%) and 8 female (13.6%) patients in Group 1 with an average age of 29.23±6.53 years (range: 19-42). In Group 2, there were 55 male (84.6%) and 10 female (15.4%) patients and the average age was 33.04±6.84 years (range: 20-56). The demographic properties of the two groups are listed in Table 1 in detail.

Using the ROC curve analysis, we determined a cut-off value of 2.48 for NLR (sensitivity: 83.1%, specificity: 88.1%, area under curve (AUC): 0.916 and

95% confidence interval). Statistical analysis using chi-square test revealed significant relationship between recurrence and NLR (X^2=62.752, *P*=.000) and cigarette smoking (X^2=8.116, *P*=.004). We did not find any significant relationship between recurrence and presence of bullae or gender. Chi-square analysis results are listed in Table 2.

Table 2: Chi-square analysis of variables

Variable compared with recurrence	X^2 value	*P*-value
NLR	62.752	.000*
Smoking	8.116	.004*
Bullae	2.360	.124
Gender	0.83	.773

NLR: neutrophil to lymphocyte ratio; *statistically significant.

All patients in Group 1 were treated with tube thoracostomy with various caliber tubes changing from 14 FR to 32 FR. In Group 2, the recurrence was on the same side of the first episode in 38 patients (58%), and on the other side in 27 patients (42%). Of these patients, 18 had the second episode and the remaining patients had multiple episodes treated in other institutions. We preferred bullae resection and apical pleural abrasion in 47 (72.3%) of these patients using video assisted thoracic surgery or thoracotomy. The length of hospital stay was 6.01±1.30 days (range 4-8) in Group 1 and 7.52±2.64 days (range 4-18) in Group 2.

DISCUSSION

PSP is a benign disorder recurrent in nature[17]. Various studies suggest anatomical measurements such as Numune index and presence of bullae[1,18]. Others advocate tobacco consumption as the primary

indicator of recurrence[2]. In literature, male gender, slender body shape, cigarette smoking and presence of bullae in thorax computed tomography scan are reported as indicators[1,3,17,18]. Similar to former reports, in our groups, there was a male predominance and cigarette smoking was present in 52.5% in Group 1 and 76.9% in Group 2. In both groups, the average age was in the early 30's. We did not find any statistically significant relation between recurrence and these parameters, except for cigarette smoking (X^2=8.116, P=.004).

Presence of bullae at presentation is usually accepted as an indicator of recurrence, although there are some reports suggesting the opposite[4,18]. In our series, bullae were present in 54.2% in Group 1 and 66.2% in Group 2. We did not find any statistically significant relation between bullae presence and recurrence.

The time interval between the first and the second episode is reported as ranging from 2 to 18 months. In our study, the interval was 5.60±3.45 months (range: 1-18 months). This finding also is similar to former reports.

Neutrophil to lymphocyte ratio has become a popular indicator of poorer outcome in various disorders. In studies published recently, NLR is reported to be related to bad prognosis in seriously ill patients in the intensive care units, tuberculosis, COPD, inflammatory diseases, vascular diseases and malignancies[5-16]. Dilektasli et al revealed that an NLR greater than 8.19 and 7.92 are independent indicators of in-hospital mortality at days 2 and 5, respectively, in the critically ill trauma patients treated in intensive care units[6]. Gunay et al calculated a NLR value of 1.71±0.65 in control, 2.59±1.79 in stable and 4.28±4.12 in exacerbated COPD patients[8]. Iliaz et al reported that NLR value of 2.55 was effective in distinguishing between tuberculosis and sarcoidosis[7]. Choi et al studied the preoperative NLR levels in lung cancer patients as a predictor of survival in addition to non-steroid anti-inflammatory drug use postoperatively[13]. They reported that preoperative NLR ≥5 is an indicator of shorter overall survival. Takahashi et al studied preoperative NLR as an indicator of prognosis in a selected group of lung cancer patients[15]. Using the ROC curve analysis, they determined a cut off value for NLR as 2.498 with 66.7% sensitivity, 58.5% specificity and AUC=0.684. They used this value to discriminate the preoperative NLR as low, i.e. less than 2.498, and high. They concluded that low NLR values had a chance of 89.2% overall survival calculated using the Kaplan-Meier method. We calculated the NLR values of each patient from the initial complete blood count results from the emergency department. The average NLR values were 2.13±0.27 (range: 1.58-2.85) in Group 1 and 3.16±0.81 (range: 1.83-5.33) in Group 2.

We used ROC analysis to determine the cut-off value in our series as 2.48 with 83.1% sensitivity, 88.1% specificity, AUC=0.916 and 95% confidence interval. The statistical analysis revealed a significant relationship between NLR value and recurrence in PSP patients (X^2=62.752, P=.000). This result implies that a NLR value over 2.48 calculated from the complete blood count at the initial episode indicates a recurrence.

CONCLUSION

According to these results, we suggest that NLR may be an indicator of recurrence in PSP. To our knowledge, this is the first study evaluating NLR in relation to recurrence in PSP to be published in literature. The limitations of our study include the limited number of patients in both groups, the presence of various hospitals in the area patients may seek medical assistance from and thus resulting in loss of patients under our clinical surveillance.

We conclude that further multi-center clinical studies including larger number of patients will help to assess the value of NLR as an indicator of recurrence in PSP.

REFERENCES

1. Akkas Y, Peri NG, Kocer B, Kaplan T, Alhan A. A novel structural risk index for primary spontaneous pneumothorax: Ankara Numune Risk Index. Asian J Surg 2017; 40(4):249-253.
2. Haynes D, Baumann MH. Management of pneumothorax. Semin Respir Crit Care Med 2010; 31(6):769-780.
3. Huang YF, Chiu WC, Chou SH, Su YH, Chen YW, Chai CY, et al. Association of MMP-2 and MMP-9 expression with recurrences in primary spontaneous pneumothorax. Kaohsiung J Med Sci 2017; 33(1):17-23.
4. Haynes D, Baumann MH. Pleural controversy: aetiology of pneumothorax. Respirology 2011; 16(4):604-610.
5. Zahorec R. Ratio of neutrophil to lymphocyte counts rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy 2001; 102(1):5-14.
6. Dilektasli E, Inaba K, Haltmeier T, Wong MD, Clark D, Benjamin ER, et al. The prognostic value of neutrophil-to-lymphocyte ratio on mortality in critically ill trauma patients. J Trauma Acute Care Surg 2016; 81(5):882-888.

7. Iliaz S, Iliaz R, Ortakoylu G, Bahadir A, Bagci BA, Caglar E. Value of neutrophil/lymphocyte ratio in the differential diagnosis of sarcoidosis and tuberculosis. Ann Thorac Med 2014; 9(4):232-235.

8. Gunay E, Ulasli SS, Akar O, Ahsen A, Gunay S, Koyuncu T, *et al*. Neutrophil-to-lymphocyte ratio in chronic obstructive pulmonary disease: a retrospective study. Inflammation 2014; 37(2):374-380.

9. Ahsen A, Ulu MS, Yuksel S, Demir K, Uysal M, Erdogan M, *et al*. As a new inflammatory marker for familial Mediterranean fever: neutrophil-to-lymphocyte ratio. Inflammation 2013; 36(6):1357-1362.

10. Korkmaz M, Korkmaz H, Kucuker F, Ayyildiz SN, Cankaya S. Evaluation of the association of sleep apnea-related systemic inflammation with CRP, ESR, and neutrophil-to-lymphocyte ratio. Med Sci Monit 2015; 21:477-481.

11. Tamhane UU, Aneja S, Montgomery D, Rogers EK, Eagle KA, Gurm HS. Association between admission neutrophil to lymphocyte ratio and outcomes in patients with acute coronary syndrome. Am J Cardiol 2008; 102(6):653-657.

12. Yanartas M, Kalkan ME, Arslan A, Tas SG, Koksal C, Bekiroglu N, *et al*. Neutrophil/lymphocyte ratio can predict postoperative mortality in patients with chronic thromboembolic pulmonary hypertension. Ann Thorac Cardiovasc Surg 2015; 21(3):229-235.

13. Choi JE, Villarreal J, Lasala J, Gottumukkala V, Mehran RJ, Rice D, *et al*. Perioperative neutrophil: lymphocyte ratio and postoperative NSAID use as predictors of survival after lung cancer surgery: a retrospective study. Cancer Med 2015; 4(6):825-833.

14. Zhao QT, Yang Y, Xu S, Zhang XP, Wang HE, Zhang H, *et al*. Prognostic role of neutrophil to lymphocyte ratio in lung cancers: a meta-analysis including 7,054 patients. Onco Targets Ther 2015; 8:2731-2738.

15. Takahashi Y, Horio H, Hato T, Harada M, Matsutani N, Morita S, *et al*. Prognostic significance of preoperative neutrophil-lymphocyte ratios in patients with Stage I non-small cell lung cancer after complete resection. Ann Surg Oncol 2015; 22 Suppl 3:S1324-S1331.

16. Takahashi Y, Kawamura M, Hato T, Harada M, Matsutani N, Horio H. Neutrophil-lymphocyte ratio as a prognostic marker for lung adenocarcinoma after complete resection. World J Surg 2016; 40(2):365-372.

17. Kepka S, Dalphin JC, Parmentier AL, Pretalli JB, Gantelet M, Bernard N, *et al*. Primary spontaneous pneumothorax admitted in emergency unit: Does first episode differ from recurrence? A cross-sectional study. Can Respir J 2017; 2017:2729548.

18. Casali C, Stefani A, Ligabue G, Natali P, Aramini B, Torricelli P, *et al*. Role of blebs and bullae detected by high-resolution computed tomography and recurrent spontaneous pneumothorax. Ann Thorac Surg 2013; 95(1):249-255.

Original Article

Oral hygiene habits and storage method impact on toothbrush contamination among participants in Kuwait

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ABSTRACT

Objective(s): To investigate oral hygiene habits and the impact of different storage methods on bacterial growth on toothbrushes among participants in Kuwait
Design: Prospective controlled study
Setting: Health Science Centre, Kuwait University, and Asnan Tower (private dental clinic) between December 2013 and January 2014
Subjects: The study was conducted among 240 participants (students, staff and patients) that were selected by convenience sampling and allocated randomly into five groups according to storage method.
Intervention(s): Each participant was provided with a new toothbrush to use for five continuous days. A questionnaire regarding the oral hygiene habits was also given.
Main Outcome Measure(s): Oral hygiene habits and bacterial growth on toothbrushes were identified.
Result(s): Of the participants, 38%, 27%, and 25% brush their teeth twice a day, after meals, and once a day (morning) respectively. Most participants store their toothbrushes in the bathroom after use but only 5% store them outside the bathroom. Ten different bacterial strains were isolated from 182 toothbrushes and one type of yeast, *Candida Spp.* was identified. *Neisseria spp.* and *Staphylococcus epidermidis* were shown to be present in all groups regardless of the toothbrush storage method. *Streptococcus viridans*, *Bacillus spp.*, *Klebsiella pneumoniae* and *Enterobacter agglomerans* have been identified solely in specific groups only.
Conclusion(s): The participants showed satisfactory standards of oral hygiene habits and were definitely in need of raising the awareness of implementing better oral health. Soaking the toothbrush in chlorhexidine has shown to be the best storage method in terms of the presence of bacterial strains.

KEY WORDS: microbial contamination, oral hygiene, toothbrushes

INTRODUCTION

The oral cavity contains many different complex surfaces providing various environments for microorganisms' growth and colonization. These microorganisms usually live in the oral cavity in several habitats, which include the teeth with its supragingival and subgingival surfaces, crevicular epithelium, dorsum of the tongue, buccal mucosa, hard and soft palate, tonsils and prosthodontic and orthodontic appliances, if present[1]. Up to 700 species of bacteria, which are commensals and pathogens, have been identified in the oral cavity[2]. The microorganisms in the oral cavity are the main causes of oral diseases, such as dental caries, gingivitis and periodontitis. Hence, toothbrushing is vital to remove these microorganisms

as a part of oral hygiene regime for the prevention of oral diseases. However, it is not about toothbrushing alone, but using fluoridated toothpastes, as its efficacy in preventing dental caries has been shown with clear evidence by a Cochrane systematic review with meta-analyses[3]. Another Cochrane systematic review with meta-analyses also showed that using powered toothbrush may result in 21% reduction in plaque after three months of use and 11% reduction in gingivitis when compared with a manual toothbrush[4]. Although toothbrushing is important to remove plaque and provides good oral hygiene, toothbrushes might be the source of repeated oral infections[5]. Several studies show that toothbrushes serve as a reservoir for bacteria that can be extremely contaminating after use[6].

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Since the oral cavity could carry several opportunistic pathogens, immunocompromised or elderly people might suffer from serious lung infections[7]. Moreover, it has been shown that periodontitis can increase the risk of atherosclerotic cardiovascular diseases, which include fatal and non-fatal coronary heart diseases (angina, myocardial infarction), ischemic cerebrovascular disease (stroke/transient ischemic attack) and peripheral arterial disease[8]. Therefore, microorganisms contaminating or accumulating on toothbrushes are not only affecting the oral health, but the general health of an individual as well[9].

The purpose of this study is to investigate the oral hygiene habits and the impact of different storage methods on bacterial growth on toothbrushes.

SUBJECTS AND METHODS
Sampling and allocation of participants

A convenience sampling method was implemented in this study, followed by allocating the participants randomly into five different groups using a random numbers table. New toothbrushes and toothpastes were distributed to the five groups, each with the same toothbrushing instructions but different storage methods. All participants were advised to use the toothbrush twice a day and rinse them with tap water before and after use, but without using the covering cap. Moreover, participants were asked to brush their teeth surfaces, and tongue if possible, using a pea-sized amount of toothpaste with avoidance of rinsing with water after brushing unless necessary.

Groups were assigned as group A, B, C, D and E. Group A was the control group where toothbrushes were stored on toilet sink and group B stored toothbrushes on bedside tables. Group C stored the toothbrushes in a closed toilet cupboard, whereas group D soaked toothbrushes in oral rinse (without changing it during the whole study period) and stored them on the toilet sink. However, participants in group E were instructed to soak toothbrushes in oral rinse similar to group D, but the oral rinse was changed daily.

All participants were provided with new CURAPROX 5460 ultra soft manual toothbrushes (Curaden International, Kriens, Switzerland), CURAPROX Enzycal toothpaste (Curaden International, Kriens, Switzerland), plastic containers to stand the toothbrush and sterile plastic bags for recollection. Additionally, participants in groups D and E were provided with CURASEPT ADS® chlorhexidine oral rinses to soak the toothbrushes after rinsing them with tap water until next use. The toothbrushes were recollected after five days of use

in sterile plastic bags. All samples were collected from staff members and students of the Health Science Centre of Kuwait University. Toothbrush collection was also including the staff members and regular attendees of a private dental clinic in Kuwait, which funded this study.

In addition, a questionnaire was distributed to the participants to investigate their oral hygiene habits, such as the use of toothbrushes and other aspects of dental hygiene, including frequency of dental visits, frequency of brushing teeth, using dental floss and method of toothbrush storage.

Bacterial/microorganism identification

Samples were processed at room temperature (~23°C) within 24 hours once collected at the Microbiology Laboratory in the Medical Laboratory Sciences Department, Faculty of Allied Health Sciences, Health Sciences Centre, Kuwait University, Kuwait. Bacterial strains depositing on toothbrushes and rinses were isolated as pure cultures using different media: blood agar, MacConkey agar, chocolate agar and Sabouraud agar (Oxoid Ltd., UK). Blood agar was used for the identification of Gram-positive bacteria, while MacConkey agar was used for identifying Gram-negative bacteria. Moreover, chocolate agar was used for the identification of *Neisseria* and *Hemophilus* spp., whereas Sabouraud agar was used for identifying fungi. All isolates were incubated for 24 hours at 37 °C aerobically and anaerobically. Identification of bacterial species was done at the same laboratory following the "gram stain" flow chart of the Practical Handbook of Microbiology[10]. The obtained bacterial colonies were used for gram staining with gram stain solution (Sigma-aldrich Company, Poole, Ltd, UK). Following Gram staining results, identification schemes of target organisms incorporating biochemical tests were followed[10]. For gram-positive cocci identification, catalase test was used either via API Staph or API Strep (Oxoid) while identification of gram-negative cocci was accomplished by API NH (Oxoid). Whereas identification of target gram positive bacilli, motility test by hanging drop method was used, gram-negative bacilli was done by oxidase test and API 20E (Oxoid). Confirmation of identification of bacterial strains was done using the automated system MicroScan WalkAway-40 System (Dade Behring, West Sacramento, California, USA).

Ethical approval

This study has been granted an ethical approval from Health Sciences Centre Ethical Committee, Kuwait University.

Table 1: The participant's characteristics and their oral hygiene habits

| Variable | Gender | | Total (%) |
	Male n(%)	Female n(%)	
Age			
16-25	100 (74.6)	68 (68)	168 (71.8)
26-35	24 (17.9)	22 (22)	46 (19.7)
36-45	10 (7.5)	10 (10)	20 (8.5)
Total	134 (57.3)	100 (42.7)	234 (100)
Last visit to the dentist			
Within the last 3 months	62 (46.3)	46 (46)	108 (46.2)
Within the last 6 months	30 (22.4)	34 (34)	64 (27.4)
Last year or more	42 (31.3)	20 (20)	62 (26.5)
Total	134 (57.3)	100 (42.7)	234 (100)
Smoking status			
Smoker	48 (35.8)	0 (0)	48 (20.5)
Non-smoker	78 (58.2)	92 (92)	170 (72.6)
Occasional smoking	8 (6)	8 (8)	16 (6.8)
Total	134 (57.3)	100 (42.7)	234 (100)
Experiencing halitosis (bad breath)			
Yes	10 (7.5)	4 (4)	14 (6)
No	96 (71.6)	74 (74)	170 (72.6)
Occasionally	28 (20.9)	22 (22)	50 (21.4)
Total	134 (57.3)	100 (42.7)	234 (100)
Tongue brushing			
Yes	84 (62.7)	58 (58)	142 (60.7)
No	28 (20.9)	26 (26)	54 (23.1)
Occasionally	22 (16.4)	16 (16)	38 (16.2)
Total	134 (57.3)	100 (42.7)	234 (100)
Experiencing bleeding during toothbrushing			
Yes	34 (25.4)	22 (22)	56 (23.9)
No	62 (46.3)	42 (42)	104 (44.4)
Occasionally	38 (28.4)	36 (36)	74 (31.6)
Total	134 (57.3)	100 (42.7)	234 (100)
Do you floss?			
Yes – Regularly	26 (19.4)	20 (20)	46 (19.7)
No	64 (47.8)	38 (38)	102 (43.6)
Occasionally	44 (32.8)	42 (42)	86 (36.8)
Total	134 (57.3)	100 (42.7)	234 (100)
Mouthwash use			
Yes - Regularly	40 (29.9)	20 (20)	60 (25.6)
No	50 (37.3)	46 (46)	96 (41)
Occasionally	44 (32.8)	34 (34)	78 (33.3)
Total	134 (57.3)	100 (42.7)	234 (100)
Toothbrushing timing			
Once (morning)	40 (29.9)	18 (18)	58 (24.8)
Once (before sleeping)	14 (10.4)	6 (6)	20 (8.5)
Twice a day	44 (32.8)	44 (44)	88 (37.6)
After meals	32 (23.9)	32 (32)	64 (27.4)
Other	4 (3.0)	0 (0)	4 (1.7)
Total	134 (57.3)	100 (42.7)	234 (100)
Placing the toothbrush with other toothbrushes			
Yes	74 (55.2)	76 (76)	150 (64.1)
No	52 (38.8)	16 (16)	68 (29.1)
Occasionally	8 (6.0)	8 (8)	16 (6.8)
Total	134 (57.3)	100 (42.7)	234 (100)
Frequency of toothbrush replacement			
Every 3 months	36 (26.9)	52 (52)	88 (37.6)
Every 6 months	72 (53.7)	38 (38)	110 (47)
Every year	22 (16.4)	6 (6)	28 (12)
Other	4 (3.0)	4 (4)	8 (3.4)
Total	134 (57.3)	100 (42.7)	234 (100)

| Variable | Gender | | Total (%) |
	Male n(%)	Female n(%)	
Toothbrush storage place			
Bathroom – on the sink	102 (76.1)	96 (96)	198 (84.6)
Bathroom – away from the sink	22 (16.4)	2 (2)	24 (10.3)
Outside the bathroom	10 (7.5)	2 (2)	12 (5.1)
Total	134 (57.3)	100 (42.7)	234 (100)
Do you cover your toothbrush with a cap after use?			
Yes	10 (7.5)	8 (8)	18 (7.7)
No	124 (92.5)	92 (92)	2016(92.3)
Total	134 (57.3)	100 (42.7)	234 (100)
Are you anxious about dental treatment?			
Yes	18 (13.4)	18 (18)	36 (15.4)
No	98 (73.1)	60 (60)	158 (67.5)
Occasionally	18 (13.4)	22 (22)	40 (17.1)
Total	134 (57.3)	100 (42.7)	234 (100)
Are you happy with your teeth and smile?			
Yes	78 (58.2)	40 (40)	118 (50.4)
No	28 (20.9)	22 (22)	50 (21.4)
Occasionally	28 (20.9)	38 (38)	66 (28.2)
Total	134 (57.3)	100 (42.7)	234 (100)
How do you evaluate your oral health?			
Good	60 (44.8)	56 (56)	116 (49.6)
Fine	56 (41.8)	38 (38)	94 (40.2)
Bad	18 (13.4)	6 (6)	24 (10.3)
Total	134 (57.3)	100 (42.7)	234 (100)

RESULTS

Participants' characteristics and oral hygiene habits

Two hundred and forty participants were enrolled in the study (n=240) and distributed equally into five different groups (n=48*5). Table 1 shows the participants characteristics and their oral hygiene habits (six participants refused to fill the questionnaire).

Bacterial/microorganism identification

For each group, 48 new toothbrushes were distributed for re-collection after use. However, not all of the participants returned their toothbrushes back. Only 44, 40, 24, 36 and 38 toothbrushes were returned in groups A, B, C, D and E respectively, which in total was 182 toothbrushes. Multiple attempts were made to contact the participants who did not return the toothbrushes by text messages and phone calls. The majority of those participants could not be reached either by providing incorrect contact details or not responding at all, while the rest have lost their provided equipment, no longer want to be included in the study or werer not available in Kuwait. Therefore, 58 toothbrushes were not re-collected. Ten different bacterial strains and candida species were isolated from the used toothbrushes (Table 2). These microorganisms were *Neisseria spp.* (37%), *Staphylococcus epidermidis* (35.6%), *Aerococcus*

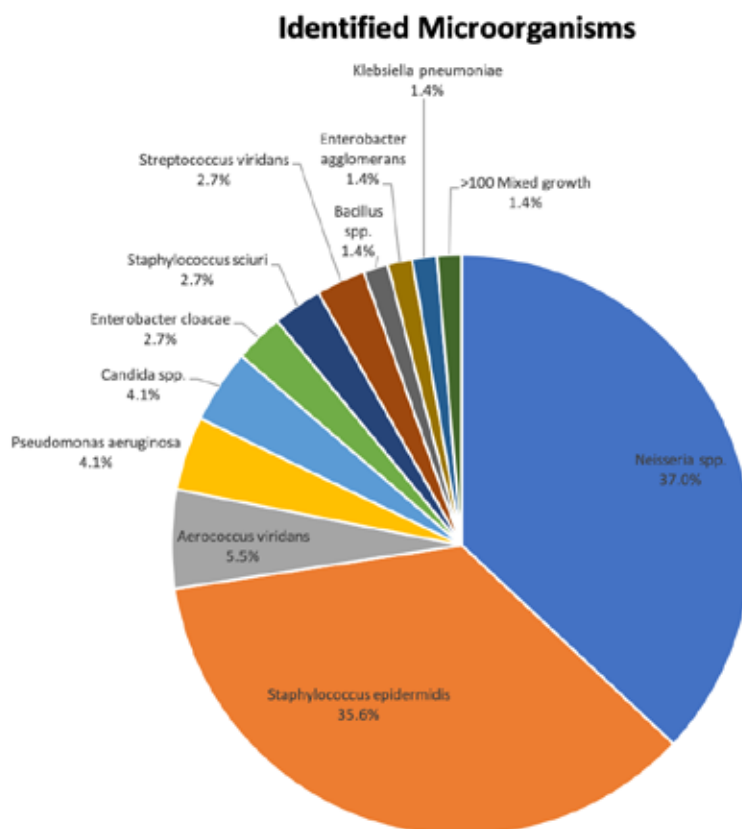**Table 2:** Isolated microorganisms from each group

Group/Microorganism	A	B	C	D	E
Neisseria spp.	√	√	√	√	√
Staphylococcus epidermidis	√	√	√	√	√
Aerococcus viridans	√	√	X	X	√
Pseudomonas aeruginosa	√	√	√	X	X
Candida spp.	√	X	√	X	X
Enterobacter cloacae	√	X	√	X	X
Staphylococcus sciuri	√	√	X	X	X
Streptococcus viridans	√	X	X	X	X
Bacillus spp.	√	X	X	X	X
Enterobacter agglomerans	X	X	X	X	√
Klebsiella pneumoniae	X	X	X	√	X
>100 Mixed growth	X	X	X	X	√

viridans (5.5%), *Pseudomonas aeruginosa* (4.1%), *Candida spp.* (4.1%), *Enterobacter cloacae* (2.7%), *Staphylococcus sciuri* (2.7%), *Streptococcus viridans* (2.7%), *Bacillus spp.* (1.4%), *Enterobacter agglomerans* (1.4%), *Klebsiella pneumoniae* (1.4%) and more than 100 mixed growth (1.4%) as shown in Figure 1. Table 3 shows the number of microorganisms found in each toothbrush according to gender.

Neisseria spp. and *Staphylococcus epidermidis* were the most common and only two microorganisms that have been identified in all of the five groups. As expected, the control group A had the greatest variation of microorganisms harbouring the toothbrushes compared to other groups. However, group D had the least variation of bacterial strains identified in the collected toothbrushes. Some bacterial strains were identified solely in specific groups such as *Streptococcus viridans* and *Bacillus spp.* in group A, *Klebsiella pneumoniae* in group D, and *Enterobacter agglomerans* in group E. Surprisingly, three different bacterial strains, which are *Enterobacter cloacae*, *Bacillus spp.*, and *Enterobacter agglomerans*, were identified only in males' toothbrushes; while *Klebsiella pneumoniae* was found only in females' toothbrushes. (Appendix 1: Detailed microorganisms' identification in each group according to gender).

DISCUSSION

The use of toothbrushes is considered as an essential part of oral hygiene. However, toothbrushes were repeatedly reported to be a source of various oral infections[4,5]. Microorganisms anchoring the tufts and bristles of toothbrushes can cause different localized and systemic diseases[6,11]. The results obtained from this study support previous studies in terms of bacterial strains, which are contaminating regularly

Identified Microorganisms

Fig 1: The percentages of the identified microorganisms isolated from the used toothbrushes

APPENDICES

Appendix 1: Detailed microorganisms' identification in each group according to gender.
1. Group A: Control

Parameters	Gender		Total
	Male	Female	
Number of microorganisms identified in one toothbrush			
0	0	2	2
1	20	14	34
2	6	2	8
Total number of toothbrushes	26	18	44
Microorganisms identified	1. Aerococcus viridans 2. Bacillus spp 3. Candida spp 4. Enterobacter cloacae 5. Neisseria spp 6. Pseudomonas aeruginosa 7. Staphylococcus epidermidis 8. Staphylococcus sciuri 9. Streptococcus viridans	1. Aerococcus viridans 2. Candida spp 3. Neisseria spp 4. Staphylococcus epidermidis 5. Streptococcus viridans	

Microorganism	Frequency of presence [M-F]
No growth	2 [0-2]
1. Neisseria spp	18 [8-10]
2. Staphylococcus epidermidis	12 [10-2]
3. Aerococcus viridans	4 [2-2]
4. Candida spp	4 [2-2]
5. Streptococcus viridans	4 [2-2]
6. Enterobacter cloacae	2 [2-0]
7. Pseudomonas aeruginosa	2 [2-0]
8. Staphylococcus sciuri	2 [2-0]
9. Bacillus spp	2 [2-0]
Total (9 microorganisms)	50 [32-18]

2. **Group B:** Bedside table

Parameters	Gender		Total
	Male	Female	
Number of microorganisms identified in one toothbrush			
0	4	12	16
1	14	6	20
2	0	4	4
Total number of toothbrushes	18	22	40
Microorganisms identified	1. Staphylococcus epidermidis	1. Aerococcus viridans 2. Neisseria spp 3. Pseudomonas aeruginosa 4. Staphylococcus epidermidis 5. Staphylococcus sciuri	

Microorganism	Frequency of presence [M-F]
No growth	16 [4-12]
1. Staphylococcus epidermidis	16 [14-2]
2. Neisseria spp	6 [0-6]
3. Aerococcus viridans	2 [0-2]
4. Pseudomonas aeruginosa	2 [0-2]
5. Staphylococcus sciuri	2 [0-2]
Total (5 microorganisms)	28 [14-14]

3. **Group C:** Closed cupboard:

Parameters	Gender		Total
	Male	Female	
Number of microorganisms identified in one toothbrush			
0	0	6	6
1	12	4	16
2	0	2	2
Total number of toothbrushes	12	12	24
Microorganisms identified	1. Enterobacter cloacae 2. Neisseria spp 3. Staphylococcus epidermidis	1. Candida spp 2. Neisseria spp 3. Pseudomonas aeruginosa 4. Staphylococcus epidermidis	

Microorganism	Frequency of presence [M-F]
No growth	6 [0-6]
1. Staphylococcus epidermidis	10 [8-2]
2. Neisseria spp	4 [2-2]
3. Enterobacter cloacae	2 [2-0]
4. Pseudomonas aeruginosa	2 [0-2]
5. Candida spp	2 [0-2]
Total (5 microorganisms)	20 [12-8]

4. **Group D:** immersed in mouth wash (same amount):

Parameters	Gender		Total
	Male	Female	
Number of microorganisms identified in one toothbrush			
0	10	10	20
1	10	4	14
2	0	2	2
Total number of toothbrushes	20	16	36
Microorganisms identified	1. Neisseria spp 2. Staphylococcus epidermidis	1. Klebsiella pneumoniae 2. Neisseria spp	

Microorganism	Frequency of presence [M-F]
No growth	20 [10-10]
1. Neisseria spp	14 [8-6]
2. Staphylococcus epidermidis	2 [2-0]
3. Klebsiella pneumoniae	2 [0-2]
Total (5 microorganisms)	18 [10-8]

5. **Group E:** immersed in mouth wash (changed daily):

Parameters	Gender		Total
	Male	Female	
Number of microorganisms identified in one toothbrush			
0	4	8	12
1	14	8	22
2	2	2	4
Total number of toothbrushes	20	18	38
Microorganisms identified	1. Aerococcus viridans 2. Enterobacter agglomerans 3. Neisseria spp 4. Staphylococcus epidermidis	1. >100 Mixed growth 2. Neisseria spp 3. Staphylococcus epidermidis	

Microorganism	Frequency of presence [M-F]
No growth	12 [4-8]
1. Neisseria spp	12 [8-4]
2. Staphylococcus epidermidis	12 [6-6]
3- >100 Mixed growth	2 [0-2]
4. Aerococcus viridans	2 [2-0]
5. Enterobacter agglomerans	2 [2-0]
Total (5 microorganisms)	30 [18-12]

Table 3: The number of microorganisms found in each toothbrush according to gender

Variable/ participant	Gender		Total (%)
	Male	Female	
Number of microorganisms (n(%))			
0	18 (18.75%)	38 (44.19%)	56 (30.77%)
1	70 (72.92%)	36 (41.86%)	106 (58.24%)
2	8 (8.33%)	12 (13.95%)	20 (10.99%)
Total	96 (100%)	86 (100%)	182 (100%)
Bacterial strains/microorganisms identified (n)			
Aerococcus viridans	4	4	8
Bacillus spp.	2	0	2
Candida spp.	2	4	6
Enterobacter agglomerans	2	0	2
Enterobacter cloacae	4	0	4
Klebsiella pneumoniae	0	2	2
Neisseria spp.	26	28	54
Pseudomonas aeruginosa	2	4	6
Staphylococcus epidermidis	40	12	52
Staphylococcus sciuri	2	2	4
Streptococcus viridans	2	2	4
>100 Mixed growth	0	2	2

when using toothbrushes. Some of these strains are of pathogenic nature, such as *K. pneumoniae* and *P. aeruginosa*, while *S. viridans* is an opportunistic strain. Others were reported to be of faecal nature such as *E. cloacae* and *Neisseria* spp.[12,13]. Thereby, toothbrushes can be considered as a possible source of contamination to their users.

In terms of oral hygiene standards, female participants showed more diligence than male participants. This could be concluded from the results of isolating more bacterial strains in males' toothbrushes, while fewer bacterial strains were isolated in females' toothbrushes. Nevertheless, the questionnaires reflect that females tend to brush their teeth more often than males. Regarding storage methods, some bacteria such as *Neisseria* spp. and *S. epidermidis* were reported from all groups with different storage means. This might rule out the hypothesis of the transmission of *Neisseria* strains from toilet flushing. It might be transmitted from poor hand hygiene, as supported by the finding of *S. epidermidis*, which is thought to be a part of skin commensals. As for yeasts, *Candida* was isolated from the toothbrushes of both genders, stored on toilet sinks and in females' toothbrushes that were immersed in unchanged mouthwashes. This could also point out to the existence of these yeasts as normal flora of the mouth and not related to storage means. Another interesting finding from this study is the isolation of fewer bacterial strains from storing the toothbrushes in an unchanged mouthwash compared to those that were stored in a daily changed mouthwash. The detection of bacterial strains in the changed mouthwash might be due to cross contamination of the toothbrushes during handling and changing the mouthwash. This indicates the effectiveness of storing toothbrushes into

chlorhexidine and supports previous studies[14,15].

Therefore, we can conclude that soaking toothbrushes in mouthwashes that are not changed on a daily basis after use is good in reducing the number of types of bacteria. Further studies are needed to investigate and to compare between the bacteria that are residing in the oral cavity of healthy individuals and individuals with underlying medical condition such as diabetes, cardiovascular diseases, immunocompromised patients and to evaluate its relationship with dental and periodontal diseases.

CONCLUSION

In conclusion, oral hygiene performs an integral part of our bodily hygiene. If neglected, different diseases can occur and affect the mouth and other organs of the body. Females were proved to show higher standards of oral hygiene than males, which reflected on bacterial growth in their oral cavities. However, greater bacterial growth on males' toothbrushes may also indicate a better tooth brushing in which bacteria have been removed properly and sufficiently, compared to females. It is not necessarily that fewer bacteria present on toothbrushes means poor oral hygiene and vice versa. Other biologic factors might play a role in the survival of certain bacterial strains in the oral cavities of different genders. Storing methods of toothbrushes may also contribute to the growth of different bacteria on toothbrushes. This was witnessed in previous reports and further supported in the results obtained from this study, which indicated the better-advised storage method. Nevertheless, the absence of bacterial deposits on the bristles of toothbrushes soaked in chlorhexidine indicated the effectiveness of storage into mouthwash. However, the effect of chlorhexidine

on toothbrush bristles and its effectiveness was not investigated in this study. More studies should be made focusing on other factors that might play a role in toothbrushes storage and contamination, such as seasons and temperatures.

REFERENCES

1. Samaranayake L. Essential microbiology for dentistry. 4th ed. Edinburgh: Churchill Livingstone/Elsevier; 2012. p. 270-3.
2. Lamont R, Jenkinson H. Oral microbiology at a glance. Chichester, West Sussex, U.K.: Wiley-Blackwell; 2010. p. 2-4.
3. Marinho V, Higgins J, Logan S, Sheiham (deceased) A. Fluoride toothpastes for preventing dental caries in children and adolescents. Cochrane Database of Systematic Reviews [Internet]. 2003 [cited 25 November 2018]. Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD002278/full
4. Yaacob M, Worthington HV, Deacon SA, Deery C, Walmsley A, Robinson PG, *et al*. Powered versus manual toothbrushing for oral health. Cochrane Database of Syst Rev 2014; 2014(6):CD002281.
5. Cobb CM. The tooth brush as a cause of repeated infections of the mouth. Boston Med Surg J 1920; 183:263-264.
6. Frazelle MR, Munro CL. Toothbrush contamination: A review of the literature. Nursing Research and Practice 2012; Article ID 420630.
7. Marsh P, Martin M, Lewis M, Williams D. Oral Microbiology. 5th ed. London: Elsevier Health Sciences UK; 2009. p. 5-6.
8. Tonetti MS, Van Dyke T, working group 1 of the joint EFP/AAP workshop. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Periodontol 2013; 84(4 Suppl):S24-S29.
9. Karibasappa GN, Nagesh L, Sujatha BK. Assessment of microbial contamination of toothbrush head: An in vitro study. Indian J Dent Res 2011; 22(1):2-5.
10. Goldman E, Green L. Practical handbook of microbiology. 3rd ed. New York: Taylor and Francis group; 2015. p. 71-92.
11. Saini R, Kulkarni V. Toothbrush: A favorable media for bacterial growth. Int J Experiment Dent Sci 2013; 2(1):27-28.
12. Keller R, Pedroso MZ, Ritchmann R, Silva RM. Occurrence of virulence-associated properties in *Enterobacter cloacae*. Infect Immun 1998; 66(2):645-649.
13. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 2005; 43(11):5721-5732.
14. Aysegul O, Elgin IE, Gulcin A, Nedim S. The efficacy of chlorhexidine spray vs mouthwash in the microbial contamination of child toothbrushes. J Dent Child (Chic) 2007; 74(3):177-181.
15. Naik R, Mujib BR, Telagi N, Anil BS, Spoorthi BR. Contaminated tooth brushes-potential threat to oral and general health. J Family Med Prim Care 2015; 4(3):444-448.

Original Article

Arthroscopic Bankart repair by using the antero-superior portal for visualization

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ABSTRACT

Objective: The posterior portal was commonly utilized as the primary visualization portal in Arthroscopic Bankart repair surgery, however the pathology was mainly based on the anterior part of the glenoid. We aimed to evaluate the early results of Bankart lesions repaired by using an anterosuperior portal for visualization.
Design: Retrospective study
Setting: Clinic of Orthopaedics and Traumatology, Istanbul Education and Research Hospital, Istanbul
Subjects: A total of 37 (30 male and seven females with a mean age of 28.4 years (range: 17-45)) consecutive patients with anterior shoulder instability
Intervention: Arthroscopic bankart repair for anterior shoulder instability by using the antero-superior portal for visualization
Main outcome measures: The clinical assessment was based on the evaluation of the shoulder range of motion, shoulder laxity test (Apprehension, Jobe relocation) and clinical outcomes were assessed by using the American Shoulder and Elbow Surgeons (ASES), as well as the Rowe shoulder scoring system.
Results: The mean follow-up period was 32.5 months (range: 24-48). The mean ASES score and Rowe score increased from 71.3±7.0 (range: 59-93) and 43.5±6.5 (range: 30-50) preoperatively, to 92.8±3.1 (range: 85-98) and 92.2±6.2 (range: 75-100) postoperatively respectively (P<.05). Redislocation was observed in two patients.
Conclusions: It was observed that the anterior-superior portal was sufficient and reliable for visualization during the entire procedure as a safe and effective alternative to the generally accepted classic posterior portal visualization.

KEY WORDS: antero-superior portal, arthroscopic bankart repair, instability, shoulder dislocation

INTRODUCTION

The shoulder is considered to be the joint with the highest mobility in the human body; however, it is also a fact that this wide range of motion might have the potential to compromise its stability and to make it prone to dislocations[1,2].

Shah *et al* reported that the incidence of shoulder dislocation was 15.5 in females and 40.4 in males per 100,000 person-years. It was observed that the highest incidence was 80.5 in men per 100,000 person-years between the ages of 16-20 years in the United Kingdom[3].

The majority of dislocations are noted to be in the anterior direction and therefore, anterior structures including the anterior glenoid, labrum, capsule or anterior inferior glenohumeral ligament are mostly torn or injured. The Bankart lesion, which was defined as the detachment of the anteroinferior glenoid labrum from the glenoid, presented in more than 90% of cases with dislocation[4,5]. However, other associated injuries must be identified carefully and addressed appropriately for adequate treatment in order to obtain favorable clinical and functional outcomes. Due to the low rate of complications and satisfactory outcomes, arthroscopic surgery has been widely performed and is recommended for the treatment of Bankart lesions[6,7]. To achieve better results, many technical steps have been published in the literature[8-10]. However, no

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single standard technique was reported as the standard treatment of this condition and different surgeons prefer to use variable patient positions, portals, anchors and suture techniques with comparable results[6-12].

The posterior portal was commonly utilized as the primary visualization portal by many authors[9,13-15]. However, since the pathology was mainly based on the anterior part of the glenoid, we believe that direct visualization from the antero-superior portal allows the careful identification of associated injuries and might be a good alternative to the posterior portal during the entire procedure. In the present study, we aimed to evaluate the early results of Bankart lesions repaired by using an antero-superior portal for visualization.

SUBJECTS AND METHODS

A total of 53 consecutive patients who were operated arthroscopically between 2013 and 2015 for anterior instability were retrospectively reviewed. The inclusion criteria comprised having purely anterior instability and having a minimum follow-up duration of 24 months. The exclusion criteria included having a revision surgery prior to operation (two patients), having voluntary or multidirectional instability (two patients) and having pure posterior instability (two patients). Furthermore, five patients were lost to follow-up, three patients refused to participate in the study and re-dislocation was observed in another two patients. After the exclusion of 16 patients, the remaining 37 patients were enrolled in the study. All data was collected in a retrospective manner from the hospital records and the intraoperative data were collected based on surgical reports and video records taken during the surgery. Informed consent was taken

from all patients, so that their pre, intra and post-operative data including the photos and videos could be used for publication by ensuring that all identities remained hidden. This retrospective study received the approval of the institutional review board (No. 1436; 28 Sep 2018).

The clinical assessment was based on the evaluation of the shoulder range of motion (ROM) and shoulder hyperlaxity, which were assessed through the Apprehension test and the Jobe Relocation test performed by a senior orthopedic surgeon pre-operatively and post-operatively. Each test was compared to the other shoulder of the patient. Clinical outcomes were assessed by using the American Shoulder and Elbow Surgeons (ASES) scoring system[16], as well as the Rowe shoulder scoring system[17], as validated scoring systems. Both pre-operative and post-operative data at the final follow up were collected and analyzed. Failure was defined as a re-dislocation or a subluxation after the surgery.

Surgical technique

All operations were performed at our clinic by the senior surgeons with different accompanying assistant doctors and specialist surgeons under hypotensive general anesthesia in the beach chair position. Adjustable pressure and flow speed arthropump (Karl Storz) were used at an initial pressure of 40 mmHg. After establishment of the standard posterior portal, a low anterior portal adjacent to the upper border of the subscapularis was opened. Diagnostic arthroscopy was performed and the anterior-superior portal was opened anterior to the supraspinatus and adjacent to the lateral border of the rotator interval (Fig. 1).

Fig 1: A: Location of portals. P: posterior, AI: antero-inferior, AS: antero-superior. B: Image of visualization from antero-superior portal.
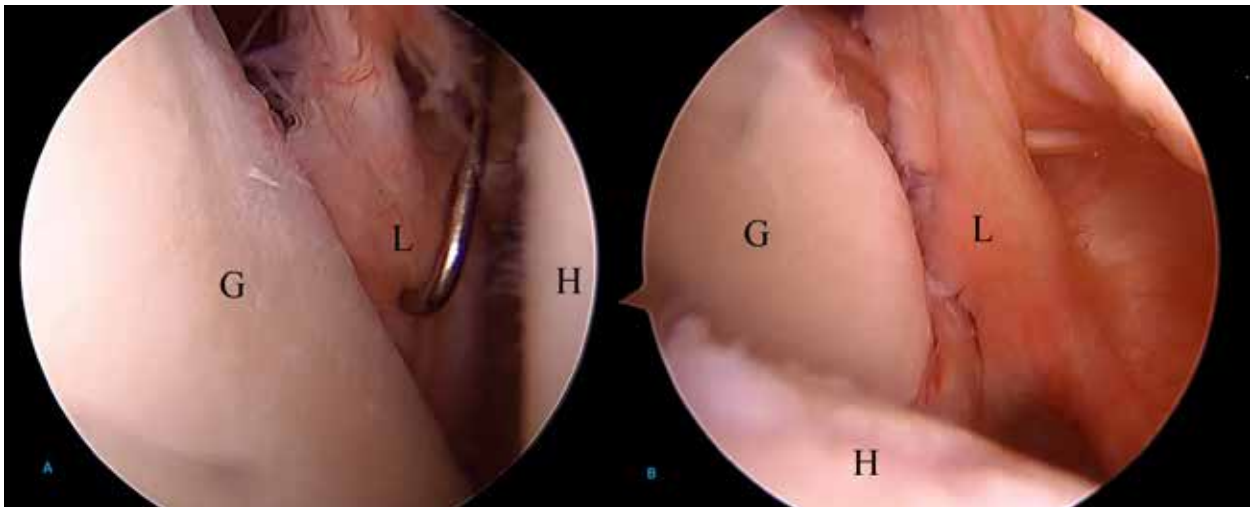
Fig 2: A: Visualization from posterior portal; a very small amount of labrum can be visualized. L: labrum, G: glenoid, SS: subscapularis, H: humeral head. B: Visualization from antero-superior portal; the entire labrum that has been medially displaced can be visualized. G: glenoid, L: labrum, H: humeral head.

The scope was then moved to this antero-superior portal to evaluate the anterior structures. The damaged glenohumeral ligament-labrum complex was mobilized from the glenoid neck as far inferiorly as the 6 to 7 o'clock position with an elevator and a radio frequency ablation device. The preparation of the medial glenoid bone was also performed with an arthroscopic shaver and elevator through the anterior portal while viewing from the antero-superior portal (Fig. 2).

We used a minimum amount of two double loaded bioabsorbable knotless 2.9 suture anchor (mean 3.1±6) starting from the most inferior part of glenoid directed anteriorly. A soft tissue penetrator or an arthroscopic suture passer was used to pass the sutures starting from the inferior aspect. After capsular plication and proximal shift of the capsulolabral complex, a Samsung Medical Centre sliding knot was tied on the capsulolabral side of the repair. Neither rotator interval closure nor remplissage was performed in any of the cases; however, SLAP repair was performed in 7 of 37 shoulders.

Rehabilitation protocol

All patients were immobilized during the first two weeks in a sling and were encouraged to perform pendulum exercises. In the following two weeks, active anterior elevation movements up to 90° flexion were allowed and complete shoulder mobilization was allowed after six weeks. Excessive external rotation was not allowed until the third month postoperatively and return to sport was allowed after the third six postoperative months.

Statistical analysis

Mean, standard deviation, median lowest, highest, frequency and ratio values were used in the descriptive statistics of the data. The distribution of the variables was measured by the Kolmogorov-Smirnov test. The Mann-Whitney U test was used in the analysis of quantitative independent data. The Wilcoxon test was used for the analysis of the dependent quantitative data and the McNemar test was used for the analysis of the dependent qualitative data. Chi-square test was used in the analysis of qualitative independent data. The SPSS 22.0 program was used in all analyses.

RESULTS

There were 30 male and seven female patients, with a mean age of 28.4±7.1 years (range: 17-45) at the time of surgery. A total of 26 patients underwent stabilization of their dominant shoulder (70.3%). In terms of employment status, 17 patients had a sedentary office job, 11 were students and nine were manual workers. The mean time between the first dislocation episode and the surgical procedure was 30.9±26.1 months (range: 1-108). The mean number of instability episodes was 8.0±5.9 (range: 1-30), where 29 patients had more than three episodes. The mean preoperative instability severity index score (ISIS) score was 2.7±1.3 (range: 0-5) (Table 1).

The mean ASES score and Rowe score increased from 71.3±7.0 (range: 59-93) and 43.5±6.5 (range: 30-50) preoperatively to 92.8±3.1 (range: 85-98) and 92.2±6.2 (range: 75-100) postoperatively respectively (P <.05) (Table 2). The shoulder joint function at the final follow-up was rated as excellent, good, fair and poor

Table 1: Patient demogrphics and intraoperative findings

Patient variables	Min-Max	Median	Mean±SD / n(%)
Age	17, 45	29	28.46±7.152
Sex			
Female			7 (18.9)
Male			30 (81.1)
Dominant			
No	1, 108	30	11 (29.7)
Yes	0, 5	3	26 (70.3)
Work			
Office			17 (45.9)
Student			11 (29.7)
Worker	1, 30	7	9 (24.3)
Time to surgery (month)			30.9±26.1
ISIS Score			2.7±1.3
0			3 (8.1)
I			3 (8.1)
II			10 (27)
III			9 (24.3)
IV			10 (27)
V			2 (5.4)
Instability episode			8.0±5.9
X-Ray Hill Sachs			
No			28 (75.7)
Yes			9 (24.3)
Intra-operative findings			
Alpsa			2 (5.4)
Bankart			24 (64.9)
Bankart+Slap I			5 (13.5)
Bankart+Slap II			1 (2.7)
Bankart+Slap IV			1 (2.7)
Osseos Bankart			4 (10.8)
Number of anchor	2, 5	3	3.1±0.6
II			5 (13.5)
III			26 (70.3)
IV			5 (13.5)
V			1 (2.7)
Follow-up time (month)	24, 48	30	32.5±7.2
Forward flexion difference	0, 18	7	7.2±4.1
External rotation ın adduction difference	5, 18	9	10.2±3.6
External rotation ın 90 abduction difference	7, 20	12	12.8±3.4

Table 2: Pre-and post-operative physical examination findings and functional outcome scores

Physical examination	Min-Max	Median	Mean±SD / n(%)	P
Apprehension Test				0.000[N]
Pre-Op				
(-)			0 (0)	
(+)			37 (100)	
Post-Op				
(-)			34 (91.9)	
(+)			3 (8.1)	
Jobe Relocation Test				0.000[N]
Pre-Op				
(-)			0 (0)	
(+)			37 (100)	
Post-Op				
(-)			37 (100)	
(+)			0 (0)	
ASES				0.000[w]
Pre-Op	59, 93	71	71.3±7.0	
Post-Op	85, 98	93	92.8±3.1	
Rowe				0.000[w]
Pre-Op	30, 50	45	43.5±6.5	
Post-Op	75, 100	95	92.2±6.2	

[w] Wilcoxon test; [N] Mc Nemar test; ASES: American Shoulder and Elbow Surgeons

Sachs lesions or glenohumeral ligament lesions were not detected. When compared to the results of the contralateral side, a mean loss of 7.2°±4.1° (range: 0-18°) in forward flexion, 10.2°±3.6° (range: 5-18°) in external rotation in adduction and 12.8°±3.4° (range: 7-20°) in external rotation in 90° abduction were detected in the study group at the final follow-up (Table 1). Among the 37 patients included in the series, all patients confirmed that they were subjectively satisfied with the final result.

In the group with Instability Episode <10 and Instability Episode ≥10, the preoperative postoperative ASES and Rowe score did not differ significantly (P >.05) (Table 3). In the group with isolated Bankart and

according to the Rowe shoulder scoring system. All of the patients had good or excellent results.

Redislocation was observed in two patients. The first patient was seen after falling down at the 28th month postoperatively, while the second patient was seen after experiencing problems during swimming at the 34th month postoperatively. One of these patients underwent open treatment with the Latarjet method and the other did not accept surgery. The functional outcomes of these two patients were not included in the study. Recurrent dislocation was not observed until the final follow-up. The results of the preoperative and final follow-up clinical examinations are summarized in Table 2.

A SLAP lesion was identified in three of the cases and a small partial rotator cuff tear was seen in one of the cases, which were all repaired. Engaging Hill-

Table 3: Functional outcome comparison of instability episode <10 and instability episode ≥10 groups of patient

Functional outcome	Instability episode < 10		Instability episode ≥ 10		P
	Mean±SD	Median	Mean±SD	Median	
ASES					
Pre-Op	72.6±6.7	73	67.8±6.9	67.5	0.125[m]
Post-Op	93.0±2.6	93	92.2±4.3	92.5	0.847[m]
Intra Group P	0.000 w		0.008[w]		
Rowe					
Pre-Op	44.6±6.0	45	40.5±7.2	45	0.097[m]
Post-Op	93.1±5.6	95	89.5±7.2	90	0.114[m]
Intra Group P	0.000[w]		0.002[w]		

[m] Mann-whitney u test; [w] Wilcoxon test

Table 4: Comparison of functional outcomes of patients with isolated Bankart lesions and those with Bankart + SLAP

Functional outcome	Bankart		Bankart+SLAP		P
	Mean±SD	Median	Mean±SD	Median	
ASES					
Pre-Op	72.1±7.3	3	67.7±4.1	68	0.082[m]
Post-Op	92.7±2.9	93	92.9±4.3	94	0.781[m]
Intra					
Group P	0.000 [w]		0.008 [w]		
Rowe					
Pre-Op	44.5±5.9	45	39.3±7.9	40	0.086[m]
Post-Op	92.3±6.1	95	91.4±6.9	95	0.794[m]
Intra					
Group P	0.000[w]		0.008[w]		

[m]Mann-whitney u test; w Wilcoxon test

Bankart + SLAP, the preoperative - postoperative ASES and Rowe scores did not differ significantly (P >.05) (Table 4). The number of Anchors used in the Bankart + SLAP group (3.7±0.8) was significantly higher (P <.05) than that of the isolated Bankart group (2.9±0.5).

DISCUSSION

Arthroscopic treatment of glenohumeral instability has rapidly evolved over the past few years. In most recent series, the results of arthroscopic treatment were noted to be equal or superior compared to the results of open series[11]. The ASES shoulder score and the Rowe scores, which are patient reported validated outcome scales, are frequently used methods of clinical and functional assessment following arthroscopic Bankart repair[16,17]. While the ASES score was reported to primarily focus on pain level and functional ability both at work and leisure activity, the Rowe score was reported to be based on stability, motion and function[16,17]. In the present study, the ASES scores following arthroscopic Bankart repair ranged from 85 to 98, while the Rowe scores ranged from 75 to 100 at a minimum follow-up of two years, indicating a high level of motion and excellent function outcome following arthroscopic repair. This was reported to be similar to other series in the literature[7-10]. To assess the effectiveness of an arthroscopic stabilization procedure for anterior shoulder instability using the Rowe score, Park et al[18] calculated the minimal clinical important difference as 9.7. In our series, the difference was 43.5 and over minimal clinical important difference in all patients.

The major risk factors for failure and re-dislocation following arthroscopic repair consist of the younger age of patients, participation in contact sports activities, presence of an engaging Hill-Sachs or osseous Bankart lesion, ipsilateral rotator cuff or deltoid muscle insufficiency and underlying ligamentous laxity[19,20]. Balgand Boileau proposed an ISIS to determine which patients were good candidates for arthroscopic surgery and advised open procedures in cases with high scores[19]. In the present study, patients with high risk factors such as a large Hill-Sachs lesion, a glenoid defect greater than 25% or a score of more than six points in ISIS were not operated on using arthroscopic bankart repair techniques, but were treated by using the open Latarjet method instead. At the final follow up, just two patients had re-dislocation after surgery, while the functional scores were noted to be improved in all of the cases. Consequently, we detected that if the spectrum of surgical indications for arthroscopic Bankart repair was narrowed down, good clinical outcomes could be obtained. In addition to patient selection, effective surgery with the appropriate technique was reported to be the key factor for successful outcomes. In the literature, important steps with regard to surgical technique have been published by experienced authors[8-10]. Among them, visualization and identification of the lesions were noted to be of paramount importance[21,22]. We believe that direct visualization from the anterior portal might help the surgeon to identify not only a classic Bankart lesion, but also other variants, including a glenolabral articular disruption or anterior labroligamentous periosteal sleeve avulsion or a osseous Bankart lesion, which could also be possible reasons for instability.

Another important technical step is to mobilize the inferior glenohumeral ligament and labrum complex such that it could be shifted superiorly and laterally. Management of the inferior component of the pathology was determined to be critical in order to achieve a successful outcome and could only be performed with impeccable visualization. Additionally, it was also reported that untreated or unrecognized capsular tears and deformation could represent the most common cause of failure after arthroscopic Bankart repair[13,23]. All anterior structures and pathology were better evaluated while viewing through the antero-superior portal in comparison to the view obtained from the posterior portal[24]. Therefore, visualization directly through the anterior superior portal could prevent this kind of failure and give surgeons a better understanding of the lesion[24]. However, in a study conducted on shoulder and elbow surgeons in USA, it was reported that only 7% of them performed arthroscopic Bankart repair while viewing entirely from an anterior portal[25].

Meyer et al[26] reported in their study of 23 patients that there was an average loss of shoulder external rotation in adduction of 14.1° and an average loss of

shoulder external rotation in 90° of shoulder abduction of 12.8° average 3.3 years after arthroscopic Bankart repair via single anterior portal. In the present study, compared to the contralateral side, patients were detected to have an average loss of 7.2° in forward flexion, 10.2° in external rotation in adduction and 12.8° in external rotation in 90°abduction at the last follow-up. The results of the present study were found to be similar to other single anterior portal series in terms of loss of shoulder ROM[14,15,26]. This study reported that double portal to the anterior does not have a negative effect on the patient's shoulder ROM.

Using both working and viewing portals close to each other might result in obstruction and sticking of the instruments. However, we established in our cases that the anterior superior portal was located much more laterally and far from the location of the standard antero-superior portal. The distance between the anterior portals we used was larger than the distance between the standard portals (Figure 1). In our small series, we had various sizes of shoulders, varying from basketball players to small females; nonetheless, in all cases, we were able to easily establish two anterior portals without any congestion. Therefore, we believe that the anterior-superior lateral portal can be established easily and it may be a reliable arthroscopic technique with regard to visualization during the entire procedure.

Some authors reported that in the arthroscopic Bankart procedure, the single anterior portal is superior to the double anterior portal in terms of cosmetics, cost-effectiveness and postoperative analgesia, as well as similar clinical outcomes[14,15]. It may be preferable to obtain similar results with a less invasive procedure, but it should be considered that the posterior portal visualization may cause inadequate evaluation of the entire anterior structures, pathology and inadequate release of the labrum and inferior glenohumeral ligament[24].

Our study demonstrated that the number of instability episodes and accompanying SLAP lesions did not adversely affect the clinical outcomes as emphasized in the literature[8,27,28]. The main weaknesses of this study comprised the retrospective design, absence of a control group and the short duration of follow up. However, since it is a rarely used technique, we aimed to present the short-term preliminary results while the study continues with the aim of presenting the mid to long-term results in the near future.

Using the anterior portal for visualization is indeed not a new technique, but this study demonstrates that the usage of the anterior portal during the entire operation was a highly safe and effective method, since the main pathology was located at the anterior part of the glenoid and direct visualization could be achieved by using this technique.

CONCLUSION

In the current study, it was observed that the anterior-superior portal was sufficient and reliable for visualization during the entire procedure as a safe and effective alternative to the generally accepted classic posterior portal visualization.

REFERENCES

1.   Hovelius L. The natural history of primary anterior dislocation of the shoulder in the young. J Orthop Sci 1999; 4(4):307-317.
2.   Kroner K, Lind T, Jensen J. The epidemiology of shoulder dislocations. Arch Orthop Trauma Surg 1989; 108(5):288-290.
3.   Shah A, Judge A, Delmestri A, Edwards K, Arden NK, Prieto-Alhambra D, et al. Incidence of shoulder dislocations in the UK, 1995-2015: a population-based cohort study. BMJ Open 2017; 7(11):e016112.
4.   Owens BD, Agel J, Mountcastle SB, Cameron KL, Nelson BJ. Incidence of glenohumeral instability in collegiate athletics. Am J Sports Med 2009; 37(9):1750-1754.
5.   Taylor DC, Arciero RA. Pathologic changes associated with shoulder dislocations. Arthroscopic and physical examination findings in first-time, traumatic anterior dislocations. Am J Sports Med 1997; 25(3):306-311.
6.   Sperber A, Hamberg P, Karlsson J, Sward L, Wredmark T. Comparison of an arthroscopic and an open procedure for posttraumatic instability of the shoulder: a prospective, randomized multicenter study. J Shoulder Elbow Surg 2001; 10(2):105-108.
7.   Ikemoto RY, Murachovsky J, Nascimento LGP, Bueno RS, Almeida LHO, Kojima C. Evaluation of surgical

treatment of patients with shoulder instability. Acta Ortop Bras 2017; 25(6):266-269.

8. Castagna A, Markopoulos N, Conti M, Rose GD, Papadakou E, Garofalo R. Arthroscopic bankart suture-anchor repair: radiological and clinical outcome at minimum 10 years of follow-up. Am J Sports Med 2010; 38(10):2012-2016.

9. Gartsman GM, Roddey TS, Hammerman SM. Arthroscopic treatment of anterior-inferior glenohumeral instability. Two to five-year follow-up. J Bone Joint Surg Am 2000; 82-A(7):991-1003.

10. Kim SH, Ha KI, Cho YB, Ryu BD, Oh I. Arthroscopic anterior stabilization of the shoulder: two to six-year follow-up. J Bone Joint Surg Am 2003; 85(8):1511-1518.

11. Petrera M, Patella V, Patella S, Theodoropoulos J. A meta-analysis of open versus arthroscopic Bankart repair using suture anchors. Knee Surg Sports Traumatol Arthrosc 2010; 18(12):1742-1747.

12. Kocaoglu B, Guven O, Nalbantoglu U, Aydin N, Haklar U. No difference between knotless sutures and suture anchors in arthroscopic repair of Bankart lesions in collision athletes. Knee Surg Sports Traumatol Arthrosc 2009; 17(7):844-849.

13. Castagna A, Conti M, Mouhsine E, Rose GD, Massazza G, Garofalo R. A new technique to improve tissue grip and contact force in arthroscopic capsulolabral repair: the MIBA stitch. Knee Surg Sports Traumatol Arthrosc 2008; 16(4):415-419.

14. Cicek H, Tuhanioglu U, Ogur HU, Seyfettinolglu F, Ciloglu O, Beyzadeoglu T. Single anterior portal: A better option for arthroscopic treatment of traumatic anterior shoulder instability? Acta Orthop Traumatol Turc 2017; 51(4):298-302.

15. Armangil M, Basat HC, Akan B, Karaduman M, Demirtas M. Arthroscopic stabilization of anterior shoulder insatbility using a single anterior portal. Acta Orthop Traumatol Turc 2015; 49(1):6-12.

16. Richards RR, An KN, Bigliani LU, Friedman RJ, Gartsman GM, Gristina AG, *et al*. A standardized method for the assessment of shoulder function. J Shoulder Elbow Surg 1994; 3(6):347-352.

17. Rowe CR, Zarins B. Recurrent transient subluxation of the shoulder. J Bone Joint Surg Am 1981; 63(6):863-872.

18. Park I, Lee JH, Hyun HS, Lee TK, Shin SJ. Minimal clinically important differences in Rowe and Western Ontario Shoulder Instability Index scores after arthroscopic repair of anterior shoulder instability. J Shoulder Elbow Surg 2018; 27(4):579-584.

19. Olds M, Ellis R, Donaldson K, Parmar P, Kersten P. Risk factors which predispose first-time traumatic anterior shoulder dislocations to recurrent instability in adults: a systematic review and meta-analysis. Br J Sports Med 2015; 49(14):913-922.

20. Balg F, Boileau P. The instability severity index score. A simple pre-operative score to select patients for arthroscopic or open shoulder stabilisation. J Bone Joint Surg Br 2007; 89(11):1470-1477.

21. Angelo RL. Arthroscopic bankart repair for unidirectional shoulder instability. Instr Course Lect 2009; 58:305-313.

22. Millett PJ, Clavert P, Warner JJ. Arthroscopic management of anterior, posterior, and multidirectional shoulder instability: pearls and pitfalls. Arthroscopy 2003; 19 Suppl 1:86-93.

23. Mologne TS, McBride MT, Lapoint JM. Assessment of failed arthroscopic anterior labral repairs. Findings at open surgery. Am J Sports Med 1997; 25(6):813-817.

24. Todor A. Single or double anterior portal shoulder arthroscopy for Bankart repair? That is the question. Acta Orthop Traumatol Turc 2018; 52(6):485-486

25. Burks RT, Presson AP, Weng HY. An analysis of technical aspects of the arthroscopic Bankart procedure as performed in the United States. Arthroscopy 2014; 30(10):1246-1253.

26. Meyer A, Klouche S, Bauer T, Rousselin B, Hardy P. Residual inferior glenohumeral instability after arthroscopic Bankart repair: radiological evaluation and functional results. Orthop Traumatol Surg Res 2011; 97(6):590-594.

27. Hantes ME, Venouziou AI, Liantsis AK, Dailiana ZH, Malizos KN. Arthroscopic repair for chronic anterior shoulder instability: a comparative study between patients with Bankart lesions and patients with combined Bankart and superior labral anterior posterior lesions. Am J Sports Med 2009; 37(6):1093-1098.

28. Aydin N, Unal MB, Asansu M, Tok O. Concomitant SLAP repair does not influence the surgical outcome for arthroscopic Bankart repair of traumatic shoulder dislocations. J Orthop Surg (Hong Kong) 2017; 25(2):2309499017718952.

Original Article

Relationship with serum bilirubin levels and mortality in critically ill patients

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ABSTRACT

Objectives: To examine associated factors of bilirubin levels at admission to general intensive care unit (ICU) and their effects on outcomes

Design: Retrospective, observational study

Setting: Medical records of patients older than 18 years who were hospitalized during one year (2016-2017) at the general ICU in Ankara Numune Training and Research Hospital, Turkey were reviewed.

Subjects: Identifying the change of serum bilirubin level is important for determination of diagnostic and prognostic strategies in critically ill patients.

Intervention: The study group data was obtained from hospital medical records.

Main Outcome Measure: SOFA and APACHE II scores, total blood bilirubin levels, need for mechanical ventilation and

renal replacement therapy, length of hospital and ICU stay, and mortality at ICU were evaluated.

Results: Patients with normal bilirubin levels constituted 79.3% of the study population, whereas those with mild elevation and high levels were found to be less common (14% and 6.7%, respectively). Among patients in the high bilirubin group, 40.9% of patients were admitted to the ICU with primary diagnosis of infection, which was detected as 13% and 21.7% in normal and mildly elevated group ($P <.01$). Mortality rate was the same in all groups ($P >.05$).

Conclusions: Patients who were admitted to ICU due to infection were likely to have more elevated bilirubin levels and higher APACHE II and SOFA scores. High levels of bilirubin did not change mortality in ICU patients despite prolonged ICU stay.

KEY WORDS: bilirubin, critical illness, infection, intensive care, mortality

INTRODUCTION

Bilirubin is the end-product of heme metabolism and 80% of bilirubin originates from the breakdown of hemoglobin from aged red blood cells in the bloodstream. This is the indirect form of bilirubin, which is transformed into direct bilirubin in the liver and excreted via the biliary route. The sum of direct and indirect bilirubin in serum is called as total bilirubin. In normal healthy adults, serum total bilirubin levels vary from 0.3-1.2 mg/dL. Though bilirubin levels are within normal ranges in critically ill patients at admission to intensive care unit (ICU), hyperbilirubinemia and even hepatic dysfunction may develop during ICU follow-up secondary to several conditions such as sepsis, fluid gap, medications,

adverse effects of parenteral nutrition, steatosis and ischemic or sclerosing cholangitis[1]. In fact, hepatic dysfunction and jaundice may lead to sepsis and other critical conditions, further causing ICU complications and prolonged length of stay.

To the best of our knowledge, there exists no specific physiological parameter that helps the early identification of the mortality effects of hepatic dysfunction developed in the ICU. Bilirubin is a well-studied biomarker for hepatic dysfunction. Hyperbilirubinemia may be seen within 2-3 days after hepatic injury[2]. Serial bilirubin measurements may reflect a transient and adaptive condition during critical illness, and it is a prognostic biomarker particularly for existing infection[3]. Therefore, serial

bilirubin measurements have been incorporated into scoring systems that assess organ dysfunction such as Sequential Organ Failure Assessment Score (SOFA), Simplified Acute Physiology Score and Logistic Organ Dysfunction Score[4].

Earlier studies reported elevated serum bilirubin levels to have anti-inflammatory activity, hence exert favorable protective effects against devastating impact of acute hepatic injury and sepsis. It was even suggested that administration of exogenous bilirubin might reduce sepsis mortality[5-8]. Contradictory clinical evidence has been published afterwards. In patients with bilirubin levels higher than 3 mg/dL in the surgical ICU, the risk of infection was reported to be higher, near threefold, compared to that in those with bilirubin level ≤3 mg/dL, as well as relation with sepsis-related hepatic dysfunction and increased risk of infection[9]. However, Pierrakos et al reported that mild or intermediate hyperbilirubinemia was associated with poor prognosis[10].

There have been limited studies assessing the relation between bilirubin levels and mortality in critically ill patients in the general ICU. In this study, we aimed to identify the factors associated with bilirubin levels at admission to ICU and investigate their effects on prognosis.

SUBJECTS AND METHODS

This retrospective study was performed after obtaining the approval from ethics committee of Health Sciences University (approval no: E-17-1521).

Medical records of patients older than 18 years who were hospitalized between 1st September, 2016 and 31st August, 2017 at the general ICU were reviewed. Those with a known etiology of hyperbilirubinemia including cirrhosis, acute toxic or alcoholic hepatitis with or without hepatic failure, cholecystitis alone or with cholangitis, acute pancreatitis, known hemolysis, or history of hepatic or pancreatic surgery were excluded from the study. The demographic data of the patients, primary diagnosis at admission to ICU, comorbidities, SOFA and APACHE II scores, total blood bilirubin levels, need for mechanical ventilation and renal replacement therapy, length of hospital and ICU stay, and mortality at ICU were evaluated. Patients were divided into three groups as those with normal bilirubin group (≤1 mg/dL), mildly elevated bilirubin group (1.1-2 mg/dL) and high bilirubin group (2.1-6 mg/dL).

Statistical analysis

IBM SPSS Statistics for Windows v.21.0 (IBM Corp., Armonk, NY) was used. Descriptive statistics were expressed as mean, median, standard deviation, minimum-maximum for numerical variables and as number and percentage for categorical variables. The assumption of normality was examined with the Kolmogorov-Smirnov test, which failed to meet the assumption. Kruskal-Wallis test was used to determine whether there was a difference between bilirubin groups. In the case of differences between the groups, paired comparison tests were used to

Table 1: Comparison of baseline characteristics of the study population

Parameters	Bilirubin levels			P-value
	≤1 mg/dL (n=261, 79.3%)	1.1-2 mg/dL (n=46, 14%)	2.1-6mg/dL (n=22, 6.7%)	
Age (years)	67.0±18.1	71.9±15.4	70.3±16.8	.2
Sex				.4
Female	141 (54.0)	22 (47.8)	9 (40.9)	
Male	120 (46.0)	24 (52.2)	13 (59.1)	
Body mass index	26.2±5.4	25.2±3.8	25.4±3.7	.7
APACHE II	19.7±11.3	21.3±10.1	28.4±13.1	<.01*
SOFA	4.95±3.1	6.2±2.9	10.6±4.8	<.01*
Primary diagnosis				
Central nervous	68 (26.1)	15 (32.6)	4 (18.2)	.4
Respiratory	48 (18.4)	12 (26.1)	3 (13.6)	.4
Cardiovascular	11 (4.2)	1 (2.2)	0 (0)	.3
Gastrointestinal	8 (3.1)	1 (2.2)	1 (4.5)	.9
Renal	17 (6.5)	1 (2.2)	2 (9.1)	.4
Infection	34 (13.0)	10 (21.7)	9 (40.9)	<.01*
Multi-trauma	21 (8.0)	4 (8.7)	1 (4.5)	.8
Postoperative, general surgery	22 (8.4)	2 (4.3)	0 (0)	.1
Postoperative, orthopedics	48 (18.4)	6 (13.0)	2 (9.1)	.4
Drug overdose, suicide	7 (2.7)	2 (4.3)	1 (4.5)	.8
Others	6 (2.3)	0 (0)	0 (0)	.2

Results are demonstrated as mean ± standard deviation or n (%).
SOFA: Sequential Organ Failure Assessment Score

Table 2: Comparison of patients' comorbidities, supportive therapies, length of hospitalization and mortality

Parameters	Bilirubin levels			P-value
	≤1 mg/dL (n=261, 79.3%)	1.1-2 mg/dL (n=46, 14%)	2.1-6mg/dL (n=22, 6.7%)	
Comorbid conditions				
Diabetes mellitus	71 (27.2)	12 (26.1)	3 (31.8)	.5
Neurologic diseases	66 (25.3)	11 (23.9)	7 (41.2)	.6
Endocrine diseases	13 (5.0)	5 (10.9)	0 (0)	.2
Renal diseases	31 (11.9)	3 (6.5)	2 (11.8)	.7
Chronic obstructive pulmonary disease	43 (16.5)	11 (23.9)	5 (29.4)	.2
Hematological diseases	6 (2.3)	2 (4.3)	2 (11.8)	.1
Malignancy	46 (17.6)	2 (4.3)	2 (11.8)	.07
Others	16 (6.2)	2 (4.4)	1 (5.9)	.3
Mechanical ventilation	100 (38.3)	20 (43.5)	12 (54.5)	.3
Renal replacement therapy	26 (10.0)	3 (6.5)	3 (13.6)	.6
Total parenteral nutrition	50 (19.2)	8 (17.4)	4 (18.2)	.9
Length of stay at intensive care unit (days)	11.4±18.4	15.4±21.8	15.5±14.9	.04
Length of hospital stay (days)	19.6±22.5	20.5±23.8	25.00±21.28	.1
Fatal events at intensive care units	100 (38.3)	16 (34.8)	12 (54.5)	.3

Results are demonstrated as mean ± standard deviation or n (%).

determine the group that drove the difference. The differences between categorical variables were examined by chi-square test. The significance level was accepted as $P < .05$.

RESULTS

Among the 410 patients internalized to general ICU, 329 patients were found to meet study inclusion criteria. Baseline characteristics of the study population according to their bilirubin level groups is given in Table 1. Patients with normal bilirubin levels constituted 79.3% of the study population, whereas those with mild elevation and high bilirubin level were found to be less common (14% and 6.7%, respectively). Mean ages in these groups were 67±18.1, 71.9±15.4 and 70.3±16.8, respectively. Mean age, sex and body mass index did not significantly differ by bilirubin level groups of patients ($P > .05$; Table 1).

Mean APACHE II within the first 24 hours of ICU admission was significantly higher (28.4±13.1) in high bilirubin group, compared to those in mild (21.3±1.10) and normal bilirubin groups (19.7±11.3; $P < .01$). Similarly, those in high bilirubin group had significantly higher mean SOFA score (10.6±4.8) compared to those in mild (6.2±2.9) and normal bilirubin groups (4.95±3.10; $P < .01$). Among patients in the high bilirubin group, 40.9% of patients were admitted to the general ICU with primary diagnosis of infection, which was detected as 13% and 21.7% in normal and mild bilirubin group ($P < .01$; Table 1).

There was no statistically significant difference among different groups of bilirubin in terms of comorbidities at admission to ICU. Mechanical ventilation was found to be applied to 54.5% of those in the high bilirubin group, compared to 43.5% and

38.3% of patients with mild and normal bilirubin groups; where the difference was not statistically significant ($P > .05$). These groups either did not differ with respect to the renal replacement therapy or total parenteral nutrition administered. The length of hospital stay was also detected to be similar between patients with different bilirubin levels ($P > .05$). Length of stay at intensive care unit was found to be shorter in patients with normal bilirubin levels ($P < .05$). Mortality rate in high, mild and normal bilirubin groups at admission were found as 54.5%, 34.8% and 38.3%, where the difference was not statistically significant ($P > .05$; Table 2).

DISCUSSION

This study shows that patients with higher levels of bilirubin were more likely to have infection during admission to ICU. Similarly, these patients had higher levels of APACHE II and SOFA scores. There was no relationship between bilirubin levels and mortality, but high levels of bilirubin were found to be associated with long-term ICU stay.

The threshold values for hyperbilirubinemia were found to vary among different studies. Brienza et al reported to accept the threshold of bilirubin level in critically ill postoperative or sepsis patients as 2 mg/dL, showing poorer prognosis above this level[11]. Field et al considered the threshold level of bilirubin to be higher than 3 mg/dL within the first 48 hours of admission to surgical ICU[9]. Kramer et al reported that bilirubin levels of 2 mg/dL and above was an early predictor of hepatic dysfunction in critically ill patients[4]. In this study, we used the threshold values used by Pierrakos et al[10]. Accordingly, we did not include patients with bilirubin levels 6 mg/dL and

above, as this might be accompanied with marked hepatic dysfunction. We regarded the patients with bilirubin values between 2.1 and 6 mg/dL as having high bilirubin level. Patients with bilirubin levels below 1 mg/dL were found to constitute 79% of our study population.

The impact of high bilirubin levels on the body and its association with infection is not clearly established, though it is known to be accompanied with increased mortality and morbidity. Owing to its anti-oxidative effect, bilirubin is thought to disturb bactericidal effect of neutrophils, improve endothelial functions and decrease organ injury by protecting against oxidative stress[12,13]. In this study, although the comorbidities of our patients are similar, 40.9% of patients with high bilirubin levels at admission to ICU had a primary diagnosis of infection. These patients also had higher SOFA and APACHE II scores that we used for assessing organ functions, and calculated within the first 24 hours of admission, respectively. There were no differences between the groups in terms of clinical course (number of days of mechanical ventilation or stay at ICU, or renal replacement therapy) and mortality. We thought that absence of increased mortality rate despite elevated APACHE II and SOFA scores in the group with the high bilirubin group might be explained by such protective effects of bilirubin. Although increased levels of bilirubin caused by infection may be associated with such protective mechanisms, it may also be associated with secondary cholestasis secondary to the toxicity of the infectious microorganism and consequently with the host response. In addition, hemolysis that occurs during infection could also be a contributing factor[14-17]. Kramer et al reported that 11% of patients had bilirubin levels higher than 2 mg/dL within the first 48 hours of admission to ICU, and the mortality rates were higher in this subgroup[4]. Field et al reported an association between bilirubin levels higher than 3 mg/dL and infection, and positive correlation of bilirubin level to the risk of infection[9]. Patel et al showed that severe sepsis led to hyperbilirubinemia, which was related to cholestasis, concluding with relation of bilirubin elevation to mortality[13]. Vanwijngaerden et al suggested hyperbilirubinemia to be an adaptive response to critical illness, and even to protect against harmful cellular effects[18]. We detected that the infection and organ function deterioration associated with bilirubin elevation could be controlled and had no effect on mortality.

In critically ill patients, devastating clinical picture secondary to multi-organ failure accompanied with respiratory, cardiovascular or renal impairment may mask newly-emerging hepatic dysfunction. Furthermore, serial monitoring of bilirubin levels is not a routine practice in critically ill patients. While half of the patients with hepatic dysfunction had sepsis, bilirubin elevation was reported to be 3-fold higher in ICU patients when admitted due to sepsis. It is likely that dysfunction of intrahepatic cells and their impaired interactions to each other in sepsis influence liver functions[13,14]. Yamano et al reported elevated bilirubin levels to be associated with poor prognosis in prolonged sepsis[19]. However, we did not assess the impact of hyperbilirubinemia that developed after ICU admission, as our aim was to investigate the effects of bilirubin elevation that existed at admission.

Liver has a high blood supply, both from hepatic artery and the portal venous system. Hepatic dysfunction can be driven by a decrease in hepatic blood flow and failure to meet metabolic needs. Such conditions that may reduce visceral blood flow, including parenteral nutrition, sepsis, trauma, major surgery, shock, antibiotic use, high positive end-expiratory pressure as well as use of mechanical ventilation may disrupt portal and hepatic arterial hemodynamics, and hence underlie impaired liver function[20-24]. It has been reported that hepatic dysfunction that developed in post-injury setting had higher mortality[25]. In contrast to our study, Kramer et al reported a linear relationship between elevated bilirubin levels and mortality[4]. Fuhrmann et al reported hypoxic hepatitis in 11% of patients admitted to medical ICU. Patients with hypoxic hepatitis had a longer length of ICU stay and shorter ICU survival[26]. Jaundice increases rates of complications and mortality in patients with hypoxic hepatitis[27]. Pierrakos et al, in their one-day international prevalence study of adult patients admitted to ICU, reported that hospital mortality linearly increased as long as the bilirubin level raised, among patients who had bilirubin level between 1.1 to 6 mg/dL. Serum bilirubin level higher than 1 mg/dL was reported to be an independent risk factor for mortality[10].

Limitations of the study

Small sample size might reduce the likelihood of the available findings to reflect actual outcomes. Actual sepsis incidence was not found in inpatients due to infection because no diagnosis of sepsis was found in the ICD-9 coding system. In addition, the retrospective study design mostly hampers consideration of many confounding factors, which may influence observed patient outcomes.

CONCLUSION

Patients who were admitted to ICU due to infection were likely to have more elevated bilirubin levels and higher APACHE II and SOFA scores. Although the mortality rate was higher in the high-level bilirubin

group, the difference between the groups was not significant. We conclude that bilirubin levels were associated with infection but not mortality in critically ill patients. Nonetheless, we believe that prospective studies are warranted on this subject.

REFERENCES

1. Woznica EA, Inglot M, Woznica RK, Lysenko L. Liver dysfunction in sepsis. Adv Clin Exp Med 2018; 27(4):547-551.
2. Roche SP, Kobos R. Jaundice in the adult patient. Am Fam Physician 2004; 69(2):299-304.
3. Franson TR, LaBrecque DR, Buggy BP, Harris GJ, Hoffmann RG. Serial bilirubin determinations as a prognostic marker in clinical infections. Am J Med Sci 1989; 297(3):149-152.
4. Kramer L, Jordan B, Druml W, Bauer P, Metnitz PGH, ASDI Study Group. Incidence and prognosis of early hepatic dysfunction in critically ill patients-A prospective multicenter study. Crit Care Med 2007; 35(4):1099-1104.
5. Bansal V, Schuchert VD. Jaundice in the intensive care unit. Surg Clin North Am 2006; 86(6):1495-1502.
6. Wang WW, Smith DLH, Zucker SD. Bilirubin inhibits iNOS expression and NO production in response to endotoxin in rats. Hepatology 2004; 40(2):424-433.
7. Sarady-Andrews JK, Liu F, Gallo D, Nakao A, Overhaus M, Ollinger R, et al. Biliverdin administration protects against endotoxin-induced acute lung injury in rats. Am J Physiol Lung Cell Mol Physiol 2005; 289(6):L1131-1137.
8. Overhaus M, Moore BA, Barbato JE, Behrendt FF, Doering JG, Bauer AJ. Biliverdin protects against polymicrobial sepsis by modulating inflammatory mediators. Am J Physiol Gastrointest Liver Physiol 2006; 290(4):G695-703.
9. Field E, Horst HM, Rubinfeld IS, Copeland CF, Waheed U, Jordan J, et al. Hyperbilirubinemia: a risk factor for infection in the surgical intensive care unit. Am J Surg 2008; 195(3):304-306.
10. Pierrakos C, Velissaris D, Felleiter P, Antonelli M, Vanhems P, Sakr Y, et al. EPIC II investigators. Increased mortality in critically ill patients with mild or moderate hyperbilirubinemia. J Crit Care 2017; 40:31-35.
11. Brienza N, Dalfino L, Cinnella G, Diele C, Bruno F, Fiore T. Jaundice in critical illness: promoting factors of a concealed reality. Intensive Care Med 2006; 32(2):267-274.
12. Dhainaut JF, Marin N, Mignon A, Vinsonneau C. Hepatic response to sepsis: interaction between coagulation and inflammatory process. Crit Care Med 2001; 29(7 Suppl):S42-S47.
13. Patel JJ, Taneja A, Niccum D, Kumar G, Jacobs E, Nanchal R. The association of serum bilirubin levels on the outcomes of severe sepsis. J Intensive Care Med 2015; 30(1):23-29.
14. Moseley RH. Sepsis and cholestasis. Clin Liver Dis 2004; 8(1):83-94.
15. Chand N, Sanyal AJ. Sepsis-induced cholestasis. Hepatology 2007; 45(1):230-241.
16. Fuchs M, Sanyal AJ. Sepsis and cholestasis. Clin Liver Dis 2008; 12(1):151-172.
17. Kobashi H, Toshimori J, Yamamoto K. Sepsis-associated liver injury: Incidence, classification and the clinical significance. Hepatol Res 2013; 43(3):255-266.
18. Vanwijngaerden YM, Langouche L, Brunner R, Debaveye Y, Gielen M, Casaer M, et al. Withholding parenteral nutrition during critical illness increases plasma bilirubin but lowers the incidence of biliary sludge. Hepatology 2014; 60(1):202-210.
19. Yamano S, Shimizu K, Ogura H, Hirose T, Hamasaki T, Shimazu T, et al. Low total cholesterol and high total bilirubin are associated with prognosis in patients with prolonged sepsis. J Crit Care 2016; 31(1):36-40.
20. Träger K, Radermacher P, Georgieff M. PEEP and hepatic metabolic performance in septic shock. Intensive Care Med 1996; 22(11):1274-1275.
21. Harbrecht BG, Doyle HR, Clancy KD, Townsend RN, Billiar TR, Peitzman AB. The impact of liver dysfunction on outcome in patients with multiple injuries. Am Surg 2001; 67(2):122-126.
22. Esteban A, Anzueto A, Frutos F, Alia I, Brochard L, Stewart TE, et al. Mechanical Ventilation International Study Group. Characteristics and outcomes in adult patients receiving mechanical ventilation. A 28-day international study. JAMA 2002; 287(3):345-355.
23. Kredel M, Muellenbach RM, Brock RW, Wilckens HH, Brederlau J, Roewer N, et al. Liver dysfunction after lung recruitment manoeuvres during pressure-controlled ventilation in experimental acute respiratory distress. Crit Care 2007; 11:R13.
24. Kredel M, Muellenbach RM, Johannes A, Brederlau J, Roewer N, Wunder C. Hepatic effects of lung-protective pressure-controlled ventilation and a combination of high-frequency oscillatory ventilation and extracorporeal lung assist in experimental lung injury. Med Sci Monit 2011; 17(10):BR275-281.
25. Harbrecht BG, Zenati MS, Doyle HR, McMichael J, Townsend RN, Clancy KD, et al. Hepatic dysfunction increases length of stay and risk of death after injury. J Trauma 2002; 53(3):517-523.
26. Fuhrmann V, Kneidinger N, Herkner H, Heinz G, Nikfardjam M, Bojic A, et al. Impact of hypoxic hepatitis on mortality in the intensive care unit. Intensive Care Med 2011; 37(8):1302-1310.
27. Jäger B, Drolz A, Michl B, Schellongowski P, Bojic A, Nikfardjam M, et al. Jaundice increases the rate of complications and one-year mortality in patients with hypoxic hepatitis. Hepatology 2012; 56(6):2297-2304.

<center>Original Article</center>

Evaluation of the flexible laryngeal mask airway in 120 patients undergoing tympanoplasty

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ABSTRACT

Objective: Although the flexible laryngeal mask airway (FLMA) has been used widely in dental, head and neck-related surgery, information regarding its use during tympanoplasty has been limited. The study was done to evaluate the FLMA in patients undergoing tympanoplasty.
Design: A prospective clinical trial was conducted.
Setting: Department of Anesthesiology, EENT Hospital of Fudan University, Shanghai, China.
Subjects: Current study included 120 patients undergoing elective tympanoplasty after informed written consent.
Intervention: After the induction of anesthesia, the FLMA was inserted using an index finger. Fiberoptic assessment was performed to assess optimal placement.
Main outcome measures: We recorded the number of attempts required to successfully insert the FLMA, cuff pressure, tidal volume <15 cmH$_2$O airway pressure, fiberoptic assessment before/after the head position, the

partial pressure of carbon dioxide at the end of expiration during tidal breathing, duration of anesthesia and complications.
Results: FLMA insertion was successful at the first attempt in 109 patients and required a second attempt in 11. The cuff pressure and/or fiberoptic assessment did not differ between the frontal and lateral head positions. The tidal volume was significantly lower in the lateral than in the frontal head position (men: 408.78±56.73 vs. 453.35±68.66 mL, respectively; women: 362.41±59.78 vs. 396.58±63.82 mL, respectively, P<.05). Of note, 9.2%, 1.7%, 1.7%, 2.5%, 5.83% and 4.17% of patients reported a sore throat, hoarseness, dysphagia, tongue numbness, headache, and postoperative nausea and vomiting, respectively.
Conclusions: Use of FLMA was effective and safe during tympanoplasty and showed a high success and a low complication rate.

KEY WORDS: complications, cuff pressure, flexible laryngeal mask airway, tidal volume, tympanoplasty

INTRODUCTION

The laryngeal mask airway (LMA) has gained wide acceptance as an alternative to traditional tracheal tube intubation owing to the ease of insertion and a possibly lower risk of tracheal trauma[1]. Lately, the LMA (LMA Flexible, Laryngeal Mask Co. Limited, Mahe, Seychelles) is used worldwide in millions of patients undergoing dental, head and neck-related surgery[2,3]. The flexible laryngeal mask airway (FLMA) enables more hands-free anesthesia than a facemask, and omits the use of a laryngoscope, thereby reducing morbidities/stress associated with tracheal intubation

and ensures faster recovery that does not require muscle relaxation[4]. Several studies have shown its advantages such as a lower incidence of hoarseness, coughing and oxygen desaturation[5,6]; however, a few others have emphasized that the results of the LMA were related to the method of selection of the device size and cuff inflation[7]. Surgical procedures requiring muscle relaxation require mechanical positive-pressure ventilation to secure airway ventilation[8], and various types of LMAs have been developed for better convenience[9]. The FLMA was first used successfully in 1990 during tonsillectomies and dental surgeries to

prevent the obstruction and kinking observed with the use of classical LMA tubes[10]. The cuff is identical to the classic LMA; however, the long and nonrigid flexometallic tube of the FLMA makes insertion more challenging. This unique design facilitates greater movement of the tube without displacement of the cuff or loss of seal. The head of a patient undergoing tympanoplasty needs to rotate 80 degrees toward the contralateral side. There exists limited information regarding the use of an FLMA during tympanoplasty. We designed this study to determine the incidence of postoperative manifestations of laryngopharyngeal discomfort, such as sore throat, hoarseness, dysphagia and ventilation efficiency associated with the use of an FLMA during tympanoplasty.

MATERIALS AND METHODS
Inclusion and exclusion criteria

Ethical approval was obtained from the Institutional Review Board of Shanghai Eye, Ear, Nose and Throat Hospital (No.KY2014-036) prior to the start of the study, and informed consent was obtained from all patients enrolled in the study. We studied 120 patients who underwent elective tympanoplasty between June 2016 and December 2016. Patients aged 18-65 years, belonging to the American Society of Anesthesiologists category 1-2, with expected uncomplicated airway upon assessment, and with no gastroesophageal reflux disease or other known risk factors for aspiration were included. Patients with a preoperative sore throat, hoarseness, dysphagia, asthma, gastroesophageal reflux, obesity (body mass index >30 kg/m^2), symptoms of upper respiratory infection two weeks prior to surgery, or with history of previous surgeries of the oral cavity or pharynx were excluded. The study included 63 women and 57 men. The FLMA was inserted in all patients by a single anesthetist who was not involved in the data collection or analysis and had at least three years' experience with using LMAs, including an FLMA. The size of the FLMA was chosen based on a patient's body weight - in patients weighing <50 kg, 50-70 kg and >70 kg, the FLMA sizes chosen were 3, 4 and 5, respectively.

Surgical procedure

No patient received any preoperative premedication. Upon arrival at the operating room, patients underwent standard monitoring, including electrocardiography, noninvasive arterial pressure assessment and pulse oximetry. Pre-oxygenation was performed for three minutes. Subsequently, intravenous access was established, and all patients were administered sufentanil 0.3 µg/kg and propofol 2-3 mg/kg for induction of anesthesia followed by cisatracurium 0.2 mg/kg to facilitate muscle relaxation.

The mean arterial pressure and the heart rate were recorded at one minute intervals during induction of anesthesia and five minute intervals intraoperatively. Anesthesia was maintained using sevoflurane at a minimal alveolar concentration of 0.9-1.1 in an oxygen-air mixture and remifentanil 0.1 µg/kg/min to maintain a bispectral index (10083122 Rev A, COVIDIEN, USA) of 40-60. The heart rate and mean blood pressure were maintained within 20% of their preoperative values. Additional sufentanil and cisatracurium were administered as required.

The FLMA was inserted with the cuff fully deflated and digital pressure was applied at the flexometallic tube near its cuff, flattening it against the hard palate and advancing it along the posterior palatopharyngeal curve with the operator's index finger placed at the junction of the mask and the tube until resistance could be felt against the finger. The cuff was then inflated with air to achieve an intracuff pressure <40 cmH$_2$O using a hand pressure gauge (VBM Medizintechnik GmbH, Germany, 5-118X). The ventilator (Dräger Fabius® Plus) was set to a pressure-controlled ventilation mode with pressure maintained at 15 cmH$_2$O. Successful ventilation was defined as visible chest movements, a square-wave capnogram and the ability to achieve an expired tidal volume of 6 mL/kg. A tympanoplasty procedure requires the patient's head to be turned to the healthy (contralateral) side. Thus, after the head was rotated, the cuff pressure measurement was repeated and the tidal volume is determined. If the initial attempt at insertion was unsuccessful, we discontinued further attempts temporarily, and the patient's lungs were manually ventilated before re-attempting FLMA insertion. Tracheal intubation was performed in patients after three failed attempts at FLMA insertion, and these patients were excluded from the study.

An ideal anatomical positioning of the LMA minimizes the need for intraoperative airway adjustment. Fiberoptic assessment provides an objective confirmation of FLMA placement. It was performed before surgery by passing a flexible fiberoptic bronchoscope (Olympus LF-GP, Olympus Optical Co. Ltd., Tokyo, Japan) through the flexometallic tube of the LMA to a position 1 cm proximal to the end of the tube and was also assessed at any arbitrary point intraoperatively at the discretion of the supervising anesthetist. The laryngeal view was classified based on a previously described scoring system: Grade 1: clear view of the vocal cords; Grade 2: view of only the arytenoids; Grade 3: view of only the epiglottis; and Grade 4: no laryngeal structures visible[11]. Fiberoptic assessment was performed in both, the frontal and the lateral head positions.

The number of attempts required to insert the FLMA, the cuff pressure, tidal volume <15 cmH_2O airway pressure, fiberoptic assessment before and after the required head position, partial pressure of carbon dioxide at the end of expiration during tidal breathing and the duration of anesthesia were recorded.

Postoperatively, the residual neuromuscular blockade was reversed using neostigmine 0.04 mg/kg and atropine 0.02 mg/kg. Extubation was performed after gentle suctioning of the oral cavity and deflation of the cuff when the patient had completely recovered and was awake. Patients were then transferred to the post-anesthesia care unit. Pain scores were assessed by nurses who had been blinded to the proposal immediately prior to discharge in all patients using the visual analog scale (VAS 1-10). Patients were asked to rate their degree of sore throat, jaw and/or neck soreness and any other complications were also recorded.

Statistical analysis

Statistical analyses were performed using the SPSS software version 11.5 (SPSS, Chicago, IL). Continuous data were presented as mean±standard deviation and compared using the Student's t-test. Categorical data were analyzed using the Pearson's Chi-square test. A P value <.05 was considered statistically significant.

RESULTS
General information

All 120 patients completed the study and the ventilation mode was not changed intraoperatively in any patient. Demographic information such as age, gender, weight, body mass index and other information such as type and duration of anesthesia, FLMA-related parameters and complications have been shown in Table 1. We studied 57 men and 63 women. The FLMA and subsequent ventilation could be performed successfully in all 120 patients. The FLMA was successfully inserted at the first attempt in 109 (50 men, 59 women) and at the second attempt in 11 patients (7 men, 4 women). No patient required a third attempt for FLMA insertion.

Surgical indices

Patients undergoing tympanoplasty need to have their head rotated 80 degrees toward the contralateral side. We observed that the cuff pressure did not significantly differ in the frontal and lateral head positions. Tidal volume was significantly lower in the lateral than in the frontal head position (408.78±56.73 vs. 453.35±68.66 mL respectively in men and 362.41±59.78 vs. 396.58±63.82 mL respectively in

Table 1: Patient characteristics and results for all flexible laryngeal mask airways

Index	Male	Female
Number	57	63
Age (year)	43.41±13.7	41.43±13.55
Weight (kg)	68.07±10.53	57.27±8.34
Body mass index	23.44±3.02	22.55±3.06
Anesthesia duration (min)	87.89±32.27	91.32±34.53
LMA size (No.)		
Size 3	0	5
Size 4	10	54
Size 5	47	4
Insertion		
1st attempt	50	59
2nd attempt	7	4
≥3rd attempt	0	0
Cuff pressure (cmH_2O)		
Frontal head position	33.32±10.41	31.72±6.27
Lateral head position	33.64±8.97	33.68±9.73
Tidal volume (mL)		
Frontal head position	453.35±68.66	396.58±63.82
Lateral head position	408.78±56.73*	362.41±59.78*
End-tidal CO_2 pressure (mmHg)	33.64±8.97	32.42±9.36
Overall pain score (VAS)	1.98±0.56	2.02±0.89
Sore throat		
No.	5	6
VAS	3.56±1.07	3.14±1.23
Hoarseness	1	1
Dysphagia	1	1
Tongue numbness	2	1
Headache	3	4
PONV	2	3

*Compared to frontal head position, P <.05. LMA: laryngeal mask airway; VAS: visual analog scale; PONV: postoperative nausea and vomiting.

women, P <.05). An expired tidal volume of 6 mL/kg, a square-wave capnogram and stable arterial oxygen saturations were observed in all patients.

Complications of surgery

Early postoperative complications were reported in 37 patients. Sore throat was reported in 9.2% patients (5 men, 6 women) and the mean VAS was <4. Hoarseness was reported in 1.7% patients (1 man, 1 woman), dysphagia in 1.7% (1 man, 1 woman), tongue numbness in 2.5% (2 men, 1 woman), headache in 5.83% (3 men, 4 women) and postoperative nausea and vomiting (PONV) in 4.17% (2 men, 3 women). When interviewed 24 hours post procedure, all patients reported that these early complications were self-limited in nature.

Results of fiberoptic assessment

Fiberoptic assessment through the flexometallic tube was performed in all patients. The vocal cords were visible in 61.7% (frontal head position) and 55.8% patients (lateral head position). Details of the fiberoptic

Table 2: Fibre optic view via the flexometallic tube of the LMA

Head position	Number	Grade 1	Grade 2	Grade 3	Grade 4
Frontal position					
Male	57	35	12	9	1
Female	63	39	16	7	1
Lateral position					
Male	57	31	15	10	1
Female	63	36	18	8	1

Grade 1: clear view of vocal cords; Grade 2: view of arytenoids; Grade 3: view of epiglottis; Grade 4: no laryngeal structures visible.

assessment have been presented in Table 2. No significant differences were observed between the frontal and lateral head positions.

DISCUSSION

The FLMA has been widely used in cleft palate, nasal, upper chest, as well as head and neck oncoplastic surgery[5,12]. Reportedly, the insertion of an FLMA is technically a more difficult procedure than that associated with the standard LMA Classic because pressure cannot be directly transmitted along the soft flexometallic shaft[13]. Various techniques suggested for FLMA insertion include the use of a stylet[14], a spatula introducer[15], a Bosworth introducer[16] and a flexiguide introducer[17]. In our study, the FLMA was inserted using an index finger, and we observed positive results with this technique. Insertion of the FLMA was easy in most patients. The first-time insertion success rate was 90.8% (109/120) and the overall insertion success rate was 100%.

The LMA is easy to insert in patients who are placed in the supine position; however, in patients undergoing tympanoplasty, the patient's head needs to rotate 80 degrees toward the contralateral side. Reportedly, this change in head position is known to reduce the sealing and ventilation effect of the laryngeal mask such that it might not optimally meet the clinical needs[18,19]. The FLMA does not affect the surgeon because it is a sturdy device that is easy to place, and it can be easily inserted and effectively used for airway management even in children with obesity undergoing minor surgery[20].

The results of this study showed that although the tidal volume was significantly lower in the lateral than in the frontal head position, it could adequately meet the clinical ventilation requirements (>6 m/L), which would be attributable to the change in head position. Fiberoptic examination revealed a Grade 1 or 2 view of the larynx in 85% of patients (35 men, 39 women) in the frontal head position and in 83% (31 men, 36 women) in the lateral head position. Although head rotation diminished the laryngeal exposure (the Grade 1 or 2 view was not as clearly visible), no significant difference was observed between the two positions.

Sore throat is a common postoperative complaint following tracheal intubation, use of an LMA, oral airway insertion as well as mask ventilation. An LMA is known to reduce the incidence of postoperative sore throat after general anesthesia compared with an endotracheal tube[7,21]. The incidence of sore throat is highly dependent on the cuff pressure within the LMA (b). High LMA cuff pressures exert a variable pressure on pharyngeal structures[22,23]. Notably, high cuff pressures are associated not only with sore throat but a higher incidence of all complications[24-26]. Although the manufacturer guidelines for the use of the LMA propose/set a maximum recommended inflation volume, the use of the recommended maximum volume is associated with a high risk of hyperinflation and increased leakage around the LMA cuff[18]. The development of a sore throat with high cuff pressures is likely secondary to ischemic injury to the pharynx, which occurs when the laryngeal mask pressures exceed pharyngeal wall perfusion pressures[18]. Brimacombe et al have reported that no instances of sore throat were observed when cuff pressures were <40 cmH$_2$O, which is a value at which cuff pressures are unlikely to exceed the pharyngeal perfusion pressure[11]. William et al showed a 3.3% overall rate of sore throat with a cuff pressure <60 cmH$_2$O[27]. A previous study performed by the same research group reported that sore throat did not occur at a cuff pressure <40 cmH$_2$O and that the incidence of sore throat was 4.6% when the cuff pressure was 40-60 cmH$_2$O[28]. The results of our study demonstrated that the incidence of sore throat was 9.2% (5 men, 6 women) when the cuff pressures (measured using a hand pressure gauge) were <40 cmH$_2$O. These results show a higher incidence of sore throat than in previous studies[28]. This difference could be attributed to the fact that the lateral head position adopted during a tympanoplasty caused compression of the pharyngeal tissues by the laryngeal mask precipitating a sore throat.

Hoarseness has been reported in approximately 50% of intubated patients and 15% of patients undergoing LMA insertion[29]. Hoarseness is associated with low levels of patient satisfaction. Apfelbaum et al[30] suggested that the most common cause of vocal

cord paralysis could be attributed to compression of the recurrent laryngeal nerve by the endotracheal tube cuff within the endolarynx. An FLMA is positioned superior to the larynx and is rarely associated with recurrent laryngeal nerve injury. The incidence of hoarseness in our study was 1.7%, which was in agreement with previous reports[31]. Other complications included dysphagia, tongue numbness, headache and PONV. The pressure exerted by the LMA on the vocal cords and the tongue intraoperatively might lead to dysphagia and tongue numbness. Use of a postoperative head band might cause headaches. PONV is a common general anesthesia-induced complication. Fortunately, in our study, the incidence of all complications was low and no patient demonstrated severe symptoms.

Limitations of the present study

Firstly, our study included patients belonging to the American Society of Anesthesiologists category 1-2; thus, our results may not be applicable to other patients with significant comorbidities. We studied only low-risk patients who showed normal airways and most patients had not been diagnosed with obesity. Our results might not apply to patients who are diagnosed with morbid obesity and present with a potential difficulty with maneuverability in their airway or require other types of surgery. Second, we did not compare the performance of the FLMA with other likely alternatives/substitutes. This study can be regarded a feasibility study and randomized controlled studies to compare these devices are warranted. Third, post-tympanoplasty laryngopharyngeal symptoms were evaluated only through a short-term postoperative follow-up. Further studies are warranted to evaluate the long-term effects following the use of an FLMA. Lastly, the sample size of this study was relatively small. Further large-scale cohort studies comprising a greater volume of data are required in this context.

CONCLUSION

Although the heads of patients undergoing tympanoplasty need to rotate 80 degrees toward the contralateral side, the use of the LMA flexible is a good option in those undergoing tympanoplasty owing to the advantages of better placement of the airway device, decreased incidence of postoperative complications and satisfactory ventilation effects.

ACKNOWLEDGMENT

REFERENCES

1. Nicholson A, Cook TM, Smith AF, Lewis SR, Reed SS. Supraglottic airway devices versus tracheal intubation for airway management during general anaesthesia in obese patients. Cochrane Database Syst Rev 2013; (9):CD010105.

2. Tsukamoto M, Hitosugi T, Yokoyama T. Flexible laryngeal mask airway management for dental treatment cases associated with difficult intubation. J Dent Anesth Pain Med 2017; 17(1):61-64.

3. Nekhendzy V, Ramaiah VK, Collins J, Lemmens HJ, Most SP. The safety and efficacy of the use of the flexible laryngeal mask airway with positive pressure ventilation in elective ENT surgery: a 15-year retrospective single-center study. Minerva Anestesiol 2017; 83(9):947-955.

4. Hernandez MR, Klock Jr PA, Ovassapian A. Evolution of the extraglottic airway: a review of its history, applications, and practical tips for success. Anesth Analg 2012; 114(2):349-368.

5. Xu R, Lian Y, Li WX. Airway complications during and after general anesthesia: A comparison, systematic review and meta-analysis of using flexible laryngeal mask airways and endotracheal tubes. PLoS One 2016; 11(7):e0158137.

6. Jarineshin H, Kashani S, Vatankhah M, Baghaee AA, Sattari S, Fekrat F. Better hemodynamic profile of laryngeal mask airway insertion compared to laryngoscopy and tracheal intubation. Iran Red Crescent Med J 2015; 17(8):e28615.

7. van Esch BF, Stegeman I, Smit AL. Comparison of laryngeal mask airway vs tracheal intubation: a systematic review on airway complications. J Clin Anesth 2017; 36:142-150.

8. Pollack Jr CV. The laryngeal mask airway: a comprehensive review for the Emergency Physician. J Emerg Med 2001; 20(1):53-66.

9. Pajiyar AK, Wen Z, Wang H, Ma L, Miao L, Wang G. Comparisons of clinical performance of guardian laryngeal mask with laryngeal mask airway ProSeal. BMC Anesthesiol 2015; 15:69.

10. Alexander CA. A modified Intavent laryngeal mask for ENT and dental anaesthesia. Anaesthesia 1990; 45(10):892-893.

11. Brimacombe J, Keller C, Pühringer F. Pharyngeal mucosal pressure and perfusion: a fiberoptic evaluation of the posterior pharynx in anesthetizedadult patients with a modified cuffed oropharyngeal airway. Anesthesiology 1999; 91(6):1661-1665.

12. Ozmete O, Sener M, Caliskan E, Kipri M, Aribogan A. The use of flexible laryngeal mask airway for Adenoidectomies: An experience of 814 paediatric patients. Pak J Med Sci 2017; 33(4):823-828.

13. Yahaya Z, Teoh WH, Dintan NA, Agrawal R. The AMBU® Aura-i™ Laryngeal Mask and LMA Supreme™: A randomized trial of clinical performance and fibreoptic positioning in unparalysed, anaesthetised patients by novices. Anesthesiol Res Pract 2016; 2016:4717061.

14. Lee LW. Insertion of the LMA ProSeal using the Satin-Slip intubating stylet. Can J Anaesth 2007; 54(1):81-82.

15. Kil HK, Koo BN, Park JH, Kim WO. The spatula introducer for insertion of the flexible reinforced laryngeal mask airway (RLMA). Can J Anaesth 2005; 52(1):117-118.

16. Bosworth A, Jago RH. The Bosworth introducer for use with the flexible reinforced laryngeal mask airway. Anaesthesia 1997; 52(3):281-282.

17. Cook TM, Ford P, Craft TM, McCormick B, Ryder SA. An evaluation of the flexiguide introducer with the flexible laryngeal mask airway. Anaesth Intensive Care 2003; 31(2):193-195.
18. Kim WH, Gwak MS, Choi SJ, Song SH, Kim MH. Optimal head rotation and puncture site for internal jugular vein cannulation after laryngeal mask airway insertion. Singapore Med J 2015; 56(8):472-478.
19. Xue FS, Mao P, Liu HP, Yang QY, Li CW, He N, *et al*. The effects of head flexion on airway seal, quality of ventilation and orogastric tube placement using the proseal laryngeal mask airway. Anaesthesia 2008; 63(9):979-985.
20. Tian Y, Wu XY, Li L, Ma L, Li YF. A clinical trial evaluating the laryngeal mask airway-Supreme in obese children during general anesthesia. Arch Med Sci 2017; 13(1):183-190.
21. Jaensson M, Gupta A, Nilsson U. Gender differences in sore throat and hoarseness following endotracheal tube or laryngeal mask airway: a prospective study. BMC Anesthesiol 2014; 14:56.
22. Chantzara G, Stroumpoulis K, Alexandrou N, Kokkinos L, Iacovidou N, Xanthos T. Influence of LMA cuff pressure on the incidence of pharyngolaryngeal adverse effects and evaluation of the use of manometry during different ventilation modes: a randomized clinical trial. Minerva Anestesiol 2014; 80(5):547-555.
23. Keller C, Brimacombe J. Mucosal pressure and oropharyngeal leak pressure with the ProSeal versus laryngeal mask airway in anaesthetized paralysed patients. Br J Anaesth 2000; 85(2):262-266.
24. Ong M, Chambers NA, Hullett B, Erb TO, von Ungern-Sternberg BS. Laryngeal mask airway and tracheal tube cuff pressures in children: are clinical endpoints valuable to guide inflation? Anaesthesia 2008; 63(7):738-744.
25. Maino P, Dullenkopf A, Keller C, Bernet-Buettiker V, Weiss M. Cuff filling volumes and pressures in pediatric laryngeal mask airways. Paediatr Anaesth 2006; 16(1):25-30.
26. Licina A, Chambers NA, Hullett B, Erb TO, von Ungern-Sternberg BS. Lower cuff pressures improve the seal of pediatric laryngeal mask airways. Paediatr Anaesth 2008; 18(10):952-956.
27. William A, Chambers NA, Erb TO, von Ungern-Sternberg BS. Incidence of sore throat in children following use of flexible laryngeal mask airways - impact of an introducer device. Paediatr Anaesth 2010; 20(9):839-843.
28. Wong JGL, Heaney M, Chambers NA, Erb TO, von Ungern-Sternberg BS. Impact of laryngeal mask airway cuff pressures on the incidence of sore throat in children. Paediatr Anaesth 2009; 19(5):464-469.
29. Grady DM, McHardy F, Wong J, Jin F, Tong D, Chung F. Pharyngolaryngeal morbidity with the laryngeal mask airway in spontaneously breathing patients: does size matter? Anesthesiology 2001; 94(5):760-766.
30. Apfelbaum RI, Kriskovich MD, Haller JR. On the incidence, cause, and prevention of recurrent laryngeal nerve palsies during anterior cervical spine surgery. Spine (Phila Pa 1976) 2000; 25(22):2906-2912.
31. Abdi W, Amathieu R, Adhoum A, Poncelet C, Slavov V, Kamoun W, *et al*. Sparing the larynx during gynecological laparoscopy: a randomized trial comparing the LMA Supreme and the ETT. Acta Anaesthesiol Scand 2010; 54(2):141-146.

Original Article

Diagnostic value of TIMP-1, TIMP-2, TGF- ß1, YKL-40 in the evaluation of liver fibrosis in patients with chronic hepatitis B

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ABSTRACT

Objectives: To evaluate the diagnostic accuracy of noninvasive serological parameters to predict liver fibrosis in chronic hepatitis B patients
Design: Prospective clinical study
Setting: Ankara Training and Research Hospital, Department of Infectious Diseases and Clinical Microbiology, Ankara, Turkey
Subjects: This study included 88 patients with chronic hepatitis B
Intervention: Liver biopsy was performed in all patients. Fibrosis stage was determined using Ishak's scoring system. In group I, there were cases with mild or no fibrosis and group II had patients with severe fibrosis.
Main outcome measures: Serum YKL-40, TIMP-1, TIMP-2, MMP-2 and TGF-ß1 were measured by ELISA. Serological parameters were compared with histopathological fibrosis scores.

Results: Group I included 55 patients (62.5%) and group II had 33 patients (37.5%). Serum YKL-40 were found higher in group II than group I (*P*=.034). There was no statistical difference in other serological parameters. We demonstrated that YKL-40 has highest area under the receiver operating characteristic for predicting ≥F3 fibrosis. Based on the results, TIMP-1, TIMP-2, MMP-2, TGF-β1 levels are not useful in determining liver fibrosis in patients with chronic hepatitis B. However, YKL-40 values revealed correlation with histopathological parameters.
Conclusion: We found that serum YKL-40 values can be used as an important non-invasive test for diagnosis of fibrosis in patients with chronic hepatitis B. However, we think that it was not yet enough to replace liver biopsy clinically.

KEY WORDS: chronic hepatitis B, liver fibrosis, non-invasive, YKL-40

INTRODUCTION

Infections of hepatitis B virus (HBV) lead to important public health problems in Turkey, as in the rest of the world. The World Health Organization has estimated that about two billion people in the world have been exposed to HBV and are seropositive, while an average of 250 million people are currently infected with HBV. Every year, about 600,000 people in the world die from cirrhosis and hepatocellular carcinoma caused by chronic hepatitis[1]. In patients with chronic hepatitis B (CHB), the decision for therapy is generally made according to the serum HBV-DNA and alanine aminotransferase levels, the degree of necroinflammation and the stage of fibrosis in the liver biopsy[2,3].

In liver diseases, liver biopsy is the gold standard method for diagnosing fibrosis[4]. Although liver biopsy is generally accepted to be a simple and reliable procedure, its application is debatable as this procedure is an invasive method, has contraindications, may lead to complications, requires hospitalization and may cause sampling errors; it is also difficult to be replicated

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and is costly[4,5]. In recent years, studies have been made on non-invasive methods to evaluate fibrosis[6]. Some proteins like TIMP-1, TIMP-2 and MMP-2 indicating matrix degradation, TGF-B indicating fibrogenesis and YKL-40 indicating matrix accumulation was also studied.

The aim of the present study was to evaluate the diagnostic value of the serum levels of TIMP-1, TIMP-2 and MMP-2, TGF-B and YKL-40 for detection of liver fibrosis and also to assess the benefit of their clinical use.

SUBJECTS AND METHODS

The study comprised patients with HBV infection who were admitted to the Infectious Diseases and Clinical Microbiology Clinic between the dates of January 16, 2013 and July 16, 2013, who had been HBsAg positive for longer than six months and had not been treated previously. Patients with anti-HAV IgM, anti-HIV, anti-HCV or anti-HDV positivity with a chronic liver disease other than HBV (toxic hepatitis, granulomatous hepatitis, autoimmune hepatitis, cholestatic liver disease, alcoholic liver disease, Wilson's disease, alpha-1 antitrypsin deficiency and hemochromatosis), and with signs and findings of hepatocellular carcinoma (abdominal pain, weight loss, anorexia, fever, jaundice, and ascites and palpable mass at physical examination) were excluded from the study. Prior to biopsy, blood samples were collected from the patients included in the study for evaluating non-invasive serological markers. All patients underwent a percutaneous fine-needle liver biopsy using a 16-G biopsy needle. During histopathological investigations, staging and grading were made according to the Modified Knodell 'Ishak' Scoring System. Using the Ishak scoring system, stage 0 was defined as the absence of fibrosis, stages 1-2 were defined as mild fibrosis, and stages 3-4 were defined as intermediate/severe fibrosis. The patients were divided into two groups according to the degree of fibrosis as follows: Group I comprised cases with stages 0-1-2 (no fibrosis, mild fibrosis) and Group II with stages 3-4 (intermediate/severe fibrosis). The serum levels of the non-invasive serological markers MMP-2, TIMP-1, TIMP-2, TGF-B1 and YKL-40 were evaluated using the commercially available ELISA kits (EIAab Science CO, CHİNA and Aviscera Bioscience, Inc., USA), and the analyses were conducted in line with the manufacturer's instructions. The non-invasive serological markers were compared with the histopathological fibrosis scores revealed following the liver biopsy, in terms of hepatic fibrosis.

Ethical approval and consent to participate

The study was approved by our institutional review board and study number was 4097/405.

Consent for publication was obtained from all the patients.

Statistical analyses

The SPSS 16.0 for Windows program was used for statistical analysis. The descriptive statistical data were expressed as percentage distribution, mean, standard deviation and median. Comparison of percentages was made using the Chi-square test and the Fisher's exact test was used where necessary. Student's t test was used to compare two continuous variables that fit normal distribution, and Mann Whitney U test was used to compare two continuous variables that did not fit normal distribution. The correlations between these variables were evaluated using the Kendall's tau-b correlation test. A P-value less than 0.05 was accepted to be statistically significant. The receiver operating characteristic curves were drawn in order to test the efficiencies of non-invasive markers of fibrosis to indicate liver fibrosis, and the area under the receiver operating characteristic (AUROC) was calculated.

RESULTS

The study comprised 88 patients with CHB followed-up in the Infectious Diseases and Clinical Microbiology Clinic. Of the patients, 41 (46.6%) were male and 47 (53.4%) were female; the mean age of the patients was 40.03±11.9 (17-67) years. Age and gender were not found to differ significantly between the patients in Group I and Group II (P=.391 and P=.783).

The histopathological evaluation of liver biopsies conducted in line with Ishak scoring indicated that 10 (11.4%) patients were at stage 0, 45 (51.1%) were at stages 1-2 and 33 (37.5%) were at stages 3-4. No cirrhotic patients at stages 5-6 were detected. The patients' mean Knodell score and the mean stage of fibrosis were determined as 4.4 and 2.1, respectively. The distribution of the patients in according to fibrosis scores is indicated in Table 1.

Table 1: Distribution of patients according to fibrosis scores

Fibrosis scores	n	%
No fibrosis (0)	10	11.4
Mild fibrosis (1-2)	45	51.1
Intermediate-severe fibrosis (3-4)	33	37.5
Total	88	100

Patients with no/mild fibrosis were compared with patients with intermediate/severe fibrosis in terms of levels of the non-invasive serological markers such as YKL-40, TIMP-1, TIMP-2, MMP-2 and TGF-β1 (Table 2). A statistically significant difference in serum YKL-40 levels was detected in Group II patients. In this group, patients had a significantly higher YKL-40 level

Table 2: Comparison of serum levels of non-invasive fibrosis markers of Group I and Group II patients

Noninvasive serological markers	Group I (mean± SD)	Group II (mean± SD)	P
YKL-40 (0.25-16 ng/mL)	6.08±5.18	8.82±6.18	.029
TIMP-1 (62.5-4000 pg/mL)	2841±440	2880±428	.690
TIMP-2 (0.3-20 ng/mL)	2.78±2.18	3.43±2.57	.215
MMP-2 (0.31-20 ng/mL)	3.58±2.98	3.47±2.99	.865
TGF-β1 (15.6-1000 pg/mL)	446.8±317.5	362.8±305.8	.228

*YKL-40: Condrex, TIMP-1: Tissue inhibitor metalloproteinase-1, TIMP-2: Tissue inhibitor metalloproteinase-2, MMP-2: Matriks metalloproteinaz-2, TGF-β1: Transforming growth factor-beta 1

Table 3: Correlation between fibrosis stages and noninvasive fibrosis markers

Noninvasive serological markers	R	P
YKL-40 (ng/mL)	0.188	.034
TIMP-1 (pg/mL)	0.055	.532
TIMP-2 (ng/mL)	0.096	.276
MMP-2 (ng/mL)	-0.028	.750
TGF- β1 (pg/mL)	-0.128	.149

*YKL-40: Condrex, TIMP-1: Tissue inhibitor metalloproteinase-1, TIMP-2: Tissue inhibitor metalloproteinase-2, MMP-2: Matriks metalloproteinaz-2, TGF-β1: Transforming growth factor-beta 1

(P=.029). There was no statistical difference in other serological parameters.

The correlations between the stages of liver fibrosis and serum YKL-40, TIMP-1, TIMP-2, MMP-2 and TGF-B1 levels in the patients were investigated (Table 3). A positive correlation was detected between the serum YKL-40 level and stage of fibrosis (r=0.235, P=.029).

The values of the AUROC related to serum YKL-40, TIMP-1, TIMP-2, MMP-2 and TGF-B1 levels that determine the existence of fibrosis were assessed as 0.636, 0.540, 0.575, 0.480 and 0.407, respectively. YKL-40 provided the best diagnostic value for the detection of liver fibrosis. The receiver operating characteristic curves of the tests are presented in Figure 1.

DISCUSSION

Assessment of the stage of liver fibrosis in patients with CHB is paramount in determining treatment and prognosis. Liver biopsy is the gold standard method for evaluating liver fibrosis[4]. However, over recent years, studies have been undertaken with the objective of developing biochemical and other non-invasive diagnostic methods that can replace histopathological evaluation. By evaluating several parameters in combination, indices (such as Bonacini, Forns and APRI indices, ALT/AST ratio, FibroTest and ActiTest) which might be more sensitive, have been developed[7-10]. Furthermore, in recent years, some novel markers (such as laminin, hyaluronic acid, YKL-40, TIMP-1 and 2, PIIINP, MMP 1,2,3 and 9, PDGF-BB,
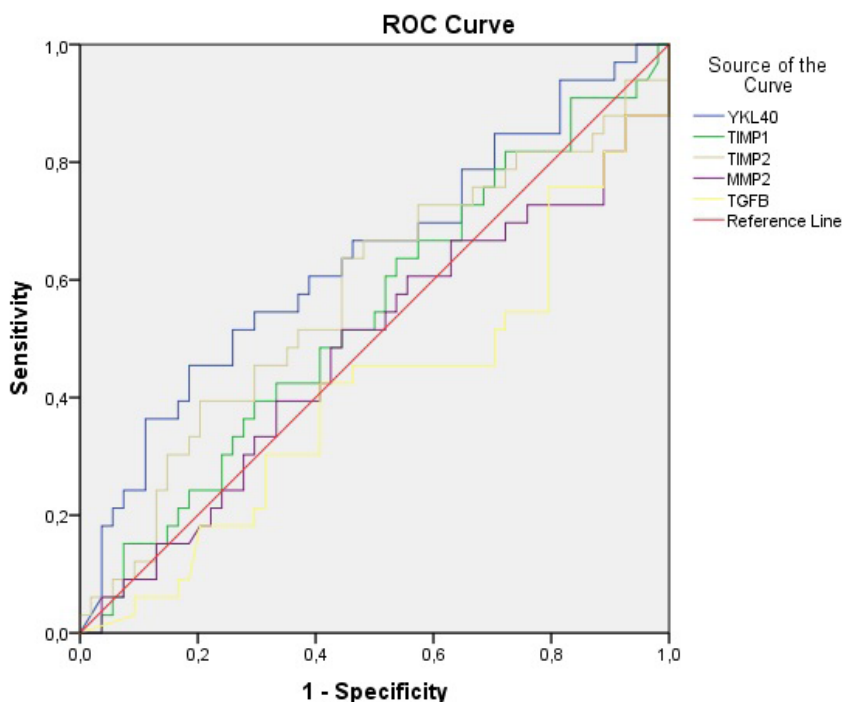
Fig 1: Receiver operating characteristic curve demonstrating diagnostic strength of non-invasive fibrosis markers on signaling fibrosis presence

TGF-B1, type 4 collagen and fibronectin), which have been linked with tissue fibrosis, have also been produced for use as commercial kits[11-13].

There are studies investigating the correlation between the level of TIMP-1, one of the non-invasive markers of fibrosis and the severity of fibrosis in patients with chronic liver disease. In a study by Zhu *et al* consisting of 159 patients with CHB, a positive correlation was determined between the stage of fibrosis and serum TIMP-1 levels (r=0.695, *P* <.01); when the threshold value was accepted as ≥174.5 ng/mL, the sensitivity, specificity, positive predictive value and negative predictive value were found to be 89.4%, 83.6%, 91.2% and 80.1%, respectively[14]. In the study of El Kamery *et al* conducted with 355 patients with chronic hepatitis C, it was revealed that the serum TIMP-1 levels increased as the stage of fibrosis advanced in a way that was statistically significant[15]. In a study by Seven *et al*, a positive correlation was identified between the serum TIMP-1 level and the stage of fibrosis in 109 patients with CHB, 33 of whom were coinfected with HDV. In this study, the AUROC for TIMP-1 has been assessed as 0.747, and it was asserted that TIMP-1 could be a good non-invasive marker of fibrosis in the patients with CHB[16]. In our study, the serum TIMP-1 level was slightly higher in the group with an intermediate/severe stage of fibrosis compared with the other group, but this difference was not found to be statistically significant (*P*=.690).

TIMP-2 was another marker evaluated in our study. In a study by Liang *et al* comprising 101 patients with CHB, the serum TIMP-2 level was found to be significantly higher in the group with a stage of fibrosis ≥2, compared with the group with stage 0-1[17]. In two separate studies comparing patients with chronic hepatitis C to healthy control groups, the serum TIMP-2 levels in the patient groups were found to be significantly higher compared with healthy groups, and no significant correlation was identified between the stage of fibrosis and serum TIMP-2 levels[18,19]. In our study, the serum TIMP-2 level was also determined to be higher in the group with an advanced stage of fibrosis; nevertheless, this difference was not statistically significant (*P*=.215) and no significant correlation was detected between the stage of fibrosis and serum TIMP-2 levels (r=0.096, *P*=.276).

There are studies demonstrating that the serum matrix metalloproteinase-2 level increases with chronic liver disease. In the study of El Kamery *et al* conducted with 355 patients with chronic hepatitis C, it was shown that the serum MMP-2 levels also increased as the stage of fibrosis increased in a way that was statistically significant[15]. The study by Liang *et al* of 101 patients with CHB, found that the serum MMP-2 level was significantly higher in the group with a stage

of fibrosis ≥2, when compared with the group with stage 0-1[17]. In the same study, 101 patients with CHB were compared to 54 healthy controls and the serum MMP-2 level was found to be significantly higher in the patient group[17]. In two different studies comprising patients with chronic hepatitis, the serum MMP-2 levels were likewise assessed to be significantly higher in the patient groups with a stage of fibrosis ≥2, compared with the groups with stages of <2[16,20]. In our study, no statistically significant difference was detected between the cases with no fibrosis/mild fibrosis and those with intermediate/severe fibrosis in terms of the serum MMP-2 levels (*P*=.865).

The TGF-B1 was the other non-invasive marker of fibrosis that we evaluated in our study. In the study of Valva *et al* that examined patients with chronic hepatitis C, the serum TGF-B1 levels were found to be significantly higher both in the adult and pediatric patient groups, when compared with healthy group. In the pediatric patient group, the serum TGF-B1 level did not differ significantly between the group with a high stage of fibrosis and the group with a low stage. In the adult patient group, the serum TGF-B1 level was seen to be significantly lower in the group with an advanced stage of fibrosis[21]. In the study by Guo *et al* comprising 131 patients with CHB, the serum TGF-B1 level was determined to be significantly lower in the group with advanced stages of fibrosis compared with the group with stage 0[22]. In another study conducted by Zhang *et al*, no statistically significant difference was identified between patients with CHB and a healthy control group in terms of serum TGF-B1 levels[23]. In our study, the serum TGF-B1 levels were also found to be lower in the group with an advanced stage of fibrosis. However, this result was not statistically significant (*P*=.228).

In recent years, the value of YKL-40 has been investigated as a marker to indicate inflammation and fibrosis. In the study of Saitou *et al* involving 109 cases of chronic hepatitis C, the YKL-40 was determined to be more sensitive than the other non-invasive parameters of fibrosis in serum, in terms of demonstrating advanced stages of fibrosis[24]. When the threshold value for YKL-40 was established as 284±8 ng/ml, the sensitivity and specificity was found to be 80% and 77%, respectively. Following antiviral treatment, the serum YKL-40 level was seen to decrease more significantly than those of the other markers. This result emphasized that the serum YKL-40 level, in addition to the severity of disease, could also be helpful for evaluating the response to therapy[24]. In a study by Nojgaard *et al* comprising 370 patients with alcohol-related chronic liver disease, the serum YKL-40 level was found to be low in the patients without steatosis and cirrhosis, whereas it was high in the patients with

fibrosis[25]. In our study, the serum YKL-40 level was seen to be higher in the group with an advanced stage of fibrosis, and this difference was found to be statistically significant (P=.029). A positive correlation was determined between the stage of fibrosis and serum YKL-40 level (r=0.188, P=.034).

Furthermore, in our study, the serum YKL-40 possessed the highest AUROC level and thus had the highest diagnostic value in demonstrating the presence of stage 3 and more severe fibrosis, and also that the YKL-40 levels were correlated with the histopathological parameters. It was considered that the evaluation of serum TIMP-1, TIMP-2, MMP-2 and TGF-B1 levels was not sufficiently effective in demonstrating the presence of fibrosis in the patients with CHB.

CONCLUSION

In conclusion, the present study suggests that the serum YKL-40 level could be used as an effective non-invasive marker of fibrosis, in terms of revealing the presence of fibrosis in the patients with CHB. Nevertheless, without more detailed and comprehensive studies, it is clear that it will not be sufficient to replace liver biopsy alone. We consider that more comprehensive studies involving the cirrhotic patient group are needed on this subject, in patients with chronic hepatitis.

REFERENCES

1. WHO, Hepatitis B. Updated July 2015. Available from: http://www.who.int/mediacentre/factsheets/fs204_Jul2014/en/

2. Lok AS, McMahon BJ, AASLD. [AASLD Practice Guidelines. Chronic hepatitis B: update of therapeutic guidelines]. Romanian Journal of Gastroenterology 2004; (2):150-154.

3. Shindo M, Hamada K, Nishioji K, Muramatsu A, Oda Y, Okuno T. The predictive value of liver fibrosis in determining the effectiveness of interferon and lamivudine therapies for chronic hepatitis B. J Gastroenterol 2004; 39(3):260-267.

4. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl J Med 2001; 344(7):495-500.

5. Bedossa P, Dargere D, Paradis V. Sampling variability of liver fibrosis in chronic hepatitis C. Hepatology 2003; 38(6):1449-1457.

6. Grigorescu M. Noninvasive biochemical markers of liver fibrosis. J Gastrointestin Liver Dis 2006; 15(2):149-159.

7. Hui AY, Chan HL, Wong VW, Liew CT, Chim AM, Chan FK, *et al.* Identification of chronic hepatitis B patients without significant liver fibrosis by a simple noninvasive predictive model. Am J Gastroenterol 2005; 100(3):616-623.

8. Halfon P, Bourliere M, Deydier R, Botta-Fridlund D, Renou C, Tran A, *et al.* Independent prospective multicenter validation of biochemical markers (fibrotest-actitest) for the prediction of liver fibrosis and activity in patients with chronic hepatitis C: the fibropaca study. Am J Gastroenterology 2006; 101(3):547-555.

9. Zeremski M, Talal AH. Noninvasive markers of hepatic fibrosis: are they ready for prime time in the management of HIV/HCV co-infected patients? J Hepatol 2005; 43(1):2-5.

10. Halfon P, Bacq Y, De Muret A, Penaranda G, Bourliere M, Ouzan D, *et al.* Comparison of test performance profile for blood tests of liver fibrosis in chronic hepatitis C. J Hepatol 2007; 46(3):395-402.

11. Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. Am J Gastroenterol 2004; 99(6):1160-1174.

12. Halfon P, Bourliere M, Penaranda G, Deydier R, Renou C, Botta-Fridlund D, *et al.* Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. Comp Hepatol 2005; 4:6.

13. Poynard T, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D, *et al.* Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comp Hepatol 2004; 3(1):8.

14. Zhu CL, Li WT, Li Y, Gao RT. Serum levels of tissue inhibitor of metalloproteinase-1 are correlated with liver fibrosis in patients with chronic hepatitis B. J Dig Dis 2012; 13(11):558-563.

15. El-Kamary SS, Mohamed MM, El-Raziky M, Shardell MD, Shaker OG, ElAkel WA, *et al.* Liver fibrosis staging through a stepwise analysis of non-invasive markers (FibroSteps) in patients with chronic hepatitis C infection. Liver International 2013; 33(7):982-990.

16. Seven G, Karatayli SC, Kose SK, Yakut M, Kabacam G, Toruner M, *et al.* Serum connective tissue markers as predictors of advanced fibrosis in patients with chronic hepatitis B and D. The Turkish Journal of Gastroenterology 2011; 22(3):305-314.

17. Liang B, Li Y, Zhao A, Xie F, Guo Z. Clinical utility of serum matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 concentrations in the assessment of liver fibrosis due to chronic hepatitis B. J Int Med Res 2012; 40(2):631-639.

18. Bruno CM, Valenti M, Bertino G, Ardiri A, Consolo M, Mazzarino CM, *et al.* Altered pattern of circulating matrix metalloproteinases -2,- 9 and tissue inhibitor

of metalloproteinase-2 in patients with HCV-related chronic hepatitis. Relationship to histological features. Panminerva medica 2009; 51(4):191-196.

19. Boker KH, Pehle B, Steinmetz C, Breitenstein K, Bahr M, Lichtinghagen R. Tissue inhibitors of metalloproteinases in liver and serum/plasma in chronic active hepatitis C and HCV-induced cirrhosis. Hepato-gastroenterology 2000; 47:812-819.

20. Cheong JY, Um SH, Seo YS, Kim DJ, Hwang SG, Lee YJ, *et al.* Non-invasive index for predicting significant liver fibrosis: comparison of diagnostic performances in patients with chronic hepatitis B and C. Dig Dis Sci 2011; 56(2):555-563.

21. Valva P, Casciato P, Diaz Carrasco JM, Gadano A, Galdame O, Galoppo MC, *et al.* The role of serum biomarkers in predicting fibrosis progression in pediatric and adult hepatitis C virus chronic infection. PloS One 2011; 6(8):e23218.

22. Guo JC, Bao JF, Chen QW, Li XO, Shi JP, Lou GQ, *et al.* [Level of serum and liver tissue TGF-beta1 in patients with liver fibrosis due to chronic hepatitis B]. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi : Chinese Journal of Experimental and Clinical Virology 2008; 22(5):354-357.

23. Zhang BB, Cai WM, Weng HL, Hu ZR, Lu J, Zheng M, *et al.* Diagnostic value of platelet derived growth factor-BB, transforming growth factor-beta1, matrix metalloproteinase-1, and tissue inhibitor of matrix metalloproteinase-1 in serum and peripheral blood mononuclear cells for hepatic fibrosis. World J Gastroenterol 2003; 9(11):2490-2496.

24. Saitou Y, Shiraki K, Yamanaka Y, Yamaguchi Y, Kawakita T, Yamamoto N, *et al.* Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease. World J Gastroenterol 2005; 11(4):476-481.

25. Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U. EMALD Group. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. J Hepatol 2003; 39(2):179-186.

Case Report

Can diabetes insipidus be used as a marker for multisystemic and progressive disease in Langerhans cell histiocytosis?

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ABSTRACT

Langerhans cell histiocytosis (LCH) is a rare disease with an yearly incidence of nine cases per a million in children and 1-2 cases per a million adults. 68.6% of LCH presented with multisystem involvement. A 40-year-old woman who was admitted to endocrinology outpatient clinic with symptoms of polyuria, polydipsia and headache was diagnosed with diabetes insipidus (DI). Desmopressin treatment was initiated, but six months after therapy, re-evaluation revealed progression in hypophyseal mass. Thoracoscopic biopsy shows LCH with multisystemic involvement. She did not respond clinically to systemic chemotherapy and external radiotherapy and died due to pneumonia.

LCH should be taken into consideration in patients diagnosed with DI. DI is almost always the hallmark of hypothalamic pituitary axis involvement and a sign of multi-systemic involvement.

KEY WORDS: diabetes insipidus, Langerhans cell histiocytosis, multisystemic disease

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare disease in childhood and adulthood which is a part of diseases caused by infiltration of Langerhans cells across multiple organs, such as the lungs, bones, skin, pituitary gland and lymph nodes[1]. The yearly incidence is 3-5 cases per million between 1 and 3 years of age[2] and 9 cases per million in children[3]. LCH is rarer in adults with the yearly incidence of 1-2 cases per a million[4,5].

The exact pathogenesis of LCH is still not defined, but identified tumor-associated mutation (BRAFV600E) indicates that LCH is more a neoplastic disease rather than a reactive disorder, although it exhibits a strong inflammatory component[6,7]. Presence of Birbeck granules in electron microscopy or S100/ CD1a positivity in immunohistochemical staining confirms the diagnosis of LCH[7].

The International LCH Study of the Histiocyte Society categorized LCH into localized (single-system disease) and disseminated forms (multisystem disease[8]. According to International Histiocyte Society Registry from January 2000 to June 2001, 68.6% of LCH presented with multisystem involvement[9].

The most commonly involved organs are lungs (58.4%) and bones (57.3%). Local pain (34%), weight loss (11%) and fever (10%) are the most common symptoms at presentation[9]. The most common endocrine manifestation of LCH in adults is diabetes insipidus (DI, 29.6%)[9].

CASE REPORT

A 40-year-old woman was admitted to endocrinology outpatient clinic with symptoms of polyuria, polydipsia and headache. In the initial examinations, there was no feature except for

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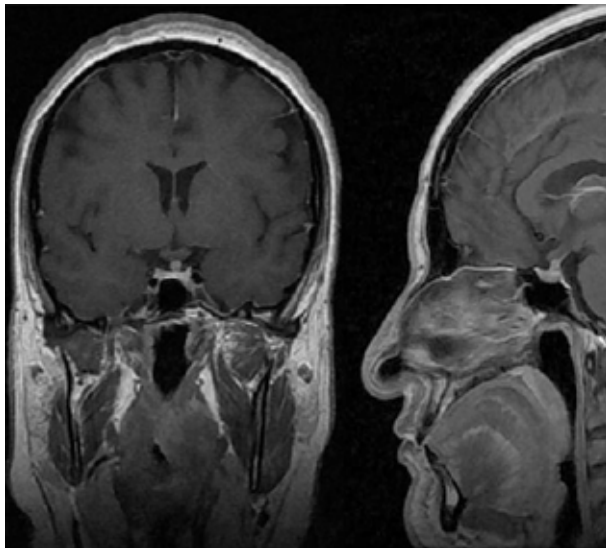
Fig 1: Magnetic resonance imaging (MRI) of the hypophysis shows an area of approximately 4x4 mm focal thickening with contrast enhancement at the distal portion of the infundibular stalk

Fig 3: High resolution computed tomography scanning of the lungs shows multiple, irregularly shaped cystic lesions at the posterior and upper lobes of both lungs, posterior reticulonodular opacities and multiple lymph nodes with max 20 mm diameter at the right paratracheal, pre-carinal region

hypoosmolar urine in routine tests. No hormonal insufficiency was detected in anterior pituitary function tests. Thirst test was compatible with DI without anterior hypophysis failure. Magnetic resonance imaging (MRI) of the hypophysis shows an area of approximately 4x4 mm focal thickening with contrast enhancement at the distal portion of the infundibular stalk (Figure 1). Chest X-ray was considered as normal. She was followed-up under desmopressin treatment for six months.

She was re-evaluated with symptoms such as

lethargy, diplopia, menstrual irregularity, headache and forgetfulness. Control hypophysis MRI shows heterogeneous, contrast enhanced, 18x15 mm nodular thickness that extends through hypothalamus (Figure 2). Hormonal evaluation reveals that her prolactin level was elevated to 92 ng/mL, cortisol response to ACTH stimulation test was normal with 33 mcg/dL highest value of cortisol response and she had secondary hypogonadism. Serum angiotensin converting enzyme, 24 hours' urine calcium excretion, complete blood count, erythrocyte sedimentation rate, ferritin, progesterone, alpha-feto protein and beta-HCG levels were within normal limits.

High resolution computed tomography scanning of the lungs showed multiple, irregularly shaped cystic lesions at the posterior and upper lobes of both lungs, posterior reticulonodular opacities and multiple lymph nodes with max 20 mm diameter at the right paratracheal, pre-carinal region (Figure 3). Positron emission tomography scan for differential diagnosis of lymphoma reveals increased osteoblastic activity at the 6th, 8th and 9th right ribs and in the central part of the left femoral shaft (Figure 4). Bone marrow biopsy was reported as normocellular. Biopsy through thoracoscopic surgery revealed pulmonary Langerhans cell histiocytosis on histopathologic examination with positive CD68, S100 and CD1a staining. No complication was observed during and after the procedure. The thickness progressed to 2.5 cm diameter in control MRI during these diagnostic processes, which lasted six months (Figure 5).

Fig 2: Control hypophysis MRI shows heterogeneous, contrast enhanced, 18x15 mm nodular thickness that extends through hypothalamus
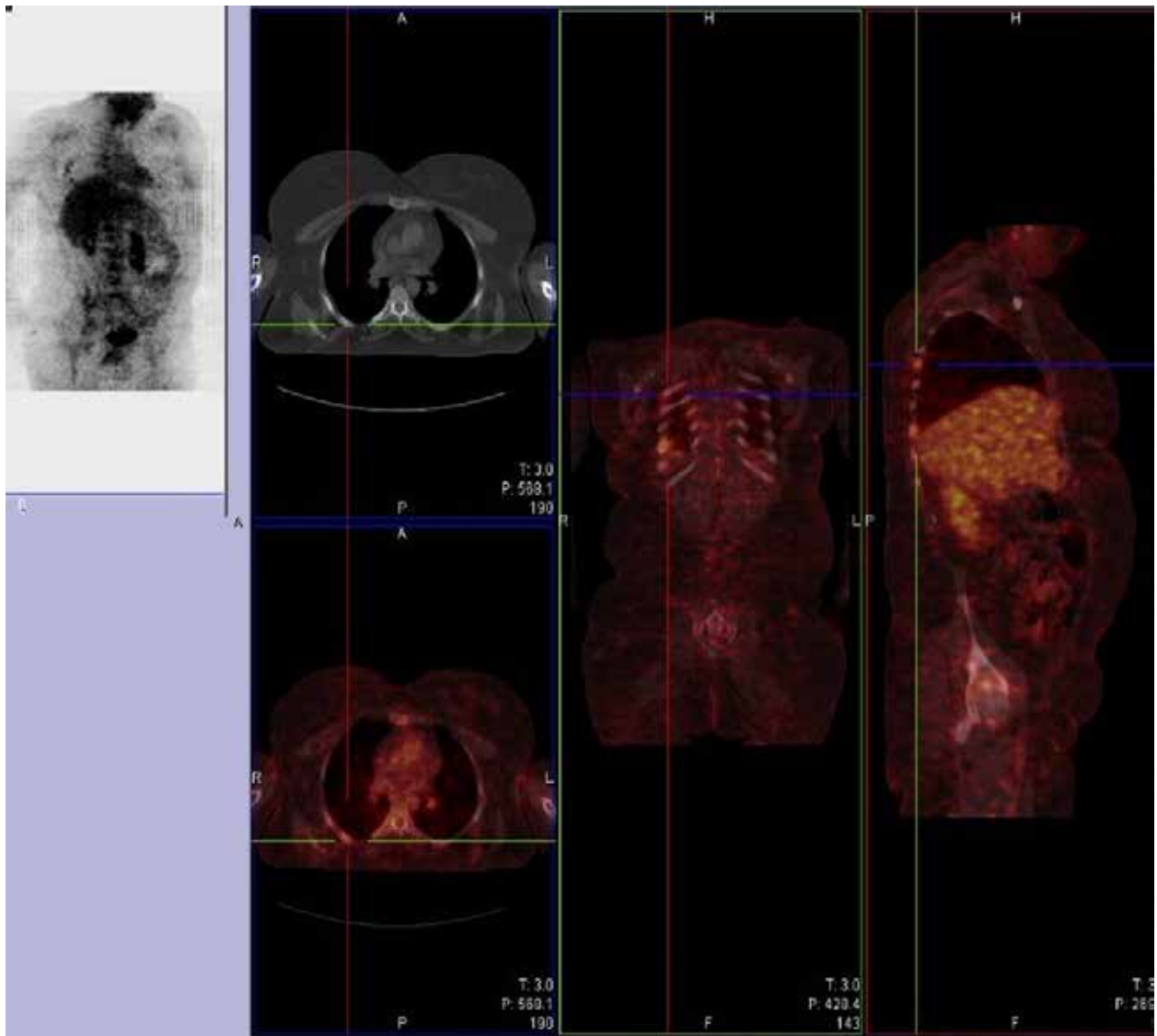
Fig 4: PET-scan for differential diagnosis of lymphoma reveals increased osteoblastic activity at the 6th, 8th and 9th right ribs and in the central part of the left femoral shaft

External radiotherapy and six-cycle of chemotherapy (vinblastine + prednisolone) was planned. In the follow-up, panhypopituitarism was well established and replacement therapy with levothyroxine and prednisolone was added in addition to desmopressin treatment. However, she did not respond clinically to systemic chemotherapy and external radiotherapy and she died due to pneumonia that developed several times.

DISCUSSION

LCH is most often multisystemic and pituitary gland involvement is particularly frequent in adults[10]. Anterior pituitary dysfunction is found in nearly 20% of patients with LCH, and is almost always associated with DI[10]. In case of pituitary gland involvement, DI is

the most common endocrine abnormality, occurring in 12% of children and 30% of adults with LCH[10]. In one of the largest series which included 274 adult patients with biopsy-proven LCH, DI existed in 81 of 188 (43.1%) patients with multisystemic involvement[9].

In a retrospective study recruited during a 50-year period with 314 LCH patients, 44 patients with pituitary-thalamic axis (HPA) LCH was observed and all of them had DI[11]. Kaltsas *et al*[12] followed-up 12 adult multisystemic LCH patients with HPA involvement for 20 years. While eight of them developed some anterior pituitary hormonal deficiencies during the follow-up, they all had DI, which is considered as the earliest hormonal deficiency. Isoo *et al* reported two patients who had DI as the first noted abnormality[13]. Asano T *et al*[14] reported a patient
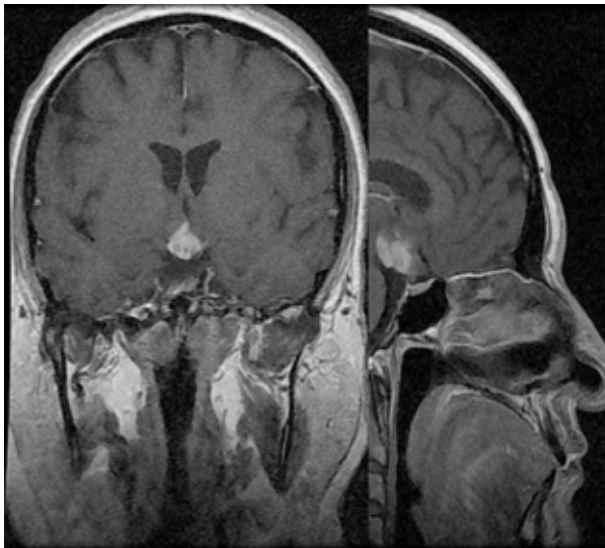
Fig 5: The thickness progressed to 2.5 cm diameter in control MRI during the diagnostic processes which lasted 6 months

in whom DI was the only HPA abnormality. There are several cases presented in the literature that had DI and anterior pituitary deficiencies (APD) at the time of LCH diagnosis[15,16]. APD occurred almost exclusively with DI in the series of seven cases[17]. Radojkovic et al[18] and Tabarin et al[19] reported a case that has pituitary LCH and hypothalamic LCH without any sign and symptoms of DI, respectively. In our case, the patient was diagnosed and treated, but DI progressed rapidly as a multisystemic and progressive disease.

Established DI is generally permanent[9,10]. In a study evaluating the efficacy of chemotherapy in childhood LCH, preexisting central DI persisted after chemotherapy in 23 patients, with the exception of two cases of partial central DI, in which the disappearance of pituitary stalk thickening was observed on MRI and desmopressin was no longer required (complete remission)[20]. This emphasizes the need for early intervention before DI is fully established. It is hypothesized that the pathogenesis of DI is related to autoantibodies against antidiuretic hormone or scarring/ infiltration of the HPA[21]. In autopsy series, granulomatous tissue has been determined on the pituitary gland and stalk, which suggests HPA axis involvement leading to pituitary hormone deficiencies[22]. The progression of hypophyseal mass can also lead to APD, as in our case.

APD is almost always seen in patients with multisystem disease. Arico et al[9] reported that while all patients with DI had multisystemic disease, 43.1% of multisystemic patients had DI. Hence, it is logical to extend diagnostic approach in LCH patients with DI.

DI may be the first clinical finding in LCH patients with systemic involvement. Kurtulmus et al[23] reported that seven of nine patients had DI initially and the other two cases developed DI within three years. Kaltsas et al[12] published that while four of 12 LCH patients had DI initially, the remaining eight patients developed DI over the next 1-20 years (median: 2 years). These studies prove that DI can be used as a clue for investigation of multisystemic disease.

CONCLUSION

LCH should be taken into consideration in patients diagnosed with DI. DI is almost always the hallmark of HPA involvement and a sign of multisystemic involvement. Since there are some cases with isolated DI in LCH, it is usually associated with multisystemic and progressive disease in which early diagnosis and appropriate treatment can affect quality of life and can also prolong life expectancy.

Infiltrative diseases should be considered in the cases presenting with diabetes insipidus. Histiocytosis is a disease with poor prognosis; therefore, early diagnosis is important. It should be kept in mind that lung involvement, one of the typical sites of involvement, cannot be ruled out by X-ray alone.

REFERENCES

1. Vassallo R, Ryu JH, Colby TV, Hartman T, Limper AH. Pulmonary Langerhans'-cell histiocytosis. N Engl J Med 2000; 342(26):1969-1978.
2. Schmitz L, Favara BE. Nosology and pathology of Langerhans cell histiocytosis. Hematol Oncol Clin North Am 1998; 12(2):221-246.
3. Stalemark H, Laurencikas E, Karis J, Gavhed D, Fadeel B, Henter JI. Incidence of Langerhans cell histiocytosis in children: a population-based study. Pediatr Blood Cancer 2008; 51(1):76-81.
4. Baumgartner I, von Hochstetter A, Baumert B, Luetolf U, Follath F. Langerhans'-cell histiocytosis in adults. Med Pediatr Oncol 1997; 28(1):9-14.
5. Malpas JS. Langerhans cell histiocytosis in adults. Hematol Oncol Clin North Am 1998; 12(2):259-268.
6. Badalian-Very G, Vergilio JA, Degar BA, MacConaill LE, Brandner B, Calicchio ML, et al. Recurrent BRAF mutations in Langerhans cell histiocytosis. Blood 2010; 116(11):1919-1923.
7. Badalian-Very G, Vergilio JA, Fleming M, Rollins BJ. Pathogenesis of Langerhans cell histiocytosis. Annu Rev Pathol 2013; 8:1-20.
8. Stockschlaeder M, Sucker C. Adult Langerhans cell histiocytosis. Eur J Haematol 2006; 76(5):363-368.

9.   Arico M, Girschikofsky M, Genereau T, Klersy C, McClain K, Grois N, *et al*. Langerhans cell histiocytosis in adults. Report from the International Registry of the Histiocyte Society. Eur J Cancer 2003; 39(16):2341-2348.

10. Makras P, Alexandraki KI, Chrousos GP, Grossman AB, Kaltsas GA. Endocrine manifestations in Langerhans cell histiocytosis. Trends Endocrinol Metab 2007; 18(6):252-257.

11. Howarth DM, Gilchrist GS, Mullan BP, Wiseman GA, Edmonson JH, Schomberg PJ. Langerhans cell histiocytosis: diagnosis, natural history, management, and outcome. Cancer 1999; 85(10):2278-2290.

12. Kaltsas GA, Powles TB, Evanson J, Plowman PN, Drinkwater JE, Jenkins PJ, *et al*. Hypothalamo-pituitary abnormalities in adult patients with langerhans cell histiocytosis: clinical, endocrinological, and radiological features and response to treatment. J Clin Endocrinol Metab 2000; 85(4):1370-1376.

13. Isoo A, Ueki K, Ishida T, *et al*. Langerhans cell histiocytosis limited to the pituitary-hypothalamic axis--two case reports. Neurol Med Chir (Tokyo) 2000; 40(10):532-535.

14. Asano T, Goto Y, Kida S, Ohno K, Hirakawa K. Isolated histiocytosis X of the pituitary stalk. J Neuroradiol 1999; 26(4):277-280.

15. Ghafoori S, Mohseni S, Larijani B, Mohajeri-Tehrani MR. Pituitary stalk thickening in a case of langerhans cell histiocytosis. Arch Iran Med 2015; 18(3):193-195.

16. Horn EM, Coons SW, Spetzler RF, Rekate HL. Isolated Langerhans cell histiocytosis of the infundibulum presenting with fulminant diabetes insipidus. Childs Nerv Syst 2006; 22(5):542-544.

17. Modan-Moses D, Weintraub M, Meyerovitch J, Segal-Lieberman G, Bielora B. Hypopituitarism in langerhans cell histiocytosis: seven cases and literature review. J Endocrinol Invest 2001; 24(8):612-617.

18. Radojkovic D, Pesic M, Dimic D, Petkovic TR, Radenkovic S, Velojic-Golubovic M, *et al*. Localised Langerhans cell histiocytosis of the hypothalamic-pituitary region: case report and literature review. Hormones (Athens) 2018; 17(1):119-125.

19. Tabarin A, Corcuff JB, Dautheribes M, *et al*. Histiocytosis x of the hypothalamus. Journal of Endocrinological Investigation 2014; 14(2):139-145.

20. Shioda Y, Adachi S, Imashuku S, Kudo K, Imamura T, Morimoto A. Analysis of 43 cases of Langerhans cell histiocytosis (LCH)-induced central diabetes insipidus registered in the JLSG-96 and JLSG-02 studies in Japan. Int J Hematol 2011; 94(6):545-551.

21. Amato MC, Elias LL, Elias J, Santos AC, Bellucci AD, Moreira AC, *et al*. Endocrine disorders in pediatric -onset Langerhans Cell Histiocytosis. Horm Metab Res 2006; 38(11):746-751.

22. Nanduri VR, Bareille P, Pritchard J, Stanhope R. Growth and endocrine disorders in multisystem Langerhans' cell histiocytosis. Clin Endocrinol (Oxf) 2000; 53(4):509-515.

23. Kurtulmus N, Mert M, Tanakol R, Yarman S. The pituitary gland in patients with Langerhans cell histiocytosis: a clinical and radiological evaluation. Endocrine 2015; 48(3):949-956.

Case Report

Parapharyngeal spindle cell rhabdomycosarcoma presenting as infra-auricular pain with vocal cord paralysis

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ABSTRACT

Spindle cell rhabdomyosarcoma is currently difficult to diagnose and is a therapeutic challenge. We report a case of parapharyngeal tumour, spindle cell rhabdomyosarcoma in a 23-year-old woman with a one-month history of infra-auricular pain, who was discovered to have vocal paralysis. Vocal paralysis due to spindle cell rhabdomycosarcoma can be a rare laryngeal problem. To the best of our knowledge, we report the first case of vocal cord paralysis in parapharyngeal spindle cell rhabdomycosarcoma to be treated successfully after surgical intervention. Our experience with this patient also highlights the importance of performing a detailed oral examination in any patient with infra-auricular pain. The unusual clinical presentation of infra-auricular pain could increase the difficulty of a clinical diagnosis. Clinicians should remember that infra-auricular pain can be a symptom of spindle cell rhabdomycosarcoma. An early, correct diagnosis can improve the patient's quality of life and may even save the patient's life.

KEY WORDS: computed tomography, diagnosis, malignant, surgical, tumour

INTRODUCTION

Spindle cell rhabdomyosarcoma (RMS) is a variant of embryonic rhabdomyosarcoma. Spindle cell RMS is a rare and aggressive malignant tumour in adults. The head and neck areas are the most commonly affected regions in adults[1]. Even though the spindle cell RMS in head and neck region had some isolated reports, parapharyngeal spindle cell RMS is a rare entity. The most common initial clinical presentation of spindle cell RMS is a painless, rapidly growing neck mass[2]. In the present report, we discuss a parapharyngeal spindle cell RMS presenting as infra-auricular pain with vocal cord paralysis successfully treated after surgical intervention.

CASE REPORT

A 23-year-old Chinese female with a medical history of glucose-6-phosphate dehydrogenase deficiency was referred to our hospital due to left infra-auricular pain (duration of one month). The patient had been well until one month before admission, when she had left infra-auricular pain, especially when swallowing. Acute parotiditis was initially diagnosed by her primary physician. Oral antibiotics and analgesic medication were prescribed by her primary physician. One month later, she still had left infra-auricular pain with increased pain severity. She was referred to our hospital. A physical examination revealed left infra-auricular tenderness. It revealed no neck swelling and no erythematous skin. Upon oral examination, medial displacement of the left, lateral pharyngeal wall was noted. The laboratory tests were within normal limits. No leukocytosis or anemia was noted, but the differential white blood cell count showed elevated neutrophil segments up to 82.8% (the healthy range is 40%-74%). The C-reactive protein concentration was within the normal limit. Two weeks later, she developed progressive hoarseness and was easily choked. A laryngoscopy revealed left vocal cord paralysis.

A computed tomography scan was performed, which revealed a space occupying lesion in the left parapharyngeal space. The hypo-intense tumour was approximately 3.3*5.2*4.0 cm in size and infiltrated the surrounding structures (Figure 1). No enlarged

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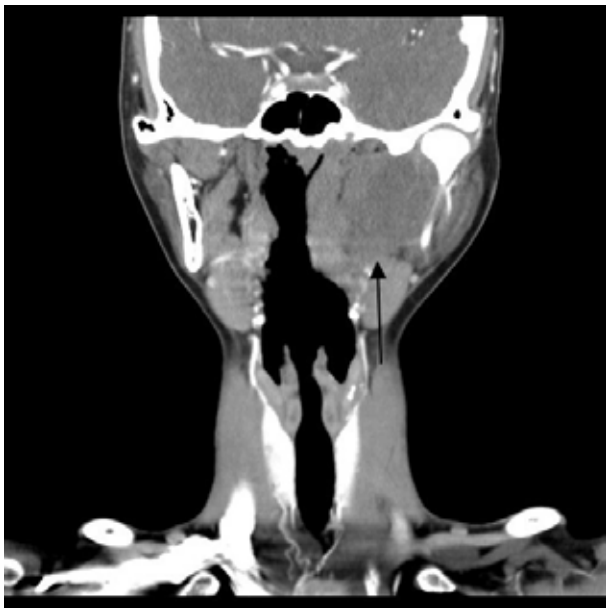
Fig 1: By hypo-intensity, computed tomography scan revealed that the tumor was occupying the left para-pharyngeal space and infiltrated the surrounding structures.

Fig 2: Highly cellular tumour was composed of spindle-shaped cells with hyperchromatic and increased mitotic activity (hematoxylin and eosin, original magnification x 100)

lymph nodes were found in the head or neck region. The left parapharyngeal tumour connected to the deep lobe of the parotid gland. The patient received surgical intervention with a left total parotidectomy, and an external cervical approach extirpated the left parapharyngeal tumour. The operative findings revealed an elastic, fragile tumour located in the left parapharyngeal space and extending toward the skull base. The vagus nerve and hypoglossal nerve were identified and were not involved with the tumour. The parotid gland was not adhered to the tumour. All branches of the facial nerve were preserved with nerve monitoring.

The pathological findings revealed that the whole parotid gland appeared normal and free of tumour invasion. The main tumour was fragile and elastic. Focal haemorrhage and tumour necrosis were observed. Microscopically, the tumour was composed of spindle-shaped cells with hyperchromatic, moderately atypical nuclei with evidence of increased mitotic activity (13/10 high power field, HPF) and a lot of eosinophilic cytoplasm (Figure 2). The arrangement was short-fasciculated or solid architecture with focally hemangiopericytoma-like vasculature. The immunohistochemical studies revealed that the tumour cells were vimentin and desmin positive. The Myo-D1 stain resulted in moderately positive nuclei, and the myogenin stain resulted in positively scattered nuclei. The other stains were negative for myosin, cytokeratin, CK8, CD34, CD99, EMA, S-100. Ki-67 staining revealed that the increased proliferative fraction was approximately 40%. Phosphahistone 3 staining resulted in 13 counts/10 HPF. Pathology

confirmed the diagnosis of grade 3 spindle cell RMS (Fedreation Nationale des Centres de Lutte Contre le Cancer, FNCLCC grading).

The postoperative course was uneventful. One week after the operation, the patient no longer had infra-auricular pain. There was no facial paralysis after the operation. Left vocal paralysis recovered after the operation. She received postoperative radiotherapy.

DISCUSSION

Rhabdomyosarcoma is now classified into three histologic subtypes (embryonic, alveolar and pleomorphic) according to the characteristics. Spindle cell RMS is a variant of embryonic RMS and was first described in 1992 in the paediatric population by the German-Italian Cooperative Sarcoma Study[3]. This subtype presents mainly in children and in some adults, especially males. It occurs predominantly in the paratesticular area followed by the head and neck region. The retroperitoneum, thigh, hand, uvula, heart, thoracic wall, abdominal wall, ureter and uterus have also been reported[1,4,5]. Following the description of this variant, a few small studies reported this tumour in the adult population, and a more invasive clinical course and poor prognosis was noted[1,6]. The clinical presentation is a rapidly growing, painless, soft tissue mass with specific symptoms related to the location and infringement on surrounding structures[2]. In the head and neck region, spindle cell RMS can present with the following symptoms: lump throat, hoarseness, dysphagia, obstructive sleep apnea syndrome, diplopia, unilateral deafness, proptosis and sinusitis[2]. However, intolerable infra-auricular pain with vocal paralysis had not been previously reported.

The parapharyngeal tumours account for approximately 0.5% of head and neck tumours. Of these, 80% are benign and 20% are malignant[7]. The

parapharyngeal space is a deep neck space extending from the skull base to the hyoid bone. The space is divided into two compartments by the styloid process and muscles. The bony structures, including the skull base, mandible and vertebrae, confine the space and the tumour only can grow in a medial and inferior direction[7]. The common symptoms of parapharyngeal tumours are lump throat, dysphagia, hoarseness, dyspnea and a painless mass[7].

The spindle cell RMS tumour is typically a firm, white to tan mass with a whorled appearance[2]. However, in this patient, the tumour was fragile and elastic. This atypical finding may have been due to the massive tumour necrosis (40%) and haemorrhage. Microscopically, the spindle cell RMS was composed of long fascicles with elongated spindle cells with hyperchromatic nuclei and pale eosinophilic cytoplasm and occasional rhabdomyoblasts[1]. Spindle cell RMS (especially in the head and neck region) should be considered during the differential diagnosis of nodular fasciitis, malignant nerve sheath tumour, spindle cell sarcoma, spindle cell malignant melanoma, leiomyosarcoma and myofibrosarcoma. The spindle cell RMS originated from the skeletal muscle, and the tumour reacted with myogenic markers such as desmin, myogenin and MyoD1. Negative reactivity for S-100, CD-99, CD-34, h-caldesmon and glial fibrillary acidic protein facilitated the differential diagnosis from other sarcomas[2].

The prognosis of patients with spindle cell RMS varies in adults and paediatric patients. The standard treatment protocol is a combination of surgery, chemotherapy and adjuvant radiotherapy[2,8]. Paediatric spindle cell RMS usually arises in the paratesticular region. With early detection and adequate surgical margins, paediatric patients have a good prognosis. However, in adults, spindle cell RMS usually arises from the head and neck region, and the patients have a poor prognosis due to difficulties in obtaining adequate surgical margins[1,2]. Adult spindle cell RMS with uncontrolled local recurrence and distant metastasis has been noted postoperatively[2]. The one-year survival rate for adults with spindle cell RMS is only 53%[1,4].

CONCLUSION

A Pubmed search (1970 to the present) revealed no reports of scenario and management similar to this patient. To the best of our knowledge, we herein report the first case of parapharyngeal spindle cell RMS presenting as infra-auricular pain with vocal cord paralysis to be treated successfully after surgical intervention. The unusual clinical presentation of infra-auricular pain could increase the difficulty of a clinical diagnosis. Patients should be carefully examined in the oral and oropharynx when presenting with intolerable infra-auricular pain to accurately diagnose these patients. Clinicians should remember that infra-auricular pain can be a symptom of spindle cell RMS. Our patient highlights the importance of performing a detailed oral examination in patients with infra-auricular pain. An early, correct diagnosis can improve the patient's quality of life and may even save the patient's life.

REFERENCES

1. Nascimento AF, Fletcher CD. Spindle cell rhabdomyosarcoma in adults. Am J Surg Pathol 2005; 29(8):1106-1113.
2. Carroll SJ, Nodit L. Spindle cell rhabdomyosarcoma: a brief diagnostic review and differential diagnosis. Arch Pathol Lab Med 2013; 137(8):1155-1158.
3. Cavazzana AO, Schmidt D, Ninfo V, Harms D, Tollot M, Carli M, et al. Spindle cell rhabdomyosarcoma. A prognostically favorable variant of rhabdomyosarcoma. Am J Surg Pathol 1992; 16(3):229-235.
4. Stock N, Chibon F, Binh MB, Terrier P, Michels JJ, Valo I, et al. Adult-type rhabdomyosarcoma: analysis of 57 cases with clinico-pathologic description, identification of 3 morphologic patterns and prognosis. Am J Surg Pathol 2009; 33(12):1850-1859.
5. Kim DW, Shin JH, Lee HJ, Hong YO, Joo JE, Kim EK. Spindle cell rhabdomyosacoma of uterus: a case study. Korean J Pathol 2013; 47(4):388-391.
6. Rubin BP, Hasserjian RP, Singer S, Janecka I, Fletcher JA, Fletcher CD. Spindle cell rhabdomyosarcoma (so-called) in adults: report of two cases with emphasis on differential diagnosis. Am J Surg Pathol 1998; 22(4):459-464.
7. Freije JE, Gluckman JL, Biddinger PW, Wiot G. Muscle tumors in the para-pharyngeal space. Head Neck 1992; 14(1):49-54.
8. Mohamed KA, Marwan NA, Kenneth CK. Rhabdomyosarcoma of the urinary bladder in an adult. Kuwait Med J 2008; 40(1):70-71.

Case Report

Treatment of bilateral patellar dislocation after bilateral total knee arthroplasty of a patient with Parkinson's disease: A case report

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ABSTRACT

We present a case of a Parkinson's disease (PD) patient who sustained bilateral lateral patellar dislocation shortly after bilateral total knee arthroplasty (TKA). Prior to admission to our hospital, he underwent several surgical reduction attempts in other health centers. His Modified Hoehn and Yahr scale was 4. Surgical procedures included medial patellofemoral ligament (MPFL) reconstruction and Insall procedure. Non-absorbable polyethylene multifilament suture was used to repair the soft tissue. Two months postoperatively, the patient sustained fracture of the right patella due to falling. Patella fracture occurred at the tunnel level that was drilled for MPFL reconstruction. The fracture was treated with the tension band technique. After two years of follow-up, he was able to walk with the help of a walker. His Knee Society Score and Womac Score improved from preoperatively 6 to postoperatively 52, and from preoperatively 5.5 to postoperatively 52.3, respectively. PD may negatively affect the medial retinaculum and tendon healing because of muscular rigidity, weakness and imbalance. Use of non-absorbable polyethylene multifilament sutures may be more helpful for healing of medial structures of patella in TKA surgery in patients with neurodegenerative diseases such as PD.

KEY WORDS: bilateral patellar dislocation, bilateral total knee arthroplasty, Parkinson's Disease, patellar fracture

INTRODUCTION

Patellar dislocation after total knee arthroplasty (TKA) surgery is a rare disease which causes difficulty in walking[1]. Dislocation of the patella may be due to insufficient surgical technique and component malposition, disruption of the extensor mechanism, high Q angle and neuromuscular disorders[2]. Patellar dislocation is one of the revision surgery causes in TKA[3]. In their study with 142 patients, Pećina *et al* reported that revision surgery was performed in three patients because of patellar instability[3]. Parkinson's disease (PD) is a neurodegenerative disorder that affects predominately dopamine-producing (dopaminergic) neurons in a specific area of the brain called substantia nigra. Tremor (mainly at rest), bradykinesia, limb rigidity, muscle weakness, gait and balance problems are the most common symptoms that patients suffer

from[4]. As it is very well known, patella is stabilized in knee joint by vastus medialis, vastus lateralis muscles and the medial patellofemoral ligament (MPFL). During TKA surgery, medial structures of patella as patellar insertion of MPFL, medial retinaculum and vastus medialis muscle tendon are cut and sutured routinely with absorbable sutures. In this study, we present a report with two years follow up of a PD patient who was surgically treated with the diagnosis of bilateral lateral patellar dislocation after TKA, and patellar fracture complication after our patella reduction surgery.

CASE REPORT

A 61-year old male patient with PD for 12 years was admitted to Orthopedics and Traumatology polyclinic with complaints of inability to walk, limited range of

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Fig. 1a-b. Bilateral patellar lateral dislocation. Arrows show the dislocated patellas. a) Preop clinical appearance b) Radiology images.

motion (ROM) of both knees, inability to lift straight legs and pain. He had bilateral TKA nine months ago (Smith & Nephew, TC-PLUS™ PRIMARY). There was no history of trauma. Within one month of TKA surgery, patellar dislocation in both knees occurred. He never had patellar dislocation prior to TKA. Due to bilateral patellar dislocation, the patient had soft tissue interventions performed for the right knee twice and to the left knee once. It was noted in his discharge summary that he had TKA in the same surgical session via medial parapatellar approach to both knees. After one month, he had medial retinacular repair in both knees. One month later, he again had patellar dislocation at his right knee. Therefore, the medial retinacular repair surgery was repeated, but his patellar dislocation at both knees repeated again within one month. As stated in his discharge summaries, the medial retinacular repairs were done via absorbable suture no:2 vicryl surgery as a routine application.

During examination, the patient could not lift his legs straight. Both patellas were laterally dislocated (Fig 1a). Passive ROM of his knees was between 30°-100° and the active ROM was 50°-90°. Passive and active ROM of the patient was observed to be painful. In clinical (Fig 1a) and radiological (Fig 1b) examinations, the patellas were fixed at lateral side of the knee. With palpation, there was a gap between the tendo vastus medialis and the patella. Based on the normal clinical examination results and laboratory values, we did not think of infection at the knees. The valgus malalignment and rotational malalignment are frequently the causes of the patella dislocation after TKA. During preoperative examinations, we observed that the patient was not able to extend his knees because of patellar dislocation and pain sensation. For this reason, we could not take a full-length X-ray of the lower limb of the patient. However, we evaluated the possible rotational malalignment through computed tomography of his knees. In radiological examination,

we observed no malrotation at the components of arthroplasty. Being a PD patient for 12 years, he had tremor, bradykinesia and limb rigidity. He did not use his medications regularly. His Modified Hoehn and Yahr scale value, a scale to present the stage of PD, was 4 (Table 1)[5]. Before surgery, his Knee Society Score was 6 and Womac Score was 5.5.

Table 1: Modified Hoehn and Yahr Scale staging of Parkinson's disease[5]

Stage	Description
0	No sign of disease
1	Unilateral disease
1.5	Unilateral plus axial involvement
2	Bilateral disease without involvement of balance
2.5	Mild bilateral disease, with recovery on pull test
3	Mild to moderate bilateral disease, some postural instability, physically independent
4	Severe disability, still able to walk or stand unassisted
5	Wheel chair bound or bed ridden unless aided

Under spinal anesthesia, both knees were entered through the prior incisions. It was observed that the musculus vastus medialis was not inserted into the patella. Both patellas had lateral retinacular release. After the lateral release, patella was placed into the trochlear groove of femoral component. Then, the Q angle between the quadriceps tendon and the patellar tendon was measured (Fig 2). The possible malalingment of lower extremity was evaluated by cable method under fluoroscopy intraoperatively[6]. We observed the alignment of lower extremities within the normal limits. Then, proximal soft tissue realignment surgery previously described by Insall et al[7] and Efe et al[8], and MPFL reconstruction were conducted. Medial parapatellar incision was performed around the patella from the upper edge of the tendo vastus medialis in the quadriceps tendon to the tibial tubercle. During the examination of patella, we noticed that the central and medial facets of the patella were crushed (Fig 1b). The thickness of

Fig 2. Intraoperative image. Lateral retinaculum loosened, patella groove reduced and tendon allograft prepared in "Y" form

Fig 3. Intraoperative image. Reconstruction of the MPFL and repaired vastus medialis

the patella was measured as 17 mm with the help of calipers intraoperatively.

The patella was placed into the groove. A fresh frozen allograft (Community Tissue Services, USA) of tibialis anterior tendon was prepared in "Y" shape (Fig 2). A tunnel was opened under fluoroscopy in the femur between the adductor tubercule and medial collateral ligament in accordance with inserting the medial collateral ligament. Two tunnels were opened to the patella for fixing it by the branches of the tendon allograft. The tendon allograft was fixed in the femoral tunnel with a bioabsorbable screw (Biorci screw, 10*25 mm, Smith & Nephew, USA).

The branches of the tendon were passed through the tunnels opened in the patella and fixed with a nonabsorbable polyethylene multifilament suture (no:2, Smith & Nephew, USA). MPFL reconstruction was performed with tibialis anterior tendon allograft. Then, medial retinaculum and tendo vastus medialis were pulled laterally and distally for at least 1-1.5 cm. Tendo vastus medialis repair was done via one 6.5 mm titanium anchor (Smith & Nephew, USA) to the patella and nonabsorbable polyethylene multifilament suture (no:2, Smith & Nephew, USA) (Fig 3). It was observed that the patella was in the groove, no maltracking was seen,
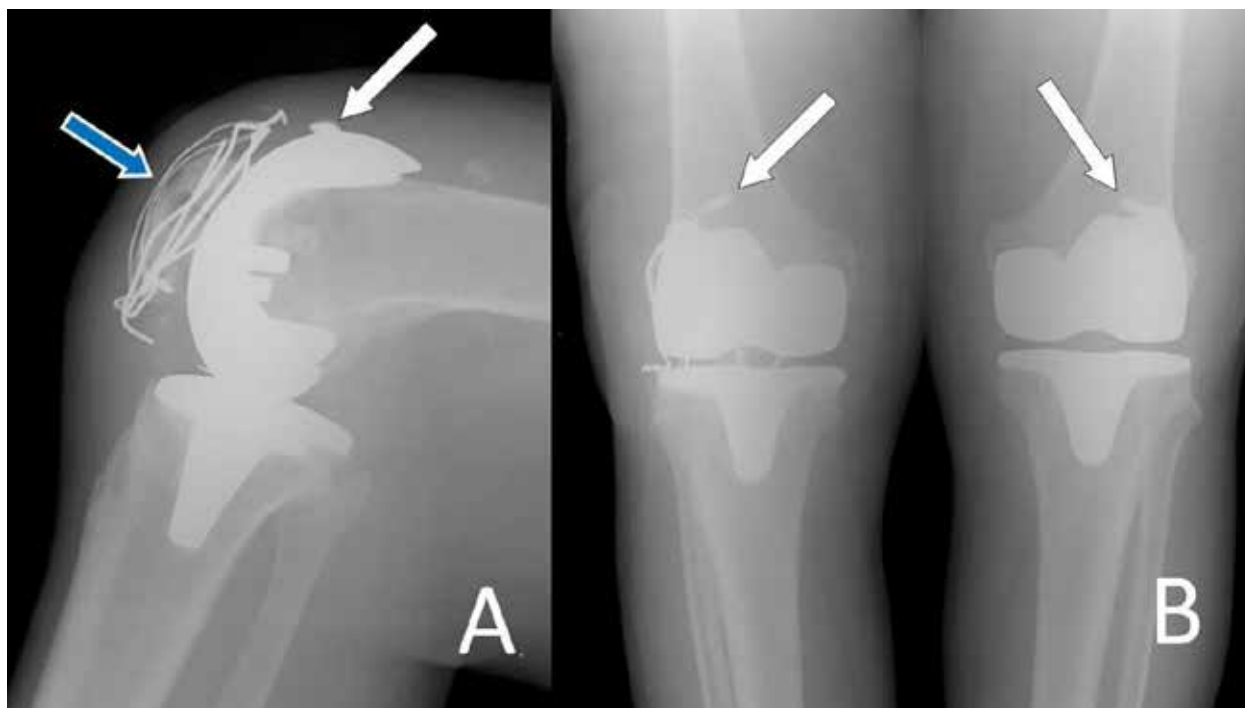
Fig 4a-b. White arrows show the dissociated anchors from patellas in both views. a) Lateral X-ray image of patella fracture treated with tension band technique after surgery. Blue arrow shows the fracture line of the patella passes through the tunnel. b) Anteroposterior view of the knees.

and there was no lateral or medial tilt (Fig 3). After surgery, rehabilitation involved an angle adjustable brace. After the surgery, knee ROM were free for rehabilitation except while walking. He was able to walk with a walker while the brace was locked at knees in extension. After the 6th week, the angle-adjustable brace was removed and he was able to walk with the walker.

Two months after the operation, the patient was referred to the polyclinic after falling on his right knee. The patient had swelling, redness and inability to lift a straight leg of the right knee. Radiological tests revealed patella fracture. The fracture line of the patella was observed to be at the tunnel level opened for the graft (Fig 4a). A tension band surgery was performed for the patella (Fig 4a, b). After the surgery, free ROM of knee was allowed. The patient was mobilized with a brace while the knee is in extension and with a walker. After six weeks, the angle-adjustable knee brace was removed.

After two-year follow-up, the patient was ambulating with a walker. Passive knee ROM was 0-110 degrees and the active knee ROM was 10-100 degrees. On the last follow-up, Knee Society Score was 52 and Womac Score was 52.3.

DISCUSSION

In the present case, medial retianculum, tendo vastus medialis and MPFL, those ensure that the patella remains in the trochlear groove, were separated from the patella, after bilateral TKA surgery. Based on laboratory and radiological findings, there was no malalignment of lower extremity, malposition of components and infection. Our patient had idiopathic PD for 12 years. PD is known for muscle weakness and imbalance[4,9]. Since medial parapatellar approach was used in TKA surgery of our patient, the force of medial vectors depended on the sutures only until the medial retinaculum and vastus medialis tendon healed.

According to discharge summary, the suture used in the TKA surgery for closing the medial structures was no:2 vicryl, and it is absorbed before complete wound healing. The patient was operated twice previously because of patellar dislocation. The technique used in previous patella reduction surgeries was only medial retinacular repair done with no:2 vicryl. The cause of fail at these surgeries may be because the no:2 vicryl was resolved before the healing of medial retinaculum and tendo vastus medialis. However, in patella reduction surgeries, non-absorbable no:2 Ethibond, which is known to be biomechanically stronger than vicryl[10], was used. The cause of fail at the patella reduction surgeries may be related to the soft tissue problems as decreased soft tissue volume is not enough for tightening. Another possible cause of fail at

medial retinaculum repair may be the short intervals (one month) between surgeries because at the early period of tissue repairs, the soft tissue weakens and its quality decreases[11]. We did not face any tissue quality decrease because we did patellar realignment surgery seven months after the last patella reduction surgery.

Based on the anamnesis and discharge summary, we decided to use MPFL reconstruction and proximal realignment surgery at the presurgical planning period as previously described[7,12], by using nonabsorbable polyethylene multifilament suture. Additionally, we used anchor for the fixation of vastus medialis tendon to patella. After the surgery, the anchor failed and dissociated from patella as seen on X-ray films (Fig 4 a, b). This dissociated anchor may be related to the muscle imbalance at PD.

TKA is not commonly suggested for PD patients with Modified Hoehn and Yahr scale score over 3[5]. In our case, however, Modified Hoehn and Yahr scale score was 4 and the surgery for reconstruction was indicated because of the extensor mechanism failure. In this case report, we present two years follow-up of the treatment of a patient with bilateral patellar dislocation after bilateral TKA in the presence of muscle imbalance accompanied by PD. Patellar dislocation or refracture of patella did not occur. The patient was able to move with a walker. A clear improvement was observed in Knee Society Score (from 6 to 52) and Womac knee score (from 5.5 to 52.3). PD is a serious disease and if the patient has this diagnosis, TKA surgery decision must be taken both by the patient and by the neurologist. A previous case report from Croatia presented a periprostethic knee recurrent dislocation at a PD patient nine months after surgery[4]. Erceg et al interpreted the dislocation of knee due to muscle weakness caused by PD and the authors treated the patient with a revision surgery[4]. We could not find any information about what sutures were used in primary and revision surgeries for closing the wound in their case report.

In our literature search, we did not encounter any report of a patient with both patella dislocated after TKA linked to muscle weakness after the neurodegenerative disease of PD and the surgical treatment results. We believe our report of the bilateral patella dislocation after TKA is a rare case presentation comparing to currently accessible literature. This case emphasizes the importance of soft tissue balance and the sturdy repair with nonabsorbable sutures in TKA surgery with PD.

CONCLUSION

Using nonabsorbable polyethylene multifilament sutures for repairing the medial patellar structures such as retinaculum/tendon during TKA surgeries on

patients with neurodegenerative diseases such as PD, may be more helpful during healing process.

ACKNOWLEDGMENT

REFERENCES

1. Rozell JC, Voleti PB, Israelite CL. Patellar dislocation after total knee arthroplasty for neglected chronic post-traumatic patellar dislocation: A case report. University of Pennsylvania Orthopaedic Journal 2015; 25:75-78.

2. Motsis EK, Paschos N, Pakos EE, Georgoulis AD. Review article: Patellar instability after total knee arthroplasty. J Orthop Surg (Hong Kong) 2009; 17(3):351-357.

3. Pećina M, Djapić T, Haspl M. Survival of cementless and cemented porous-coated anatomic knee replacements: retrospective cohort study. Croat Med J 2000; 41(2):168-172.

4. Erceg M, Maricević A. Recurrent posterior dislocation following primary posterior-stabilized total knee arthroplasty. Croat Med J 2000; 41(2):207-209.

5. Ashraf M, Priyavadhana S, Sambandam SN, Mounasamy V, Sharma OP. Total knee arthroplasty in patients with parkinson's disease- A critical analysis of available evidence. Open Orthop J 2017; 11:1087-1093.

6. Chan CK, Shanmugam R. A simple cable method for intraoperative limb alignment assessment. Malays Orthop J 2016; 10(1):69-70.

7. Insall J, Falvo KA, Wise DW. Chondromalacia patellae. A prospective study. J Bone Joint Surg Am 1976; 58(1):1-8.

8. Efe T, Seibold J, Geßlein M, Schüttler K, Schmitt J, Schofer MD, *et al.* Non-anatomic proximal realignment for recurrent patellar dislocation does not sufficiently prevent redislocation. Open Orthop J 2012;6:114-117.

9. Kakinuma S, Nogaki H, Pramanik B, Morimatsu M. Muscle weakness in Parkinson's Disease: isokinetic study of the lower limbs. Eur Neurol 1998; 39(4):218-222.

10. Vavken P, Proffen B, Peterson C, Fleming BC, Machan JT, Murray MM. Effects of suture choice on biomechanics and physeal status after bioenhanced anterior cruciate ligament repair in skeletally immature patients: a large-animal study. Arthroscopy 2013; 29(1):122-132.

11. Dargel J, Gotter M, Mader K, Pennig D, Koebke J, Schmidt-Wiethoff R. Biomechanics of the anterior cruciate ligament and implications for surgical reconstruction. Strategies Trauma Limb Reconstr 2007; 2(1):1-12.

12. Zaffagnini S, Dejour D, Grassi A, Bonanzinga T, Muccioli GM, Colle F, *et al.* Patellofemoral anatomy and biomechanics: current concepts. Joints 2013; 1(2):15-20.

<div align="center">Case Report</div>

Oliver Sacks syndrome treated with adaptation to hearing aid

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ABSTRACT

An eighty-year-old woman was diagnosed with bilateral severe sensorineural hearing loss in audiometric examination and recommended to use hearing aids. The patient could not adapt to the device and was reluctant to wear it. In the psychiatric examination, it was difficult to communicate with the patient due to hearing loss. No delusions were found in the thought content. When hallucinations were asked about, she noted resonance and humming in the early periods that later turned to helicopter, airplane sound, and as hearing loss progressed, she heard wedding music. The patient had full insight that the sound was coming from her head. The psychiatric treatment was useful for adjustment to hearing aid and maintaining acoustic stimulation. As hearing devices were used, the ambient sounds increased, and the musical hallucinations were slowed down and communication with her relatives increased.

KEY WORDS: audiometry, hearing aid, hearing loss, musical hallucination

INTRODUCTION

Auditory hallucinations can be seen either in elementary forms such as resonance and buzzing or in complex forms such as human voices or music. Musical hallucinations have been defined as Oliver Sacks syndrome; they are a form of cortical release phenomenon in hearing loss and in this context they are also described as auditory Charles Bonnet syndrome[1].

CASE REPORT

An eighty-year-old woman was brought to the psychiatry outpatient unit of the health board by her children for investigation about disability. On the wheelchair, she seemed a little bit surly. She was overweight and had been diagnosed with diabetes mellitus, essential hypertension and gonarthrosis during the last 25 years. She was well controlled with antidiabetic and antihypertensive drugs. For the past three years, she could not hear what others were saying in conversations properly and she had the television on a high volume all the time. She was brought to an otorhinolaryngologist three months ago and was diagnosed with bilateral severe sensorineural hearing loss according to the audiometry tests and it was suggested to use hearing aids. The patient could not adapt to the device and did not want to wear it. Her children noticed that she had recently become more uncomfortable and stubborn, talked less with her relatives, occasionally cried and had difficulty sleeping at night. There was no previous history of psychiatric disorders. She lost her husband five years ago. She lived with her children, their spouses and her grandchildren. Her appearance seemed compatible with her chronological age. It was difficult to communicate with the patient during the mental status examination due to hearing loss. When she could hear and understand the questions asked, she was responding a little bit reluctantly. She was oriented to place, person and time. No delusions were found in her thought content. When hallucinations were questioned, she confirmed that there were noises in

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her ears. When she was asked about the voices, it was learned that in the early periods of the hearing loss, the voices were in the form of resonating and humming. Later, they converted to a helicopter, airplane noise and progressively to halay music which is composed of drum and clarion sounds played at the weddings in the region she lived in. She said that the sounds were heard from both ears, increasing at quieter times such as nighttime.

A detailed audiometric examination including Bekesy and Sisi scores was done. Bekesy audiometry is based on a comparison of thresholds for constant and intermittent the tones. Clinical importance has been attached to the measurement of the threshold excursions. Short Increment Sensitivity Index test measures the ability of a subject to detect a 1 dB change in intensity at a level 20 dB above threshold. This test can be used at any frequency in either ear, regardless of the asymmetry of the hearing loss[2]. There was severe sensorineural hearing loss in both ears plus air conduction deficits were noted on the left (right ear speech reception threshold [SRT] 65 dB, discrimination 40% dB 90; left ear SRT 80 dB, discrimination 40% dB 100). Tympanograms were type A and acoustic reflexes were absent bilaterally. The music was less with hearing aids, but the device was very uncomfortable. The music noise could be suppressed by television or radio noise. Different melodies and folk songs were able to change her musical hallucination temporarily. Halay sound was not as uncomfortable as the device's. The patient had good insight that the sound was coming from inside her head.

The cranial magnetic resonance imaging of the patient revealed mild cortical atrophy and widening of the sulci. Routine hemogram and biochemistry values were in the normal range. Hepatitis, AIDS, syphilis, and brucellosis were excluded by serological tests. The electroencephalogram was normal. In neuropsychological tests, simple attention can be considered immediate memory or very short-term memory. Complex attention defines functions based on sustaining attention; in the event of deterioration, there is deterioration in persistence (persistence), perseverations, distractibility, reduced resistance to interference, and difficulty in suppressing the immediate, but unfavorable, tendency to react. These functions, which are based on complex considerations, are called executive or executive functions[3]. Short-term memory, simple and complex attention were preserved in our patient. There was minimal age-compatible cognitive impairment. She had a depressed mood most of the day, nearly every day, diminished interest and pleasure in almost all activities, psychomotor retardation, fatigue and insomnia, yet she did not have suicidal thoughts or tendencies. Thus,

she was diagnosed with major depressive disorder according to DSM-V. In order to exclude malingering, confirm the diagnosis of depression and to learn antidepressant treatment response, the decision of the board was postponed for six months with once a month outpatient follow-up visits.

Escitalopram 5 mg/day and trazodone 50 mg/day were prescribed for the treatment of depression and insomnia respectively. She was supported by her family to wear her hearing aids starting with one hour a day and to increase gradually. At the third-month outpatient visit, the trazodone was discontinued because the sleeping problem had disappeared. As hearing devices were used, the ambient sounds increased; thereby the sensory input suppressed myriads of nonessential information, including previously acquired memories. The musical hallucinations regressed and communication with her relatives increased. At the sixth-month follow-up visit, escitalopram was discontinued.

DISCUSSION

The diagnosis of dementia was ruled out because the patient's cognitive changes were compatible with her age. Psychotic depression was ruled out as a differential diagnosis due to the fact that the musical hallucinations reflecting past experiences were not congruent with depressive mood (Halay voice did not include a sad, pessimistic, critical or accusative theme), though she had major depressive disorder with melancholic features. It was thought that auditory hallucinations were due to hearing loss because they started at similar times with the hearing loss and they increased as the hearing loss progressed.

Sensory inputs suppress much of the information that is not necessary, such as previous memories. The 'Perceptual Release Theory' of West[4] influenced by Hughlings-Jackson's concept of disinhibition suggests that when a certain amount of external sensory stimulation does not go to the area of the cerebral cortex responsible for hearing (in the temporal region of the non-dominant hemisphere) due to deafness or isolation, the acoustic stimuli of recorded memories are transmitted and perceived again with familiar or new and strange forms[5].

Peripheral sensory organ damage by anatomical and physiologic pathways may cause central perceptions which can also be seen in Charles Bonnet and Phantom Limb syndromes. Charles Bonnet Syndrome, in which visual hallucinations occur due to loss of sight, is more similar by its origin to auditory hallucinations of patients with hearing loss. In addition, it has been reported that musical hallucinations are lateralized to the ear that cannot hear in a person with unilateral deafness[6].

The two-factor theorists who advocate that both peripheral and central dysfunction should be present, as evidence, suggest that such hallucinations occur more frequently in older adults with a high incidence of brain pathology[7].

The main risk factors for musical hallucinations are impaired hearing, tinnitus, advanced age and, perhaps, also female sex; however, the latter finding may be due to an overrepresentation of females in the literature[8].

There is no consensus for the treatment of disturbing musical hallucinations and decision is made usually according to underlying etiology. In a review, among pharmacological treatment methods investigated, antidepressants were found to be possibly more helpful than antiepileptics (which are still better than antipsychotics)[9]. The limited use of acetylcholinesterase inhibitors was promising[10]. Musical hallucinations experienced in the context of brain injuries and epilepsy tend to respond well to antiepileptics. Musical hallucinations occurring as part of a psychiatric disorder tend to respond well to psychopharmacological treatments targeting the underlying disorder.

As in our case, the hypoacusis etiological group appears to respond to hearing impairment treatment. More acoustic stimulation suppresses the hallucination (e.g. our patient increased the volume of the television or music)[9].

Not all adults provided with hearing aids use them, wear them regularly, or are satisfied with them[11].

We used antidepressant treatment in this patient for depressive disorder and easing adjustment to hearing aid use thereafter. In contrast to the report by Serrador-García *et al*, our patient had a complete response; musical hallucination did not occur when taking hearing aid out at night[12].

CONCLUSION
Adjustment to hearing loss and hearing aids become much more difficult with increasing age and psychiatrists can help these people by means of dealing with this adjustment disorder.

ACKNOWLEDGMENT
None to declare.

REFERENCES

1. Colon-Rivera HA, Oldham MA. The mind with a radio of its own: a case report and review of the literature on the treatment of musical hallucinations. Gen Hosp Psychiatry 2014; 36(2):220-224.
2. Thomas WG. Clinical assessment of auditory dysfunction. Environmental Health Perspectives 1982; 44:67-75.
3. Öktem Ö. Nöropsikolojik Testler ve Nöropsikolojik Değerlendirme. Türk Psikoloji Dergisi -33:(33)9 ;1994 44.
4. West LJ. A clinical and theoretical overview of hallucinatory phenomena. In: Siegel RK, West LJ, editors. Hallucinations: Behavior, experience and theory. New York: John Wiley; 1955; p. 301-302.
5. Miller TC, Crosby TW. Musical hallucinations in a deaf elderly patient. Ann Neurol 1979; 5(3):301-302.
6. Fisman M. Musical hallucinations: Report of two unusual cases. Can J Psychiatry 1991; 36(8):609-611.
7. Hammeke TA, McQuillen MP, Cohen BA. Musical hallucinations associated with acquired deafness. J Neurol Neurosurg Psychiatry 1983; 46(6):570-572.
8. Sacks O, Blom JD. Musical hallucinations. In: Blom JD, Sommer IEC, editors. Hallucinations. Research and Practice. NewYork: Springer; 2012; p. 133-142.
9. Coebergh JAF, Lauw RF, Bots R, Sommer IEC, Blom JD. Musical hallucinations: review of treatment effects. Front Psychol 2015; 6:814.
10. Blom JD, Coebergh JAF, Lauw R, Sommer IE. Musical hallucinations treated with acetylcholinesterase inhibitors. Front Psychiatry 2015; 6:46.
11. Knudsen LV, Oberg M, Nielsen C, Naylor G, Kramer SE. Factors influencing help seeking, hearing aid uptake, hearing aid use and satisfaction with hearing aids: A review of the literature. Trends Amplif 2010; 14(3):127-154.
12. Serrador-García M, Santos-Bueso E, Sáenz-Francés F, Díaz-Valle D, Martínez- De-La-Casa-Borrelia JM, García-Feijóo J. Charles Bonnet plus syndrome: a propos of a case. Eur J Ophthalmol 2012; 22(5):836-839.

Case Report

Hyalinizing trabecular tumor of the thyroid gland: A case report and review of the literature

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ABSTRACT

A hyalinizing trabecular tumor (HTT) is a rare and controversial thyroid gland tumor with an extremely low malignant potential. Its cytological and histological features overlap with those of papillary thyroid carcinomas (PTCs) and medullary thyroid carcinomas (MTCs). Hence, diagnosis of this entity can be challenging, especially when diagnosis is based on fine needle aspiration cytology (FNAC). Herein, we report the case of a 52-year-old woman with HTT along with the cytological, histological and immunohistochemical studies performed. A follicular origin for the tumor was confirmed based on immunohistochemistry, which showed that the tumor cells were positive for thyroglobulin and TTF-1 and negative for calcitonin. The tumor cells were also negative for HBME-1, galactin-3 and CK19, but they were positive for CD56. The preferred diagnosis based on the histological and immunohistochemistry studies was an HTT. The patient is currently undergoing regular follow-up visits and has shown no evidence of residual tumor or metastasis. This case highlights the importance of considering HTT when interpreting FNAC results when the cytological features are those of PTC or MTC, but the ultrasonography findings are not worrisome.

KEY WORDS: carcinoma, cytology, immunohistochemistry, papillary, trabecular

INTRODUCTION

The thyroid gland is commonly studied by researchers, especially after the announcement in 2016 of the new entity called non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)[1]. A hyalinizing trabecular tumor (HTT) of the thyroid gland is a rare non-invasive follicular cell-derived thyroid neoplasm[2]. HTT is included in the WHO classification of tumors of the endocrine organs, which defines this lesion as a tumor with a trabecular pattern of growth and intra-trabecular hyalinization[3]. While a RAS mutation is the most common mutation associated with NIFTP[4], a rearranged during transfection / papillary thyroid carcinoma (RET/PTC) mutation is more likely to be identified in HTT[5]. The cytological identification of this tumor based on fine needle aspiration cytology (FNAC) is extremely difficult and even impossible in some cases, as these tumors share major cytopathological features with papillary thyroid carcinomas (PTCs) and medullary thyroid carcinomas (MTCs), such as nuclear grooves, pseudo-inclusions and abundant cytoplasm. Here, I describe my experience with a case of HTT and the process by which it was diagnosed.

CASE REPORT

The patient was a 52-year-old woman with a six-year history of hypothyroidism, for which she was receiving treatment. Two years previously, she presented with a large anterior neck mass that caused difficulty in breathing and swallowing. An ultrasonography scan revealed a well-circumscribed vascular nodule in the left lobe that measured 1.8 cm. Follow-up ultrasonography performed one-and-a-half years later showed that the nodule had increased in size to 2.5 cm. A nuclear medicine thyroid scan showed a hot nodule corresponding to the nodule identified on ultrasonography. FNAC was performed, and the obtained material was prepared for air-dried and wet fixation staining with Diff-Quik and Papanicolaou

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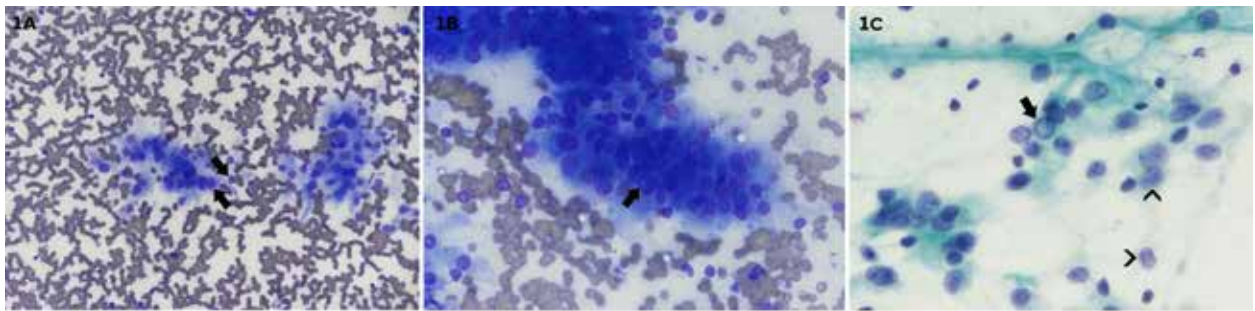
Fig 1 A, B & C: Light microscopy photographs of fine needle aspiration cytology from the left lobe of the thyroid gland nodule shows groups of thyroid follicular cells arranged in three-dimensional clusters in a haemorhagic background. Some cells display nuclear overlapping and grooving (arrowheads), and some show nuclear pseudo-inclusions (arrows). (A. Diff-Quik stain; ×200; B. Diff-Quik stain; ×400; and C. Papanicolaou stain; ×400).

stain, respectively. The stained smears were moderately cellular with a lymphocytic background and nonspecific hemorrhaging. Epithelial cells formed cohesive clusters of variable sizes with prominent cytoplasm. The nuclei were round to oval-shaped with dispersed chromatin and several pseudo-inclusions and grooves (Figure 1). No papillary architectures or significant stromal material was observed. Based on these findings, a possible diagnosis of PTC was suggested. The patient then underwent total thyroidectomy. Gross examination of the excised thyroid revealed a well-defined, firm white nodule measuring 1*1*0.9 cm with an adjacent hemorrhagic area. Histological examination showed that the nodule was well-circumscribed, thinly encapsulated and had a smooth border on a background of chronic lymphocytic thyroiditis. The tumor showed a trabecular and nested pattern with a hyalinized stroma. The cells were large with abundant eosinophilic cytoplasm. The nuclei were round or elongated with frequent nuclear pseudo-inclusions and grooves (Figure 2). No calcification or psammoma bodies were seen. A follicular origin for the tumor was confirmed based on immunohistochemistry, which showed that the tumor cells were positive for thyroglobulin and

TTF-1 and negative for calcitonin. The tumor cells were also negative for HBME-1, galactin-3 and CK19, but they were positive for CD56 (Figure 3). Congo red staining was negative. No gene mutation or rearrangement studies were performed. The preferred diagnosis based on the histological and immunohistochemistry studies was HTT. The patient is currently undergoing regular follow-up visits and has shown no evidence of residual tumor or metastasis.

DISCUSSION

HTT is a rare follicular cell-derived thyroid neoplasm with a trabecular growth pattern and hyalinization. It is a controversial entity that is markedly predominant in women. The lesion was first described in 1987 by Carney and colleagues, who called it a hyalinizing trabecular adenoma[2]. Four years later, one of a series of nine cases with a trabecular pattern and hyalinization showed a lymph node metastasis. Thus, the terminology used for the entity changed from "adenoma" to the now-preferred "tumor"[6]. There is controversy surrounding HTTs with respect to their potential malignant behavior and their possible relationship with PTC. The confusion exists due to its overlapping morphology with PTC
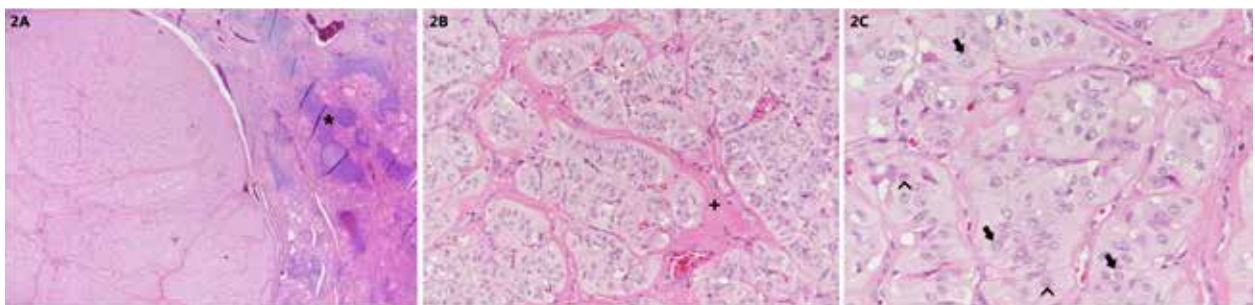
Fig 2 A, B & C: (A) Light microscopy photographs of the left lobe thyroid gland lesion shows an encapsulated nodule on a background of lymphocytic thyroiditis (star). (B and C) Higher magnifications show neoplastic follicular cells arranged in a trabecular pattern with intervening stromal hyalinization (plus). Several nuclear pseudo-inclusions (arrows) and nuclear grooves (arrowheads) are noted. (Hematoxylin and eosin stain; A ×20; B ×200; and C ×400).
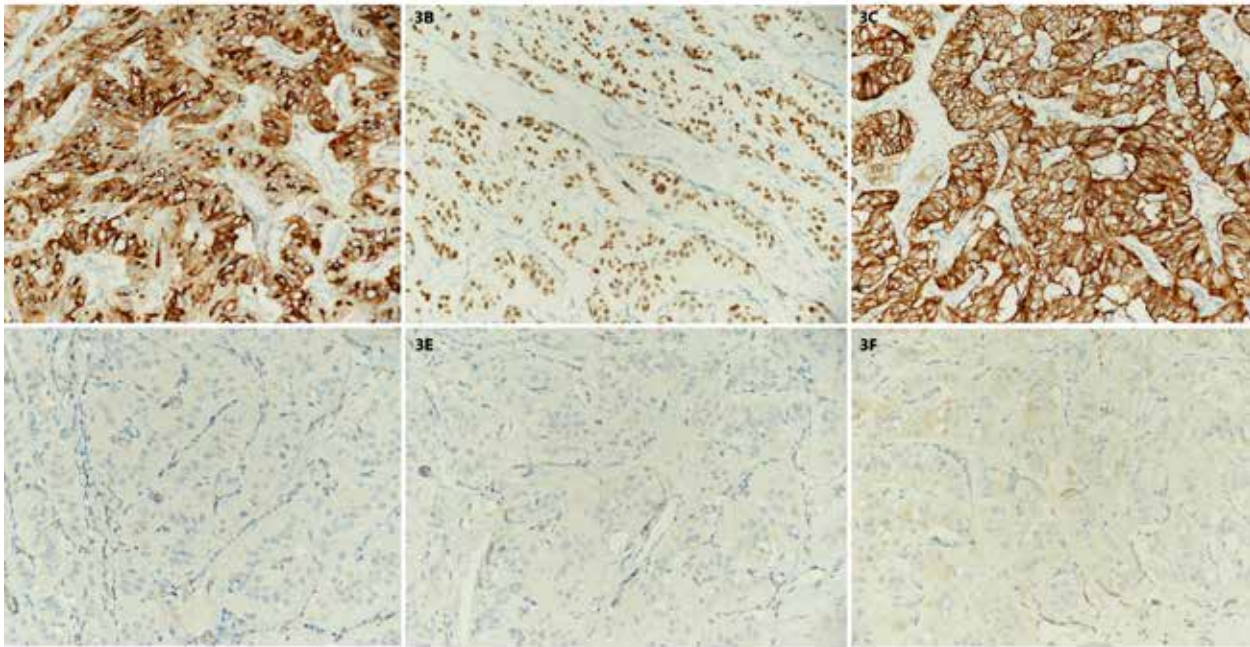
Fig 3 A to F: Light microscopy photographs of immunohistochemistry staining study of the tumor cells show (A) cytoplasmic positivity to thyroglobulin, (B) nuclear positivity to TTF-1, and (C) diffuse cell membrane positivity to CD56. The tumor cells are negative for HBME-1 (D), cytokeratin 19 (E), and galectin-3 (F). (All magnifications are ×200).

and the discovery of RET/PTC oncogene rearrangements in some cases of HTT, which is the most common genetic alteration in PTC. In a large series of HTT by Dell'Aquilla and his colleagues, they support the fact that HTT is a benign neoplasm[7]. Furthermore, most experts consider this tumor to be benign[5,8-9].

HTT's cytological features are indefinite and could lead to erroneous diagnoses of either PTC or MTC. These features include elongated cells, ill-defined cell borders, an eosinophilic cytoplasm, nuclei with prominent grooves and frequent pseudo-inclusions, hyaline material in the background, and a lack of papillary architecture[9]. Some authors have suggested that the combination of a bloody background, radially oriented cohesive cells, an abundant cytoplasm, nuclei with very frequent pseudo-inclusions and grooves, and the presence of hyaline should raise the possibility of an HTT[4]. Nonetheless, a cytological diagnosis remains challenging[10-11].

With regard to immunohistochemistry, HTTs are positive for thyroglobulin and TTF-1. They are generally negative for cytokeratin-19, whereas they show a variable pattern of galectin-3 expression[9]. Their unusual pattern of cytoplasmic immunoreactivity with MIB-1 (Ki-67) (which is prepared at room temperature) is useful in distinguishing them from PTCs[12,13]. HBME-1 negativity and CD56 positivity can be useful for confirming the benign nature of this tumor[14], and

calcitonin and Congo red staining negativity can be of great value for excluding MTC[15].

It is recommended that physicians use ultrasonography to confirm the tumor's shape, margins, consistency and echogenicity. Additionally, HTT should be considered as a differential diagnosis when a tumor's cytological features are suggestive of PTC but ultrasonography results are not worrisome[16].

In this case, the tumor's nuclear features in the cytological material were the main reason for considering the diagnosis of PTC. However, a papillary configuration and psammoma bodies, which are usually seen in PTC, were not seen. In contrast to MTCs, granular nuclear chromatin and amyloid-like amorphous material were not observed in the cytological material of this tumor.

CONCLUSION

It is difficult to impossible to differentiate HTTs from papillary and medullary thyroid carcinomas based on FNAC alone. The cytological diagnosis of HTT should be considered for thyroid lesions with nuclear grooves and pseudo-inclusions that lack other diagnostic criteria for PTCs. Histological examination is superior to cytological studies in making a diagnosis of HTT.

ACKNOWLEDGMENT

REFERENCES

1. Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LD, *et al*. Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors. JAMA Oncol 2016; 2(8):1023-1029.
2. Carney JA, Ryan J, Goellner JR. Hyalinizing trabecular adenoma of the thyroid gland. Am J Surg Pathol 1987; 11(8):583-591.
3. DeLellis RA, Lloyd RD, Heitz PU, Eng C. WHO: Pathology and genetics. Tumors of endocrine organs. In: Kleihues P, Sobin LE, editors. WHO classification of tumors. Lyon, France: IARC Press; 2004. p. 49-133.
4. Casey MB, Sebo TJ, Carney JA. Hyalinizing trabecular adenoma of the thyroid gland: Cytologic features in 29 cases. Am J Surg Pathol 2004; 28(7):859-867.
5. Papotti M, Volante M, Giuliano A, Fassina A, Fusco A, Bussolati G, *et al*. RET/PTC activation in hyalinizing trabecular tumors of the thyroid. Am J Surg Pathol 2000; 24:1615-1621.
6. Sambade C, Franssila K, Cameselle-Teijeiro J, Nesland J, Sobrinho-Simões M. Hyalinizing trabecular adenoma: a misnomer for a peculiar tumor of thyroid gland. Endocr Pathol 1991; 2:83-91.
7. Dell'Aquila M, Gravina C, Cocomazzi A, Capodimonti S, Musarra T, Sfregola S, *et al*. A large series of hyalinizing trabecular tumors: Cytomorphology and ancillary techniques on fine needle aspiration. Cancer Cytopathol 2019; 127(6):390-398.
8. LiVolsi VA. Hyalinizing trabecular tumor of the thyroid: Adenoma, carcinoma, or neoplasm of uncertain malignant potential? Am J Surg Pathol 2000; 24(12):1683-1684.
9. Papotti M, Riella P, Montemurro F, Pietribiasi F, Bussolati G. Immunophenotypic heterogeneity of hyalinizing trabecular tumors of the thyroid. Histopathology 1997; 31:525-533.
10. Kuma S, Hirokawa M, Miyauchi A, Kakudo K, Katayama S. Cytologic features of hyalinizing trabecular adenoma of the thyroid. Acta Cytol 2003; 47:399-404.
11. Saglietti C, Piana S, La Rosa S, Bongiovanni M. Hyalinizing trabecular tumour of the thyroid: fine-needle aspiration cytological diagnosis and correlation with histology. J Clin Pathol 2017; 70(8):641-647.
12. Hirokawa M, Carney JA. Cell membrane and cytoplasmic staining for MIB-1 in hyalinizing trabecular adenoma of the thyroid gland. Am J Surg Pathol 2000; 24(4):575-578.
13. Leonardo E, Volante M, Barbareschi M, Cavazza A, Dei Tos AP, Bussolati G, *et al*. Cell membrane reactivity of MIB-1 antibody to Ki67 in human tumors: fact or artifact? Appl Immunohistochem Mol Morphol 2007; 15(2):220-223.
14. Nechifor-Boila A, Catana R, Loghin A, Radu TG, Borda A. Diagnostic value of HBME-1, CD56, Galectin-3 and Cytokeratin-19 in papillary thyroid carcinomas and thyroid tumors of uncertain malignant potential. Rom J Morphol Embryol 2014; 55(1):49-56.
15. Goellner JR, Carney JA. Cytologic features of fine-needle aspirates of hyalinizing trabecular adenoma of the thyroid. Am J Clin Pathol 1989; 91(2):115-119.
16. Choi WJ, Baek JH, Ha EJ, Choi YJ, Hong MJ, Song DE, *et al*. The ultrasonography features of hyalinizing trabecular tumor of the thyroid gland and the role of fine needle aspiration cytology and core needle biopsy in its diagnosis. Acta Radiol 2015; 56:1113-1118.

Case Report

Emergency parathyroidectomy under combined superficial and intermediate cervical plexus block: A fatal case of hypercalcemic crisis due to parathyroid carcinoma

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ABSTRACT

Hypercalcemic crisis is a rare but fatal condition. This condition may occur primarily due to primary hyperparathyroidism caused by parathyroid adenoma or rarely parathyroid carcinoma. In surgical treatment, parathyroidectomy and additional procedures are often performed with general, local and regional anesthesia.
We present a case of a 71-year-old white woman with a hypercalcemic crisis associated with a possible preoperative parathyroid carcinoma. Serum calcium and parathyroid hormone levels were 18.8 mg/dl and >1900 pg/ml, respectively. Emergency left lower parathyroidectomy was performed only under locoregional anesthesia because of existing comorbidities. There was a rapid decrease in

parathyroid hormone levels immediately after surgery. Histology revealed a 3 cm parathyroid carcinoma in our patient. Definitive surgery was planned and the patient was discharged.
Excision in hypercalcemic crisis due to parathyroid adenoma, ipsilateral thyroid lobectomy and removal of abnormal lymph nodes in case of carcinoma is recommended. In our patient, emergency parathyroidectomy was preferred instead of definitive surgery in the first stage because of comorbidities. This case raises awareness of the life-threatening hypercalcemic crisis and emphasizes the importance and benefit of emergency parathyroidectomy and alternative anesthesia techniques in hypercalcemic crisis.

KEY WORDS: malignant hypercalcemia, minimal invasive parathyroidectomy, parathyroid carcinoma

INTRODUCTION

Hypercalcemic crisis caused by primary hyperparathyroidism is a rare but life-threatening condition. Symptoms of hypercalcemic crisis, also interpreted as malignant hypercalcemia, are often non-specific and may occur with multiple organ involvement. Hypercalcemic crisis of primary hyperparathyroidism should be considered in acute patients with deep dehydration, gastrointestinal symptoms, urinary symptoms, altered mental status or cardiac arrhythmias. Among these pathologies, parathyroid carcinoma is a rare malignancy with 0.5-4% of reported primary hyperparathyroidism cases[1,2]. Surgery is the most curative treatment in parathyroid

carcinoma with a high rate of 98%[1-4]. The most appropriate surgical procedure is ipsilateral thyroid lobectomy and anblock parathyroidectomy, as well as removal of enlarged or abnormal lymph nodes[3].

In this study, we present a case of emergency parathyroidectomy in hypercalcemia crisis due to primary hyperparathyroidsm under superficial and intermediate cervical plexus block.

CASE REPORT

A 71-year-old female was admitted to the Emergency Department with flutter, spasm, weakness and poor general condition. The patient who previously had chronic obstructive pulmonary disease, hypertension,

Fig. 1: Ultrasound guided determination of preoperative parathyroid localization

Fig. 3: Excised lower left parathyroid gland

cardiac failure and hyperthyroidism was hospitalized by our Endocrine Department. Laboratory results showed urea: 62 mg/dl (17-43), creatinin: 1.60 mg/dl (0.66-1.09), albumin: 2.6 g/dl (3.5-5.2), calcium: 18.8 mg/dl (8.8-10.6), phosphor: 3.2 mg/dl (2.5-4.5), free T3: 5.4 pg/ml (2.3-4.2), free T4: 4.5 ng/dl (0.89-1.76), thyroid stimulating hormone: 0.130 µU/ml (0.55-4.78) and parathyroid hormone (PTH): >1900.0 pg/ml (11.1-79.5). Neck and thyroid ultrasound showed nodular lesions smaller than 2 cm in thyroid gland and a noduler lesion approximately 3 cm was located posteroinferior of left thryoid gland. This nodular lesion was described as a parathyroid adenoma by our
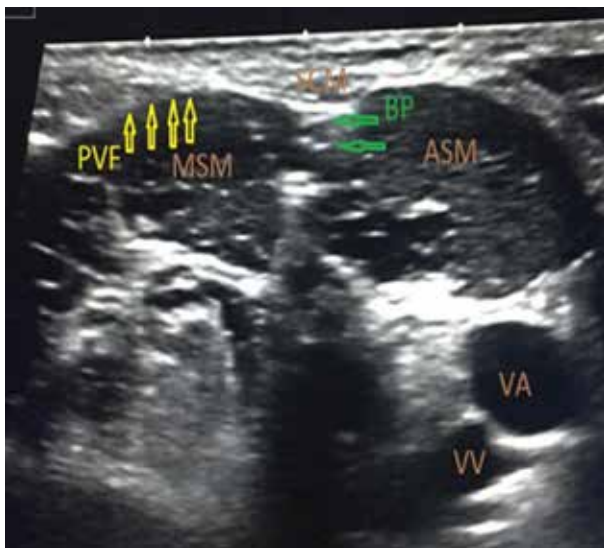
Fig. 2: Image of the region with ultrasound. Abbreviations; PVF: prevertebral fascia, MSM: middle scalen muscle, ASM: anterior scalen muscle, BP: brachial plexus, SCM: sternocleidomastoid muscle, VA: vertebral arteria, VV: vertebral vein

radiology department. Our endocrine team thought it may be a malignancy, but we did not have the option for exploration under general anesthesia due to her other co-morbidities. Due to her co-morbid factors, she did not have another option. Her hypercalcemia was under control with medical management for a short time. Immediately after, we planned the minimal invasive parathyroidectomy under locoregional anesthesia. We marked the parathyroid location with the aid of ultrasound on the operating day (Fig. 1). She was evaluated ASA-3 for operating by Anesthesia Department. Initially our anesthesia team established vascular access and began isotonic infusion over 10 ml/kg/hour. IV midazolam was applied for premedication by anesthetist. Following positioning, anesthesia team placed ultrasound probe (10-15 Ghz) and viewed investing prevertebral fascia that is located between sternocleidomastoid and middle scalene muscles (Fig. 2). Local anesthesia was applied to skin and subcutenaous tissue. Following that, they injected 20 ml 0.5% bupivacaine to infiltrate the prevertebral fascia and injected 5 ml 0.5% bupivacain through pretracheal area with the purpose of blockage of contralateral cervical nerve fibers.

Approximately twenty minutes later, we started the surgery. We did nearly five cm incision from marked area with ultrasound. In addition, 0.025 mg/kg midazolam was administered during operating time, but we did not need another anesthetic drug. We could find the parathyroid gland (Fig. 3) from this incision (Fig. 4) and parathyroidectomy was performed in about thirty minutes. Following operation, she was taken into service. She did not need to stay in intensive care. Six hours later, laboratory studies showed that PTH was 800 pg/ml and the day after, PTH was 73 pg/ml.

Fig. 4: Image of the incision during surgery

She was discharged the day after operation and she recovered fully in approximately fourteen days. Meanwhile, the parathyroid gland's definitive pathological result was described as parathyroid carcinoma by our Pathology Department and we planned further operation for carcinoma. However, her co-morbid factors should be taken into consideration.

DISCUSSION

Hypercalcemia crisis caused by primary hyperparathyroidism has been named as parathyroid storm. Hypercalcemia which develops into hypercalcemic crisis can be seen between 1% and 6%[4-6]. Nowadays, being able to easily detect hypercalcemia during routine blood analysis enables us to plan diagnosis and treatment without allowing the disease to enter into crisis. Malignancy is the most common cause of hypercalcemia in the case of decompensated hypercalcemic crisis[7]. The most common malignancies associated with hypercalcemia are squamous cell carcinoma of the lung, breast cancer, renal cell carcinoma, bladder cancer and multiple myeloma disease. The mechanism of hypercalcemia in malignancy is due to tumor production of parathyroid hormone-related polypeptides or other osteolytic factors. Bone resorption occurs due to primary disease and bone metastases[6,8].

In patients with primary hyperparathyroidism with chronic hypercalcemia, hypercalcemic crisis may develop suddenly. Sudden increase of hypercalcemia can be life-threatening. Therefore, just as in our case, hypercalcemia due to primary hyperparathyroidsm should be treated quickly, as an emergency parathyroidectomy should be performed without delaying. Although the mechanism is not yet fully understood, several factors have been blamed. The most important of these are infection, trauma, surgery and the use of calcium antagonists. A high mortality rate of 93.5% has been reported in patients who had not undergone parathyroidectomy in the hypercalcemic crisis, but this rate was reduced to 6.7% by an experienced endocrine surgeon[5,8,9].

Vital steps are recommended in the hypercalcemic crisis. Firstly, medical treatment is necessary to decrease serum calcium and stabilize the patient, the etiology of hyperparathyroidism must then be determined by measuring parathyroid hormone level and finally, emergency parathyroidectomy must be planned quickly. In case of hypercalcemic crisis, saline diuresis, bisphosphonate, glucorticoid and/or calcitonin treatments can be tried to decrease the calcium values of patients and stabilize them for surgery. If it fails, it can be considered in calcium-free dialysis[10-12].

Removing abnormal parathyroid gland is the basic surgical procedure in primary hyperparathyroidsm. Until 1990, bilateral cervical exploration of four parathyroid glands was the gold standard procedure. However, because of the extended operating time and postoperative hypocalcemia rates, this procedure was abondoned by physicians. Nowadays, minimal invasive parathyroidectomy has become popular. Evolution of parathyroid gland imaging techniques can assure a well satisfactory parathyroid surgery[13]. In addition, the evolution of local and general anesthesia increases the success of operations. In recent years, minimal invasive parathyroidectomy can often be performed under local anesthesia succesfully. The elderly population especially have many healthy problems such as cardiopulmonary diseases, renal or neural disorders. Many elderly patients who have primary hyperparathryoidsm are treated surgically. In this way, the hospital stay and hospital costs are markedly reduced with minimal morbidity and mortality rates[14].

CONCLUSION

We present a case of emergency and minimally invasive parathyroidectomy due to primary hyperparathyroidism in hypercalcemic crisis. We aimed to show that the primary hypercalcemic crisis can be resolved in a very short time in some co-morbid patients with this combination of local anesthesia technique and surgical procedure.

ACKNOWLEDGMENT

REFERENCES

1. Levin KE, Galante M, Clark OH. Parathyroid carcinoma versus parathyroid adenoma in patients with profound hypercalcaemia. Surgery 1987; 101(6):649-660.
2. Shortell CK, Andrus CH, Philips CE Jr, Schwartz SI. Carcinoma of the parathyroid gland: a 30-year experience. Surg 1991; 110(4):704-708.
3. Sheehan JJ, Hill AD, Walsh MF, Crotty TB, McDermott EW, O'Higgins NJ. Parathyroid carcinoma: diagnosis and management. Eur J Surg Oncol 2001; 27(3):321-324.
4. Singh DN, Gupta SK, Kumari N, Krishnani N, Chand G, Mishra A, *et al*. Primary hyperparathyroidism presenting as hypercalcemic crisis. Twenty-year experience. Indian J Endocrinol Metab 2015; 19(1):100-105.
5. Gurrado A, Piccinni G, Lissidini G, Di Fronzo P, Vittore F, Testini M. Hypercalcaemic crisis due to primary hyperparathyroidism – a systematic literature review and case report. Endokrynol Pol 2012; 63(6):494-502.
6. Wong P, Carmeci C, Jeffrey RB, Weigel RJ. Parathyroid crisis in a 20 year old – an unusual cause of hypercalcaemic crisis. Postgrad Med J 2001; 77(909):468-470.
7. Kuan YC, Tan F. Hypercalcemic crisis - a fatal case of primary hyperparathyroidism. Med J Malaysia 2014; 69(6):231-233.
8. Ziegler R. Hypercalcemic crisis. J Am Soc Nephrol 2001; 12 Suppl 17:S3-9.
9. Marienhagen K, Due J, Hanssen TA, Svartberg J. Surviving extreme hypercalcemia – a case report and review of the literature. J Intern Med 2005; 258(1):86-89.
10. Koo WS, Jeon DS, Ahn SJ, Kim YS, Yoon YS, Bang BK. Calcium-free hemodialysis for the management of hypercalcemia. Nephron 1996; 72(3):424-428.
11. Bentata Y, El Magharoui H, Benabdelhak M, Haddiya I. Management of hypercalcaemic crisis in adults: Current role of renal replacement therapy. Am J Emerg Med 2018; 36(6):1053-1056
12. Trabulus S, Oruc M, Ozgun E, Altiparmak MR, Seyahi N. The use of low-calcium hemodialysis in the treatment of hypercalcemic crisis. Nephron 2018; 139(4):319-331.
13. Mekel M, Mahajna A, Ish-Shalom, Barak M, Segal E, Sali AA, *et al*. Minimally invasive surgery for treatment of hyperparathyroidism. Isr Med Assoc J 2005; 7(5):323-327.
14. Suliburk JW, Perrier ND. Primary hyperparathyroidism. Oncologist 2007; 12(6):644-653.

<p style="text-align:center">**Brief Communication**</p>

The experience of Oman with establishing a field hospital during COVID-19 pandemic

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ABSTRACT

Over the past fourteen months, services in various countries across the globe have been hit hard by the COVID-19 pandemic. The outbreak of corona virus has been overwhelming on healthcare systems in particular; and this is likely to have a lasting impact on health ventures and delivery models over the coming few years.

On 31st December 2019, the World Health Organization reported the first case of COVID-19, and since then 179,553,288 cases have been reported across the sphere. In Oman, the total number of cases reported reached 250,572 as of 21st June 2021. This is against a hospital ward bed capacity of 6,034 beds across all government and private hospitals in the country. Due to the healthcare system becoming overwhelmed by the load of cases detected, a field hospital was established to act as a main hub of care for COVID-19 cases in the country.

The aim of this article is to highlight the process involved in establishing and operating the field hospital. The main aspects that will be covered are the objectives of establishing the hospital, operation, components, in addition to the management and provision of cadres in its departments. The objective is to enlighten healthcare providers and the public about this service and share the experience of establishing a field hospital during this period.

KEY WORDS: corona virus, flu pandemic, health services administration, health unit, mobile

INTRODUCTION

On the eve of the year 2020, the World Health Organisation (WHO) reported the first case of COVID-19 disease after cases of pneumonia of unknown aetiology were reported in Wuhan city, located at Hubei Province of China. In the following month, the causative agent of this newly emerging disease was identified and named as Corona-Sars-2 virus, and during the same month, the WHO declared the global situation as a public health emergency of international concern. Ten weeks following this declaration, COVID-19 disease was categorised as a global pandemic[1].

The global toll at that stage had reached 318,941 for detected cases and 10,191 for deceased ones[2]. Figure 1 summarises the main declarations made by the WHO and the country regarding COVID 19 pandemic from December 2019 up until March 2020.

Over the past twenty years, some members of the Corona family have caused coronavirus-associated diseases before, including Severe Acute Respiratory Syndrome Coronavirus and Middle East Respiratory Syndrome-related Coronavirus. However, Corona-SARS-2 virus is a newly identified member of this unwelcome family, and it is the first time for this strain of novel agents to be reported to cause ailments in the human race. Corona-SARS-2 virus causes a spectrum of symptoms, ranging from mild to moderate (80%), severe (15%) and critical (5%)[3]. The majority of patients infected appear to have an uneventful recovery (80%), without requiring further medical care. However, symptoms progress and worsen in around one out of every five people infected with the virus[1]. This group makes around 20% of all infections, and it is this group that has been overwhelming the healthcare systems across the globe[3].

In Oman, the virus was discerned for the first time on February 24th 2020, after the return of two females

from abroad and testing positive for it[4]. Following that, His Majesty issued royal orders to stipulate the formation of the Supreme Committee to study scopes for a mechanism to handle developments resulting from COVID-19 disease. The biggest burden of the virus was on the healthcare system, as it created a significant load on the Ministry of Health as well as the tertiary hospitals, following the surge in the number of confirmed and admitted cases. The surplus of patients requiring medical care created a challenge on the hospitals in terms of providing additional care for patients with COVID-19 along with the usual care for other patients with long-term illnesses. This necessitated the need to establish a field hospital as a temporary shelter for that group of patients.

The aim of this article is to discuss the process involved in establishing and operating the field hospital. The main aspects that will be highlighted are the objectives of establishing the hospital, its components, in addition to the management and provision of cadres in its departments. The objective is to educate physicians, health care workers and the public about the service, and likewise share the experience of establishing a field hospital during this period. Figures obtained in this report have been taken from WHO global dashboard, global surveillance bodies and national surveillance data reports.

DISCUSSION

Over the last few months, the number of cases diagnosed with COVID-19 has been rising locally, regionally and globally, with some countries being affected more than others. Sustaining healthcare organizations, consequently, has become a worldwide concern[5]. Up until 21st June 2021, the total number of confirmed cases in the world reached 179,553,288[2]. Of the total number of cases reported globally, 45.9% were noted to be from only three countries: United States of America, India and Brazil[2]. In Oman, the overall figure reached 250,572, out of those 220,171 recovered (87.9%) while 2,741 passed away (12.1%)[4]. This is against a hospital ward bed capacity of 6,034 beds across all government and private hospitals in the country[6].

Following the global and local surge in the number of COVID-19 cases, the supreme committee deemed it necessary to take additional measures in order to mitigate the impacts locally. A combination of proactive containment measures and suppression interventions were implemented. The former involved travel restrictions, case finding, contact tracing, isolation of confirmed cases and quarantine of exposed individuals. The latter comprised lock down measures, social distancing, closure of educational institutions and prohibition of large-scale public gatherings[4]. In

addition, an electronic surveillance system was launched, named Tarassud Plus to track the cases in the country, and ensure isolation measures are adhered to[4]. The WHO described it as one of the most powerful technological solutions deployed in the Middle East to track movement and spread of COVID-19[7].

These public health measures were improbable months ago, but the government deemed it necessary to implement them at that stage to control the situation locally. Following attenuation of control measures, another surge appeared to surface with case numbers and, consequently, the death toll making another ascent. The increase in demand on the health care system led the supreme committee to re-evaluate the role of the country's government hospitals as the main sites of care. Consequently, the committee released a decision on July 5, 2020 to establish a field hospital[8]. The Ministry of Health then made a ministerial decision (95/2020) to formulate a task force for supervising the establishment, preparation and operation of the field hospital for patients infected with COVID-19 disease[8].

During the COVID era, several countries opted to establish field hospitals, being either as seclusion sites for cases with mild to moderate severity, sites with capabilities of an Intensive Care Unit to care for severe cases, or as step-down units to provide care for recovering cases[9]. The government of Oman established a field hospital for the latter reason, with the main objective of accommodating patients infected with COVID-19 disease in one institute, following the shortage of beds in the local hospitals. This was to ultimately decrease the burden on other health care institutes in the country, and enable them to resume their usual daily services, along with provision of care to other patients with long-standing illnesses[10].

The Field Hospital established in Oman was designed to encompass two zones: Zone A and Zone B. The former has multiple departments, including medium dependency, high dependency and female zone, in addition to radiology, laboratory and pharmacy sections. The latter comprises general quarantine beds for both genders[11]. Figure 2 represents the distribution of beds in Zone A.

The total bed capacity in the field hospital is 312 beds. A plan was set to operate it over three phases, phase one encompassing 100 beds, phase two 200 beds, and phase three covering the full hospital capacity, *i.e.* 312 beds. The first phase of the hospital was inaugurated on 5th October 2020, under the auspices of The Minister of State and Governor of Muscat[11]. It started to operate on the same day of the opening ceremony. The hospital was not receiving patients directly, but only cases referred from other health care institutions and requiring intermediate level of care. The critical and

Table 1: Bed distribution in the field hospital

Zone	Sections	Number of beds	Total bed capacity	Area
A (Hospital)	Medium dependency	261	312	6,100 m²
	High dependency	16		
	Female zone	35		
B (Shelter Centre)	General beds	314	384	10,000 m²
	Female zone	70		

severe cases were sent directly to the referral hospitals in the region[12]. An admission criterion was prepared and distributed to all primary, secondary and tertiary healthcare institutions.

Table 1 outlines the bed distribution in the field hospital[10-12]. Among the sites that were proposed for establishing the hospital was Oman Convention and Exhibition Centre, Sultan Qaboos Football Stadium and Old Muscat Airport Building. The latter was ultimately selected for the project due to the site being located at the heart of the city, existing spacious building, availability of space for future expansion, ease of modifying the internal design to suit the facility and possibility of providing additional services to accommodate the new requirements[8,10]. It was planned to utilize the existing building as a temporary hospital and a shelter centre, where the latter can be converted to a hospital if the need did arise. The assigned engineering company along with the supervising committee studied the design in depth in order to ensure on utilizing the space provided and allocating the beds and services in the most convenient location, and to ease movement and transfer of patients between different departments during their stay there. The hospital was run by a medical team seconded from the Ministry's various institutes, including 30 doctors, 115 male and female nurses, 7 lab technicians, 7 pharmacist assistants, 4 radiologists and 2 physiotherapists, in addition to administrative personnel and ambulance drivers[13]. Establishing and equipping the hospital with beds, medical equipment and logistic support was achieved in collaboration with multiple authorities, including the Ministry of Health, Civil Aviation Authority, Oman Airport, Petroleum Development Oman, Royal Court Affairs and Royal Oman Police, as a means of collaborating with the committee for the public's best interest[13,14].

From the start of operating the field hospital and

Fig 1: The main declarations made by the WHO and the country regarding COVID 19 pandemic, from December 2019 up until March 2020. Courtesy Dr. Z Allawati, Muscat, Oman (January, 2021)
https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline?gclid=CjwKCAiAu8SABhAxEiwAsodSZP5gy0toe
419XFO6EdUdIlXVy6luY4njZ-El36eYS-Tqb7lJN-OVhxoChvMQAvD_BwE#event-7
[Accessed 28.01.2021]

Fig 2: The the distribution of beds in Zone A in the field hospital
https://www.omanobserver.com/sc-members-visit-field-hospital-at-old-muscat-airport/#jp-carousel-632574
[Accessed 20.02.2021]

up until this day, the majority of patients admitted there underwent an uneventful course and were discharged from the hospital. Some cases did show a clinical deterioration and were transferred to other healthcare facilities to receive appropriate level of care, as there is no intensive care unit in the hospital[14].

Figure 3 highlights the main actions that were taken by the government to combat the spread of COVID-19 disease[13-15].
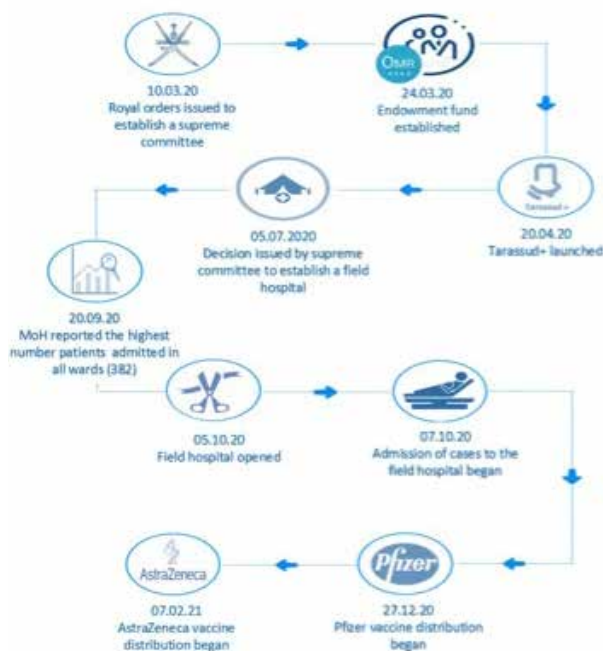
Fig 3: Summary of the main actions taken by the government to combat the spread of COVID-19 disease.
Courtesy Dr. Z Allawati, Muscat, Oman (February, 2021)

The experience Oman has witnessed with regards to responding to this pandemic has emphasized some of the strategies that should be accentuated in the sector of healthcare management, to ensure keeping the healthcare system robust, sustainable and innovative during onerous periods. These stratagems include having agile leaders, clear emergency management plans, creating makeshift hospitals during a short timeframe and subsidizing the healthcare sector[16].

CONCLUSION

This report provided a succinct summary of the experience Oman has encountered with regards to establishing a field hospital during the COVID-19 pandemic. A significant proportion of this information lies within news agency reports, thus such an article is vital to validate and document the information pertaining to this critical milestone during this era. This experience has highlighted the importance of re-evaluating the role of hospitals as main centers for care, and the lessons learnt should be reminisced after the calamity period. With the establishment of the field hospital and increased immunity among society from the vaccination programs, the load on the government is bound to decline with time. However, the battle against this virus will continue to exist and will require long-lasting harmony, both locally and internationally, to control the situation and return to normality.

ACKNOWLEDGMENT

REFERENCES

1. World Health Organization (WHO) [Internet]. Timeline: WHO's COVID-19 response; c2021 [Accessed 15.01.2021]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline.

2. Worldometer [Internet]. COVID-19 Coronavirus Pandemic; c2021 [Accessed 10.02.2021]. Available from: https://www.worldometers.info/coronavirus/

3. Balkhair AA. COVID-19 pandemic: A new chapter in the history of infectious diseases. Oman Med J 2020; 35(2):e123.

4. Ministry of Health (MOH) [Internet]. e-Health Portal: MOH Statement; c2020 [Accessed 18.02.2021]. Available from: https://www.moh.gov.om/en/-/-287-

5. Butler SM. After COVID-19—Thinking differently about running the health care system. JAMA 2020; 323(24):2450-2451.

6. Annual Health Report [Internet]. Department of Health Information & Statistics, Directorate General of Planning and Studies, Ministry of Health, Sultanate of Oman; c2019 [Accessed 20.02.2021] Available from: https://www.moh.gov.om/en/web/statistics/-/-2019

7. World Health Organization (WHO) [Internet] Oman launches technological surveillance system to track COVID-19, 17.06.2021. [Accessed 18.02.2021]. Available from: https://www.who.int/news-room/feature-stories/detail/oman-launches-technological-surveillance-system-to-track-covid-19

8. Times News Service [Internet]. Oman to set up 200-bed field hospital to treat COVID-19 patients, Times of Oman, 05.07.2020. [Accessed 17.02.2021]. Available from: https://timesofoman.com/article/oman-to-set-up-200-bed-field-hospital-to-treat-covid-19-patients

9. Yuan L, Sherryn S, hu P, Chen F. U.S. Field Hospitals: A Study on Public Health Emergency Response to COVID-19, medrxiv. c2020 [Accessed 18.02.2021]. DOI: https://doi.org/10.1101/2020.08.05.20169094.

10. Supreme Committee Members Visit COVID-19 Field Hospital, Ministry of Health (MOH) e-Health Portal, 21.09.2020. [Accessed 17.02.2021]. Available from: https://www.moh.gov.om/en/medical-alert-display/-/asset_publisher/4hProhtDQEMs/content/--1465?inheritRedirect=false.

11. Oman Observer [Internet]. Covid-19 field hospital opens at old Muscat Airport, Oman Daily Observer, 05.10.2020. [Accessed 11.02.2021]. Available from: https://www.omanobserver.om/wp-content/uploads/2020/10/Feild-H1.jpeg

12. Oommen A [Internet]. Covid-19 field hospital opens at old Muscat Airport, Construction week, 07.10.2020. [Accessed 11.02.2021]. Available from: https://www.constructionweekonline.com/projects-and-tenders/267824-oman-opens-phase-1-of-6100m2-covid-19-field-hospital

13. AH, COVID-19 Field Hospital Opens, Oman News Agency (ONA), 05.10.2020 [Accessed 11.02.2021]. Available from: https://omannews.gov.om/NewsDescription/ArtMID/392/ArticleID/21054/COVID-19-Field-Hospital-Opens

14. Ministry of Health (MOH) [Internet]. e-Health Portal: COVID-19 Field Hospital Opens, 05.10.2020. [Accessed 18.02.2021]. Available from: https://www.moh.gov.om/en/-/--34

15. AD [Internet]. Oman's Supreme Committee members visit field hospital for COVID-19 patients, Arabian Daily, 20.09.2020. [Accessed 11.02.2021]. Available from: https://arabiandaily.com/omans-supreme-committee-members-visit-field-hospital-for-covid-19-patients/

16. Al Fannah J, Al Harthy H, Al Salmi Q. COVID-19 pandemic: Learning lessons and a vision for a better health system. Oman Medical Journal (OMJ) 2020; 35(5):e169.

Letter to the Editor

Overview on outbreak investigations of 2019 novel Coronavirus disease

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ABSTRACT

The therapy of "post-COVID-19 syndrome" requires a multidisciplinary team, an individualized approach and long-term rehabilitation. Recovery and rehabilitation after COVID-19 is aimed specifically at overcoming the consequences of the disease by strengthening the respiratory, nervous and cardiovascular systems through treatment and rehabilitation procedures. The complex program includes pulmonary rehabilitation to increase lung capacity and overall metabolic effects. Complex rehabilitation is part of the whole treatment plan and includes many physical and rehabilitation activities, health care, healthy behavior, training and prevention of complications in patients after infection.

KEY WORDS: coronavirus infection, treatment plan

Dear Editor,

As like other coronavirus pneumonias such as Middle East Respiratory Disease (MERS) and Severe Acute Respiratory Syndrome (SARS), COVID-19 can also lead to acute Respiratory Distress Syndrome. This viral pneumonia was reported in early December 2019 and initially, it was linked to exposure at Huanan Seafood Market, Wuhan, Hubei province, China[1]. Data as of February 16, 2020 showed that the virus had caused 70,548 infections and 1,770 deaths in mainland China and 413 infections in Japan[2].

On 21st January 2020, a 50-year-old man was admitted in clinic for fever with other symptoms of cough, shortness of breath (SOB), chills and fatigue. On day nine of his illness, 22nd January 2020, the patient was confirmed by Beijing Centers for Disease Control through reverse real-time PCR assay to be positive for COVID-19[3].

Since the onset of the COVID-19 outbreak, infection prevention control system has played a major role in preventive and mitigation measures. In the 24-hour survey of confirmed and suspected cases of COVID-19 reported by provinces, regions and cities in China on March 13, 2020, 11 of them were confirmed cases of COVID-19, seven deaths occurred and 33 of them were suspected[4]. This outbreak of COVID-19 was then continuously under investigation with new and confirmed cases increasing day by day. On March 15, 2020, the same outbreak within the same region and same population was investigated for 24 hours and this time, the confirmed cases of COVID-19 rose to 27 individuals, deaths reached 10, and 39 individuals were suspected cases[5].

The Director General of the World Health Organization described the situation of COVID-19 as a pandemic on 16th of March 2020. A study suggested that elderly people, particularly those older than 80 years, and people with comorbidities such as cardiac disease, respiratory disease and diabetes, are at greatest risk of serious disease and increased chance of mortality[6].

Another study conducted in China reported that pregnant women with COVID-19 associated pneumonia showed a similar pattern of clinical features

to non-pregnant adult patients. Common symptoms at the onset of COVID-19 pneumonia in these women were fever and cough, whereas less common symptoms were myalgia, malaise, sore throat, diarrhea and SOB. Laboratory tests indicated that lymphopenia is also likely to occur[7]. COVID-19 is a respiratory illness that can spread from person to person and having common symptoms of fever, cough and SOB[8,9]. However, there is no evidence of person to person transmission from asymptomatic carriers with normal chest computed tomography[10]. The four genera of this viral disease are alpha, beta, gamma and delta corona virus[11]. The signs and symptoms and other pathological features of this COVID-19 is greatly similar to SARS and MERS coronavirus infections[3].

Studies in China on COVID-19 patients showed the following clinical features in them: fever in 98% of patients, cough in 76% of patients, fatigue in 44% of patients, and dyspnea and SOB. Other less common symptoms were sputum production, headache, haemoptysis and diarrhea. The blood samples of patients showed leucopenia and lymphopenia[1].

The most essential measures for prevention and control from COVID-19 was addressed by Washington State Department of Health on 13th March 2020, which were the following:

• Stay at home in a separate room, except to get medical care
• Isolate yourself from people and animals in your home
• Call your doctor just before visiting
• Wearing face mask
• Cover your mouth when sneezing or coughing
• Frequently cleaning hands
• Do not share any personal domestic items
• Clean high touch surfaces all day
• Monitor symptoms in yourself (most commonly fever, cough and SOB)
• Request your doctor to call the "state health department" for discussing your situation

The public were advised to discontinue home isolation in the following cases:

i. If you had a fever, three days after the fever ends and you see an improvement in your initial symptoms (*e.g.* cough, SOB);
ii. If you did not have a fever, three days after you see an improvement in your initial symptoms (*e.g.* cough, SOB)

In cases where fever persists with cough or SOB but the person has not been exposed to somebody with COVID-19 and have not tested positive for COVID-19, then you should stay at home away from others until 72 hours after the fever and other symptoms are

relieved[9]. China multicenter clinical trials reveal that an old drug chloroquine, which is used for the treatment of malaria, had also demonstrated marked efficacy and a possible safety in treating pneumonia associated with COVID-19. Different vitro studies suggest that chloroquine can block infection in COVID19 at low-micromolar concentrations[2].

However, the Emergency Use Authorization (EUA) mechanism permits the US Food and Drug Administration to respond fast to novel threats by approving new instrumentations and drugs. Pfizer became the first company to succeed in its pursuit of approval for its COVID-19 vaccine on 13th November 2020, making it the first sample of EUA approval for vaccine[12]. In addition to the Pfizer vaccine, on 20th January 2021, the Corona-Vac vaccine was also approved by Public Health Institute of Chile, China for emergency use[13].

Since COVID-19 is caused by an emerging new type of enveloped RNA virus, treatment is yet to be developed and needs further studies. The case fatality ratio in children and elderly people was more because of underdeveloped and degenerated immune systems respectively. However, this case fatality ratio was demonstrated from lack of data availability and the case fatality ratio might be fluctuating. On laboratory findings, lymphopenia was also common.

ACKNOWLEDGMENT

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020; 395(10223):497-506.
2. Quinn SC, Jamison AM, Freimuth V. Communicating effectively about emergency use authorization and vaccines in the COVID-19 pandemic. Am J Public Health 2021; 111(3):355-358.
3. Bedford J, Enria D, Giesecke J, Heymann DL; WHO Strategic and Technical Advisory Group for Infectious Hazards. COVID-19: towards controlling of a pandemic. Lancet. 2020 Mar 28;395(10229):1015-1018. doi: 10.1016/S0140-6736(20)30673-5. Epub 2020 Mar 17. PMID: 32197103; PMCID: PMC7270596
4. Gao J, Tian Z, Yang X. Breakthrough: Chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends 2020; 14(1):72-73.
5. Chaudhry RM, Hanif A, Chaudhary M, et al. Coronavirus Disease 2019 (COVID-19): Forecast of an Emerging Urgency in Pakistan. *Cureus*. 2020;12(5):e8346.

Published 2020 May 28. doi:10.7759/cureus.8346

6. Tripathi R, Alqahtani SS, Albarraq AA, Meraya AM, Tripathi P, Banji D, Alshahrani S, Ahsan W and Alnakhli FM (2020) Awareness and Preparedness of COVID-19 Outbreak Among Healthcare Workers and Other Residents of South-West Saudi Arabia: A Cross-Sectional Survey. *Front. Public Health* 8:482. doi: 10.3389/fpubh.2020.00482

7. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 2020; 8(4):420-422.

8. Chen H, Guo J, Wang C, Luo F, Yu X, Zhang W, *et al*. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women : a retrospective review of medical records. Lancet 2020; 395(10226):809-815.

9. Singhal T. (2020). A Review of Coronavirus Disease-2019 (COVID-19). *Indian journal of pediatrics*, *87*(4), 281–286. https://doi.org/10.1007/s12098-020-03263-6.

10. Guo, YR., Cao, QD., Hong, ZS. *et al.* The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. *Military Med Res* **7,** 11 (2020). https://doi.org/10.1186/s40779-020-00240-0

11. Dong Y, Mo X, Hu Y, Qi X, Jiang F, Jiang Z, *et al*. Epidemiological characteristics of 2143 pediatric patients with 2019 coronavirus disease in China. J Emerg Med 2020; 58(4):712-713.

12. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, *et al*. Presumed asymptomatic carrier transmission of COVID-19. JAMA 2020; 323(14):1406-1407.

13. Jara A, Undurraga EA, González C, Paredes F, Fontecilla T, Jara G, *et al*. Effectiveness of an inactivated SARSCoV-2 vaccine in Chile. N Engl J Med 2021; 385(10):875-884.

Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Handling missing data in a rheumatoid arthritis registry using random forest approach

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Missing data in clinical epidemiological research violate the intention-to-treat principle, reduce the power of statistical analysis, and can introduce bias if the cause of missing data is related to a patient's response to treatment. Multiple imputation provides a solution to predict the values of missing data. The main objective of this study is to estimate and impute missing values in patient records. The data from the Kuwait Registry for Rheumatic Diseases was used to deal with missing values among patient records. A number of methods were implemented to deal with missing data; however, choosing the best imputation method was judged by the lowest root mean square error (RMSE). Among 1735 rheumatoid arthritis patients, we found missing values vary from 5% to 65.5% of the total observations. The results show that sequential random forest method can estimate these missing values with a high level of accuracy. The RMSE varied between 2.5 and 5.0. missForest had the lowest imputation error for both continuous and categorical variables under each missing data rate (10%, 20%, and 30%) and had the smallest prediction error difference when the models used the imputed laboratory values.

Endoscopic internal drainage by double pigtail stents in the management of laparoscopic sleeve gastrectomy leaks

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BACKGROUND
Leaks and fistulas after laparoscopic sleeve gastrectomy (SG) are major adverse events of bariatric surgery. Endoscopic management of post-SG leaks has evolved from closure with covered self-expanding metallic stents to endoscopic internal drainage (EID).

OBJECTIVE
To report our experience with the management of post-SG leaks treated with EID, either as primary therapy or after failure of closure therapy with self-expanding metallic stents.

SETTING
Single-center observational study.

METHODS
A retrospective study of 20 patients treated for post-SG leaks with EID by deployment of double pigtail stents across the leak orifice, positioning one end inside the collection and the other end in the lumen of the stomach.

RESULTS
There were 13 (65%) males and 7 (35%) females with a mean age of 34.2 ± 11.6 years. EID was performed after a mean 62 days after SG. Three patients had gastrobronchial fistula. Seventeen (85%) patients had failed some form of prior therapy for the leak. The mean duration of EID was 83 days and 17 (85%) patients had complete healing of the leak with a mean follow-up of 16 months. There were 2 (10%) adverse events and no mortalities. The success of EID in healing post-SG leak was significantly associated with the absence of a gastrobronchial fistula (P < .05).

CONCLUSIONS
EID is an effective and safe endoscopic treatment of leaks after SG and is well tolerated. It allows early feeding and has fewer adverse events than other techniques. The presence of a gastrobronchial fistula is associated with higher failure rates. Long-term follow-up confirms a good outcome with no mortality.

Physical activity and sedentary behaviors among active college students in Kuwait relative to gender status

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OBJECTIVES
To determine the prevalence of physical activity (PA), sedentary behaviors (SB) and sleep duration and the associations between those variables among Kuwaiti Physical Education (PE) College students.

METHODS
A total of 418 participants (220 females) were randomly selected from the Basic Education College in the Public Authority for Applied Education and Training in Kuwait. Body weight and height were measured, and body mass index (BMI) was calculated. A validated questionnaire was used to assess lifestyle behaviors.

RESULTS
Based on BMI classification, the prevalence of overweight or obesity among males (34.9%) was significantly higher than that of females (16.7%). However, the mean (SD) of body fat percentage using bioelectrical impedance analysis was 21.3 (9.0) for males and 32.3 (7.7) for females. The proportions of highly active (> 1,200 METs-min/week) males (85.9%) and females (64.3%) were significantly (p < 0.005) different. The participants exceeding 3 hours of screen viewing time/day and insufficient sleep duration (< 7 hours/night) were 76.8% and 65.1%, respectively, with no gender differences. Logistic regression, adjusted for confounders, showed that highly active PE students had a higher proportion of SB. However, neither sleep duration nor body fat percentage exhibited any significant difference relative to PA.

CONCLUSIONS
Despite having high PA, Kuwaiti PE College students had a high prevalence of SB and insufficient sleep. Gender differences were found in PA but not in screen time or sleep duration. Efforts toward reducing SB and insufficient sleep among Kuwaiti PE College students are needed to reduce unhealthy lifestyle behaviors and promote health and well-being.

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1193rd International Conference on Recent Advances in **Medical and Health Sciences**
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1183rd International Conference on **Science, Health and Medicine**
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1223rd International Conference on **Medical and Biosciences**
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International Conference on **Science, Health and Medicine**
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1263ʳᵈ International Conference on Recent Advances in **Medical and Health Sciences**
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1122ⁿᵈ International Conference on **Pharma and Food**
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Apr 30, 2022
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Email: info.conferenceonline@gmail.com
Event website: http://www.conferenceonline.net/
Conference/293/ICOVT/

WHO-Facts Sheet

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1. BRUCELLOSIS

KEY FACTS
- Brucellosis is found globally and is a reportable disease in most countries
- The disease causes flu-like symptoms, including fever, weakness, malaise and weight loss
- Person-to-person transmission is rare
- Brucellosis is a bacterial disease caused by various Brucella species, which mainly infect cattle, swine, goats, sheep and dogs

Brucellosis is a bacterial disease caused by various *Brucella* species, which mainly infect cattle, swine, goats, sheep and dogs. Humans generally acquire the disease through direct contact with infected animals, by eating or drinking contaminated animal products or by inhaling airborne agents. Most cases are caused by ingesting unpasteurized milk or cheese from infected goats or sheep.

Brucellosis is one of the most widespread zoonoses transmitted by animals and in endemic areas, human brucellosis has serious public health consequences. Expansion of animal industries and urbanization, and the lack of hygienic measures in animal husbandry and in food handling, partly account for brucellosis remaining a public health hazard.

Who is at risk?
Brucellosis is found globally and is a reportable disease in most countries. It affects people of all ages and both sexes. In the general population, most cases are caused by the consumption of raw milk or its derivatives such as fresh cheese. Most of these cases are from sheep and goat products.

The disease is also considered an occupational hazard for people who work in the livestock sector. People who work with animals and are in contact with blood, placenta, foetuses and uterine secretions have an increased risk of contracting the disease. This method of transmission primarily affects farmers, butchers, hunters, veterinarians and laboratory personnel.

Worldwide, *Brucella melitensis* is the most prevalent species causing human brucellosis, owing in part to difficulties in immunizing free-ranging goats and sheep.

Human-to-human transmission is very rare.

Prevention and control
Prevention of brucellosis is based on surveillance and the prevention of risk factors. The most effective prevention strategy is the elimination of infection in animals. Vaccination of cattle, goats and sheep is recommended in enzootic areas with high prevalence rates. Serological or other testing and culling can also be effective in areas with low prevalence. In countries where eradication in animals through vaccination or elimination of infected animals is not feasible, prevention of human infection is primarily based on raising awareness, food-safety measures, occupational hygiene and laboratory safety.

Pasteurization of milk for direct consumption and for creating derivatives such as cheese is an important step to preventing transmission from animals to humans. Education campaigns about avoiding unpasteurized milk products can be effective, as well as policies on its sale.

In agricultural work and meat-processing, protective barriers and correct handling and disposal of afterbirths, animal carcasses and internal organs is an important prevention strategy.

Treatment and care

Brucellosis typically causes flu-like symptoms, including fever, weakness, malaise and weight loss. However, the disease may present in many atypical forms. In many patients the symptoms are mild and, therefore, the diagnosis may not be considered. The incubation period of the disease can be highly variable, ranging from 1 week to 2 months, but usually 2–4 weeks.

Treatment options include doxycycline 100 mg twice a day for 45 days, plus streptomycin 1 g daily for 15 days. The main alternative therapy is doxycycline at 100 mg, twice a day for 45 days, plus rifampicin at 15mg/kg/day (600-900mg) for 45 days. Experience suggests that streptomycin may be substituted with gentamicin 5mg/kg/daily for 7–10 days, but no study directly comparing the two regimes is currently available. The optimal treatment for pregnant women, neonates and children under 8 is not yet determined; for children, options include trimethoprim/ sulfamethoxazole (co-trimoxazole) combined with an aminoglycoside (streptomycin, gentamycin) or rifampicin.

WHO response

WHO provides technical advice to member states through provision of standards, information and guidance for the management of brucellosis in humans and animals. The Organization works to support the coordination and sharing of information between the public health and animal health sectors. In collaboration with the Food and Agricultural Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and the Mediterranean Zoonoses Control Programme (MZCP), WHO supports countries in the prevention and management of the disease through the Global Early Warning System for Major Animal Diseases (GLEWS).

2. CHILDREN: NEW THREATS TO HEALTH

KEY FACTS
- Greenhouse gas emissions leading to climate change and ecological degradation existentially threaten the lives of all children.
- Children are vulnerable to adverse health effects from indoor and outdoor air pollution which causes an estimated 7 million deaths per year (2016).
- Over 250 million children are at risk of not meeting their development potential (2017).
- There are 124 million children and adolescents affected by obesity (2016).
- Children are frequently exposed to commercial marketing promoting addictive substances and unhealthy commodities.
- Road injury is the leading cause of death for children and young people; more than 1 billion children are exposed to violence every year.

Overview

Children's survival, nutrition and education have improved dramatically over recent decades. But progress on indicators of child health and well-being is currently stalled across the Sustainable Development Goal (SDGs). No country is currently providing the conditions needed to support every child to grow up and have a healthy future.

Children (aged 0 to 18 years) today face a host of new threats linked to climate change, pollution, harmful commercial marketing, unhealthy lifestyles and diets, injury and violence, conflict, migration and inequality. Their very future is uncertain, and urgent action is needed to address these threats.

Some of the most important actions needed to protect children and ensure their future are: centering the child in every policy linked to the Sustainable Development Goal agenda;
- urgently reducing greenhouse gas emissions to fight the climate crisis;
- taking multisectoral action, coordinated at the highest levels of government;
- increasing funding and political prioritization of children; involving children and youth in the design of their future;
- enacting new national and international regulations to curb harmful commercial marketing, including an Optional Protocol to the UN Convention on the Rights of the Child; and
- improving reporting of data on children's health and well-being.

Investing in children's health, education and well-being brings substantial returns for societies. For every dollar invested in children, there is a benefit of around US$ 10 for many interventions and up to US$ 20 for some. Each dollar invested in health brings 20 times that in benefit in lower-middle income countries and nine times the benefit in low-income countries. Improving health and well-being in childhood benefits the individual throughout the life course and for generations to come.

Main health risks
Environmental threats

The lives of all children are existentially threatened by greenhouse gas emissions leading to climate change. Children's lives today, and future existence, are at risk from rising sea levels, extreme weather events, water and food insecurity, heat stress, emerging infectious diseases and large-scale population

migration. These issues are already affecting hundreds of millions of children today.

Urgent action is needed to reduce carbon emissions in order to keep warming below 1.5 °C and implement the provisions of the 2015 Paris Agreement on climate change.

Indoor and ambient (outdoor) air pollution are both responsible for an estimated 7 million deaths (2016). Air pollution is associated with poor childhood respiratory health; it impairs the lungs and the brain and increases the risk of cardiovascular disease, obesity, type 2 diabetes and metabolic syndrome across the child's lifespan.

Climate-related health risks are compounded amongst the 40% of the world's children who live in informal settlements where substandard housing, overcrowding, hazardous locations, unhealthy living conditions, poverty and poor access to basic services can harm their health and well-being.

Obesity and non-communicable diseases

The rapid rise in childhood obesity is one of the most serious public health challenges of the 21st century, with the number of children and adolescents affected by obesity increasing more than ten times from 11 million in 1975 to 124 million in 2016.

Children are frequently exposed to harmful commercial marketing, typically seeing tens of thousands of advertisements a year for addictive substances and unhealthy commodities including fast food and sugar-sweetened beverages which contribute to obesity and chronic diseases, as well as online gambling services, which can harm their relationships, school achievement, and mental health.

The marketing and inappropriate use of breastmilk substitutes (formula milk)— a US$ 70 billion industry — is associated with lowered intelligence, obesity, increased risk of diabetes and other non-communicable diseases, accounting for an estimated loss to society of US$ 302 billion.

- Evidence suggests that children in some countries see as many as 30 000 advertisements on television alone in a single year, many for harmful products.
- A review of 23 studies in Latin America reported that advertising exposure was associated with a preference for and purchase of unhealthy foods by families and children who are overweight and obese.
- In a sample of five- and six-year-olds in Brazil, China, India, Nigeria and Pakistan, 68% could identify at least one cigarette brand logo; 50% could do so in Russia and 86% in China.
- In a study of 11- to 14-year-olds from Los Angeles, United States, African-American youth were exposed to an average of four to one alcohol

advertisements per day.
- In Iran, food advertising during children's programs is dominated by food items that are potentially harmful to oral health, as are almost two thirds of food adverts during children's television in the United Kingdom.

The commercial threats to children's health are dangerously underappreciated. Children's high online exposure can also harm them when companies buy and sell their profiles for the purposes of commercial targeting. Children are also exposed to via bullying, exploitation, and contact with criminals and sexual predators.

Injuries, violence and conflict

Road injury is the leading cause of death for children and young people aged five to 29 years. And more than 1 billion children — half of all children — are exposed to violence every year.

In 2018, 1 billion people had moved or were on the move either as internally displaced persons, international migrants or refugees, including many children, as a result of conflict, violence or inequity and lack of opportunities.

WHO response

In 2010, the World Health Assembly, passed Resolution WHA63.14 Marketing of food and non-alcoholic beverages to children. In so doing, the Assembly endorsed a set of 12 recommendations which call for global action on marketing to children of food and drinks high in saturated fats, trans fatty acids, free sugars or salt.

In 2017, the WHO Western Pacific Region passed Resolution WPR/RC68.R3 Protecting children from the harmful impact of food marketing. It calls for accelerated, multisectoral and multi-stakeholder action; sharing of best practices; provision of technical support and advocacy; and more collaboration among countries on measuring and mitigating harmful impacts of food marketing.

In 2020, a WHO-UNICEF-Lancet Commission published the report A future for the world's children?, which is based on the expertise of more than 40 child health specialists from around the world. The report sets out new threats to child health and well-being and provides recommendations to ensure children's health today and in the future.

WHO and UNICEF are providing technical support to countries as well as communication and advocacy on the report's findings and messages globally. WHO will, inter alia:
- Work with Member States to foster a new legally binding and regularly monitored Optional Protocol to the Convention on the Rights of the Child to curb

harmful commercial marketing of fast foods, sugar-sweetened beverages, inappropriate use of breastmilk substitutes, alcohol and tobacco, and collate best practices;
- Develop a package of child health interventions involving other sectors as needed;
- Adopt a systematic process to generate and capture evidence for policy and programming;
- Provide integrated technical support to country child health programmes; and
- Develop a scorecard with partners for monitoring the implementation of the recommendations of the report.

3. ENDOMETRIOSIS

KEY FACTS
- Endometriosis is a disease where tissue similar to the lining of the uterus grows outside the uterus, causing pain and/or infertility (1).
- Endometriosis affects roughly 10% (190 million) of reproductive age women and girls globally (2).
- It is a chronic disease associated with severe, life-impacting pain during periods, sexual intercourse, bowel movements and/or urination, chronic pelvic pain, abdominal bloating, nausea, fatigue, and sometimes depression, anxiety, and infertility.
- The variable and broad symptoms of endometriosis mean that healthcare workers do not easily diagnose it and many individuals suffering from it have limited awareness of the condition. This can cause a lengthy delay between onset of symptoms and diagnosis (3).
- At present, there is no known cure for endometriosis, and treatment is usually aimed at controlling symptoms (4).
- Access to early diagnosis and effective treatment of endometriosis is important, but is limited in many settings, including in low- and middle-income countries.
- There is a need for more research and awareness raising around the world to ensure effective prevention, early diagnosis, and improved management of the disease (2,5).

Introduction and definition
Endometriosis is a disease characterized by the presence of tissue resembling endometrium (the lining of the uterus) outside the uterus (1). It causes a chronic inflammatory reaction that may result in the formation of scar tissue (adhesions, fibrosis) within the pelvis and other parts of the body. Several lesion types have been described (1,6):
- superficial endometriosis found mainly on the pelvic peritoneum

- cystic ovarian endometriosis (endometrioma) found in the ovaries
- deep endometriosis found in the recto-vaginal septum, bladder, and bowel
- in rare cases, endometriosis has also been found outside the pelvis

Symptoms associated with endometriosis vary, and include a combination of:
- painful periods
- chronic pelvic pain
- pain during and/or after sexual intercourse
- painful bowel movements
- painful urination
- fatigue
- depression or anxiety
- abdominal bloating and nausea

In addition to the above, endometriosis can cause infertility. Infertility occurs due to the probable effects of endometriosis on the pelvic cavity, ovaries, fallopian tubes or uterus. There is little correlation between the extent of endometrial lesions and severity or duration of symptoms: some individuals with visibly large lesions have mild symptoms, and others with few lesions have severe symptoms. Symptoms often improve after menopause, but in some cases painful symptoms can persist. Chronic pain may be due to pain centres in the brain becoming hyper-responsive over time (central sensitisation), which can occur at any point throughout the life course of endometriosis, including treated, insufficiently treated, and untreated endometriosis, and may persist even when endometriosis lesions are no longer visible. In some cases, endometriosis can be asymptomatic.

What is the cause of endometriosis?
Endometriosis is a complex disease that affects some women globally, from the onset of their first period (menarche) through menopause regardless of ethnic origin or social status. The exact origins of endometriosis are thought to be multifactorial, meaning that many different factors contribute to its development. Several hypotheses have been proposed to explain origins of endometriosis. At present endometriosis is thought to arise due to:
- Retrograde menstruation, which is when menstrual blood containing endometrial cells flows back through the fallopian tubes and into the pelvic cavity at the time that blood is flowing out of the body through the cervix and vagina during periods. Retrograde menstruation can result in endometrial-like cells being deposited outside the uterus where they can implant and grow.
- Cellular metaplasia, which is when cells change from one form to another. Cells outside the uterus change into endometrial-like cells and start to

grow.
- Stem cells giving rise to the disease, which then spreads through the body via blood and lymphatic vessels.

Other factors may also contribute to the growth or persistence of ectopic endometrial tissue. For example, endometriosis is known to be dependent on estrogen, which facilitates the inflammation, growth, and pain associated with the disease. However, the relationship between estrogen and endometriosis is complex since the absence of estrogen does not always preclude the presence of endometriosis. Several other factors are thought to promote the development, growth, and maintenance of endometriosis lesions. These include altered or impaired immunity, localized complex hormonal influences, genetics and potentially, environmental contaminants (2,7).

Health, social and economic benefits of addressing endometriosis

Endometriosis has significant social, public health and economic implications. It can decrease quality of life due to severe pain, fatigue, depression, anxiety, and infertility. Some individuals with endometriosis experience debilitating endometriosis-associated pain that prevents them from going to work or school (8,9). In these situations, addressing endometriosis can reduce absence from school or increase an individual's ability to contribute to the labour force. Painful sex due to endometriosis can lead to interruption or avoidance of intercourse and affect the sexual health of affected individuals and/or their partners (9). Addressing endometriosis will empower those affected by it, by supporting their human right to the highest standard of sexual and reproductive health, quality of life, and overall well-being.

Prevention

At present, there is no known way to prevent endometriosis. Enhanced awareness, followed by early diagnosis and management may slow or halt the natural progression of the disease and reduce the long-term burden of its symptoms, including possibly the risk of central nervous system pain sensitisation, but currently there is no cure.

Diagnosis

A careful history of menstrual symptoms and chronic pelvic pain provides the basis for suspecting endometriosis. Although several screening tools and tests have been proposed and tested, none are currently validated to accurately identify or predict individuals or populations that are most likely to have the disease. Early suspicion of endometriosis is a key factor for early diagnosis, as endometriosis can often present symptoms that mimic other conditions and contribute to a diagnostic delay. In addition to medical history, referral from the primary health care level to secondary centers where additional investigations are available may be needed. For instance, ovarian endometrioma, adhesions and deep nodular forms of disease often require ultrasonography or magnetic resonance imaging (MRI) to detect. Histologic verification, usually following surgical/laparoscopic visualization, can be useful in confirming diagnosis, particularly for the most common superficial lesions (1,2). The need for histologic/laparoscopic confirmation should not prevent the commencement of empirical medical treatment.

Treatment

Treatment can be with medications and/or surgery depending on symptoms, lesions, desired outcome, and patient choice (4). Contraceptive steroids, non-steroidal anti-inflammatory medications, and analgesics (painkillers) are common therapies. All must be carefully prescribed and monitored to avoid potentially problematic side effects. Medical treatments for endometriosis focus on either lowering estrogen or increasing progesterone in order to alter hormonal environments that promote endometriosis. These medical therapies include the combined oral contraceptive pill, progestins, and GnRH-analogues. However, none of these treatments eradicates the disease, they are associated with side effects, and endometriosis-related symptoms can sometimes - but not always - reappear after therapy discontinuation. The choice of treatment depends on effectiveness in the individual, adverse side effects, long-term safety, costs, and availability. Most current hormonal management is not suitable for persons suffering from endometriosis who wish to get pregnant, since they affect ovulation.

Surgery can remove endometriosis lesions, adhesions, and scar tissue. However, success in reducing pain symptoms and increasing pregnancy rates are often dependent on the extent of disease. In addition, lesions may recur even after successful eradication, and pelvic floor muscle abnormalities can contribute to chronic pelvic pain. Secondary changes of the pelvis, including the pelvic floor, and central sensitisation may benefit from physiotherapy and complementary treatments in some patients. Treatment options for infertility due to endometriosis include laparoscopic surgical removal of endometriosis, ovarian stimulation with intrauterine insemination (IUI), and in vitro fertilization (IVF), but success rates vary (4). Other comorbidities may occur alongside

endometriosis, requiring diagnosis and management. Multidisciplinary treatment addressing different symptoms and overall health should involve different specialists, such as pain specialists, pelvic physiotherapists, complementary and alternative medicine specialists, general practitioners, and psychologists, in addition to gynaecologists, to be most effective (2,10).

Addressing current challenges and priorities

In many countries, the general public and most front-line healthcare providers are not aware that distressing and life-altering pelvic pain is not normal, leading to a normalisation and stigmatisation of symptoms and significant diagnostic delay (2,3). Patients who could benefit from medical symptomatic management are not always provided with treatments due to limited awareness of endometriosis among primary healthcare providers. Due to diagnostic delays, prompt access to available treatment methods, including non-steroidal analgesics (painkillers), oral contraceptives and progestin-based contraceptives is often not achieved. Due to limited capacity of health systems in many countries, access to specialised surgery for those who need it is sub-optimal. In addition, and especially in low and middle-income countries, there is a lack of multi-disciplinary teams with the wide range of skills and equipment needed for the early diagnosis and effective treatment of endometriosis. Although primary health care professionals should play a role in screening and basic management of endometriosis, tools to screen and accurately predict patients and populations who are most likely to have the disease are lacking. In addition, many knowledge gaps exist, and there is need for non-invasive diagnostic methods as well as medical treatments that do not prevent pregnancy.

Subsequently, some of the current priorities related to endometriosis include:
- Raising awareness about endometriosis among health care providers, women, men, adolescents, teachers and wider communities. Local, national and international information campaigns to educate the public and healthcare providers about normal and abnormal menstrual health and symptoms are needed.
- Training all healthcare providers to improve their competency and skills to screen, diagnose, manage, or refer patients with endometriosis. This can range from basic training of primary healthcare providers to recognize endometriosis, to the advanced training of specialist surgeons and multidisciplinary teams.

- Ensuring that primary health care plays a role in screening, identifying and providing basic pain management of endometriosis, in situations where gynaecologists or advanced multidisciplinary specialists are unavailable.
- Advocating for health policies that ensure access to at least a minimum level of treatment and support for patients with endometriosis.
- Setting up referral systems and care pathways consisting of well-linked primary healthcare centres and secondary and tertiary centres with advanced imaging, pharmacologic, surgical, fertility and multi-disciplinary interventions.
- Strengthening capacity of health systems to achieve early diagnosis and management of endometriosis by enhancing availability of equipment (e.g. ultrasound or magnetic resonance imaging) and pharmaceuticals (e.g. non-steroidal analgesics, combined oral contraceptives and progestin-based contraceptives).
- Increasing research on the pathogenesis, pathophysiology, natural progression, genetic and environmental risk factors, prognosis, disease classification, non-invasive diagnostic biomarkers, personalized treatments and other treatment paradigms, role of surgery, novel targeted therapeutics, curative therapies, and preventive interventions in endometriosis (2,5).
- Accelerating collaborative global action to improve access to reproductive health care for women globally, including in low- and middle-income countries.

WHO response

The World Health Organization (WHO) recognizes the importance of endometriosis and its impact on people's sexual and reproductive health, quality of life, and overall well-being. WHO aims to stimulate and support the adoption of effective policies and interventions to address endometriosis globally, especially in low and middle-income countries. WHO is partnering with multiple stakeholders, including academic institutions, non-state actors and other organizations that are actively involved in research to identify effective models of endometriosis prevention, diagnosis, treatment, and care. WHO recognises the importance of advocating for increased awareness, policies and services for endometriosis, and collaborates with civil society and endometriosis patient support groups in this regard. WHO is also collaborating with relevant stakeholders to facilitate and support the collection and analysis of country- and region-specific endometriosis prevalence data for decision making.

REFERENCES

1. World Health Organization (WHO). International Classification of Diseases, 11th Revision (ICD-11) Geneva: WHO 2018.
2. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med 2020; 382:1244-56.
3. Agarwal SK, Chapron C, Giudice LC, et al. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol 2019(4):354-64.
4. Johnson NP, Hummelshoj L, World Endometriosis Society Montpellier Consortium. Consensus on current management of endometriosis. Hum Reprod 2013;28(6):1552-68.
5. Horne AW, Saunders PTK, Abokhrais IM, et al. Top ten endometriosis research priorities in the UK and Ireland. Lancet 2017; 389:2191-92.
6. Johnson NP, Hummelshoj L, Adamson GD, et al. World Endometriosis Society consensus on the classification of endometriosis. Hum Reprod 2017;32(2):315-24.
7. Wen X, Xiong Y, Qu X, et al. The risk of endometriosis after exposure to endocrine-disrupting chemicals: a meta-analysis of 30 epidemiology studies. Gynecol Endocrinol 2019;(35):645-50.
8. Nnoaham K, Hummelshoj L, Webster P, et al. Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries. Fertil Steril 2011;96(2):366-73.e8.
9. Culley L, Law C, Hudson N, et al. The social and psychological impact of endometriosis on women's lives: a critical narrative review. Hum Reprod Update, 2013;19(6):625-639.
10. Carey ET, Till SR, As-Sanie S. Pharmacological management of chronic pelvic pain in women. Drugs 2017;77:285-301.

4. HEPATITIS C

KEY FACTS
- Hepatitis C is an inflammation of the liver caused by the hepatitis C virus.
- The virus can cause both acute and chronic hepatitis, ranging in severity from a mild illness to a serious, lifelong illness including liver cirrhosis and cancer.
- The hepatitis C virus is a bloodborne virus and most infection occur through exposure to blood from unsafe injection practices, unsafe health care, unscreened blood transfusions, injection drug use and sexual practices that lead to exposure to blood.
- Globally, an estimated 58 million people have chronic hepatitis C virus infection, with about 1.5 million new infections occurring per year.
- WHO estimated that in 2019, approximately 290 000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).
- Antiviral medicines can cure more than 95% of persons with hepatitis C infection, but access to diagnosis and treatment is low.
- There is currently no effective vaccine against hepatitis C.

Overview
Hepatitis C virus (HCV) causes both acute and chronic infection. Acute HCV infections are usually asymptomatic and most do not lead to a life-threatening disease. Around 30% (15–45%) of infected persons spontaneously clear the virus within 6 months of infection without any treatment.

The remaining 70% (55–85%) of persons will develop chronic HCV infection. Of those with chronic HCV infection, the risk of cirrhosis ranges from 15% to 30% within 20 years.

Geographical distribution
HCV occurs in all WHO regions. The highest burden of disease is in the Eastern Mediterranean Region and European Region, with 12 million people chronically infected in each region. In the South-East Asia Region and the Western Pacific Region, an estimated 10 million people in each region are chronically infected. Nine million people are chronically infected in the African Region and 5 million the Region of the Americas.

Transmission
The hepatitis C virus is a bloodborne virus. It is most commonly transmitted through:
- the reuse or inadequate sterilization of medical equipment, especially syringes and needles in healthcare settings;
- the transfusion of unscreened blood and blood products; and
- injecting drug use through the sharing of injection equipment.

HCV can be passed from an infected mother to her baby and via sexual practices that lead to exposure to blood (for example, people with multiple sexual partners and among men who have sex with men); however, these modes of transmission are less common.

Hepatitis C is not spread through breast milk, food, water or casual contact such as hugging, kissing and sharing food or drinks with an infected person.

Symptoms
The incubation period for hepatitis C ranges from 2 weeks to 6 months. Following initial infection, approximately 80% of people do not exhibit any

symptoms. Those who are acutely symptomatic may exhibit fever, fatigue, decreased appetite, nausea, vomiting, abdominal pain, dark urine, pale faeces, joint pain and jaundice (yellowing of skin and the whites of the eyes).

Testing and diagnosis

Because new HCV infections are usually asymptomatic, few people are diagnosed when the infection is recent. In those people who go on to develop chronic HCV infection, the infection is often undiagnosed because it remains asymptomatic until decades after infection when symptoms develop secondary to serious liver damage.

HCV infection is diagnosed in 2 steps:

1. Testing for anti-HCV antibodies with a serological test identifies people who have been infected with the virus.
2. If the test is positive for anti-HCV antibodies, a nucleic acid test for HCV ribonucleic acid (RNA) is needed to confirm chronic infection because about 30% of people infected with HCV spontaneously clear the infection by a strong immune response without the need for treatment. Although no longer infected, they will still test positive for anti-HCV antibodies.

After a person has been diagnosed with chronic HCV infection, an assessment should be conducted to determine the degree of liver damage (fibrosis and cirrhosis). This can be done by liver biopsy or through a variety of non-invasive tests. The degree of liver damage is used to guide treatment decisions and management of the disease.

Early diagnosis can prevent health problems that may result from infection and prevent transmission of the virus. WHO recommends testing people who may be at increased risk of infection.

In settings with high HCV antibody seroprevalence in the general population (defined as >2% or >5% HCV antibody seroprevalence), WHO recommends that all adults have access to and be offered HCV testing with linkage to prevention, care and treatment services.

About 2.3 million people (6.2%) of the estimated 37.7 million living with HIV globally have serological evidence of past or present HCV infection. Chronic liver disease represents a major cause of morbidity and mortality among persons living with HIV globally.

Treatment

A new infection with HCV does not always require treatment, as the immune response in some people will clear the infection. However, when HCV infection becomes chronic, treatment is necessary. The goal of hepatitis C treatment is to cure the disease.

WHO recommends therapy with pan-genotypic direct-acting antivirals (DAAs) for persons over the age of 12 years. DAAs can cure most persons with HCV infection, and treatment duration is short (usually 12 to 24 weeks), depending on the absence or presence of cirrhosis.

Pan-genotypic DAAs remain expensive in many high- and upper-middle-income countries. However, prices have dropped dramatically in many countries (primarily low-income and lower-middle-income countries) due to the introduction of generic versions of these medicines.

Access to HCV treatment is improving but remains too limited. Of the 58 million persons living with HCV infection globally in 2019, an estimated 21% (15.2 million) knew their diagnosis, and of those diagnosed with chronic HCV infection, around 62% (9.4 million) persons had been treated with DAAs by the end of 2019.

Prevention

There is no effective vaccine against hepatitis C so prevention depends on reducing the risk of exposure to the virus in health care settings and in higher risk populations. This includes people who inject drugs and men who have sex with men, particularly those infected with HIV or those who are taking pre-exposure prophylaxis against HIV.

Primary prevention interventions recommended by WHO include:
- safe and appropriate use of health care injections;
- safe handling and disposal of sharps and waste;
- provision of comprehensive harm-reduction services to people who inject drugs;
- testing of donated blood for HBV and HCV (as well as HIV and syphilis);
- training of health personnel; and
- prevention of exposure to blood during sex.

WHO response

In May 2016, the World Health Assembly adopted the first *Global health sector strategy on viral hepatitis, 2016-2021*. The strategy highlights the critical role of universal health coverage and sets targets that align with those of the Sustainable Development Goals. The strategy aims to eliminate viral hepatitis as a public health problem by reducing new viral hepatitis infections by 90% and reduce deaths due to viral hepatitis by 65% by 2030.

WHO is working in the following areas to support countries in moving towards achieving the global hepatitis goals under the Sustainable Development Agenda 2030:
- raising awareness, promoting partnerships and mobilizing resources;
- formulating evidence-based policy and data for

action;
- increase health equities within the hepatitis response;
- preventing transmission; and
- scaling up screening, care and treatment services.

WHO organizes the annual World Hepatitis Day campaign (as 1 of its 9 flagship annual health campaigns) to increase awareness and understanding of viral hepatitis. For World Hepatitis Day 2021, WHO is focusing on the theme "Hepatitis Can't wait" to highlight the urgency of hepatitis elimination with a view to achieving the 2030 elimination targets.

5. MENINGITIS

KEY FACTS
- Meningitis is a devastating disease with a high case fatality rate and leading to serious long-term complications (sequelae).
- Meningitis remains a major global public-health challenge.
- Epidemics of meningitis are seen across the world, particularly in sub-Saharan Africa.
- Many organisms can cause meningitis including bacteria, viruses, fungi, and parasites.
- Bacterial meningitis is of particular concern. Around 1 in 10 people who get this type of meningitis die and 1 in 5 have severe complications.
- Safe affordable vaccines are the most effective way to deliver long-lasting protection.

The focus of this fact sheet is on the four main causes of acute bacterial meningitis:
- *Neisseria meningitidis* (meningococcus)
- *Streptococcus pneumoniae* (pneumococcus)
- *Haemophilus influenzae*
- *Streptococcus agalactiae* (group B streptococcus)

These bacteria are responsible for more than half of the deaths from meningitis globally and they cause other severe diseases like sepsis and pneumonia.

Other bacteria e.g., *Mycobacterium tuberculosis*, Salmonella, Listeria, Streptococcus and Staphylococcus, viruses such as enteroviruses and mumps, fungi especially Cryptococcus, and parasites like Amoeba are also important causes of meningitis.

Who is at risk?
Although meningitis affects all ages, young children are most at risk. Newborn babies are at most risk from Group B streptococcus, young children are at higher risk from meningococcus, pneumococcus and Haemophilus influenzae. Adolescents and young adults are at particular risk of meningococcal disease while the elderly are at particular risk of pneumococcal disease.

People all over the world are at risk of meningitis.

The highest burden of disease is seen in a region of sub-Saharan Africa, known as the African Meningitis Belt, especially recognised to be at high risk of epidemics of meningococcal but also pneumococcal meningitis.

Higher risk is seen when people are living in close proximity, for example at mass gatherings, in refugee camps, in overcrowded households or in student, military and other occupational settings. Immune deficiencies such as HIV infection or complement deficiency, immunosuppression, and active or passive smoking can also raise the risk of different types of meningitis.

Transmission
The route of transmission varies by organism. Most bacteria that cause meningitis such as meningococcus, pneumococcus and *Haemophilus influenzae* are carried in the human nose and throat. They spread from person to person by respiratory droplets or throat secretions. Group B streptococcus is often carried in the human gut or vagina and can spread from mother to child around the time of birth.

Carriage of these organisms is usually harmless and helps build up immunity against infection, but the bacteria occasionally invade the body causing meningitis and sepsis.

Signs and symptoms
The incubation period is different for each organism and can range between two and 10 days for bacterial meningitis. Since bacterial meningitis is often accompanied by sepsis, the signs and symptoms cover both conditions.

Signs and symptoms can include:
- severe headache
- stiff or painful neck
- high fever
- avoiding bright light
- drowsy, confused, comatose
- convulsions
- rash
- joint pains
- cold hands and feet
- vomiting

In babies, signs can include:
- poor feeding
- sleepy, difficult to wake, comatose
- irritable, crying when handled
- difficulty breathing, grunting
- fever
- neck rigidity
- bulging soft spot on top of head (fontanelle)
- high pitched cry
- convulsions

- vomiting
- rash
- pale or blotchy skin

Prevention

Preventing meningitis through vaccination is the most effective way to reduce the burden and impact of the disease by delivering long-lasting protection.

Antibiotics are also used to help prevent infection in those at high risk of meningococcal and group B streptococcal disease. Controlling epidemics of meningococcal meningitis relies on both vaccination and antibiotics.

1. Vaccination

Licensed vaccines against meningococcal, pneumococcal and *Haemophilus influenzae* disease have been available for many years. These bacteria have several different strains (known as serotypes or serogroups) and vaccines are designed to protect against the most harmful strains. Over time, there have been major improvements in strain coverage and vaccine availability, but no universal vaccine against these infections exists.

Meningococcus

The meningococcus has 12 serogroups, with A,B,C,W,X,and Y causing most meningitis.

There are three types of vaccine available:

- Polysaccharide-protein conjugate vaccines (conjugate vaccines) are used in prevention and outbreak response:
  - They confer longer-lasting immunity, and also prevent carriage, thereby reducing transmission and leading to herd protection.
  - They are effective in protecting children under two years of age.
  - Vaccines are available in different formulations:
    - monovalent vaccines (serogroup A or C)
    - tetravalent vaccines (serogroups A, C, W, Y).
    - in combination (serogroup C and *Haemophilus influenzae* type b)
- Protein based vaccines against serogroup B. These vaccines protect against meningitis in all ages but are not thought to prevent carriage and transmission so do not lead to herd protection.
- Polysaccharide vaccines are safe and effective in children and adults, but weakly protective in infants. Protection is short-lived and they do not lead to herd protection as they do not prevent carriage. They are still used for outbreak control but are being replaced by conjugate vaccines.

Global public health response – elimination of meningococcal A meningitis epidemics in the African meningitis belt

In the African meningitis belt, meningococcus serogroup A accounted for 80–85% of meningitis epidemics before the introduction of a meningococcal A conjugate vaccine through mass preventive campaigns (since 2010) and into routine immunization programmes (since 2016). As of April 2021, 24 of the 26 countries in the meningitis belt have conducted mass preventive campaigns targeting 1-29 year olds (nationwide or in high-risk areas), and half of them have introduced this vaccine into their national routine immunization schedules. Among vaccinated populations, incidence of serogroup A meningitis has declined by more than 99% - no serogroup A case has been confirmed since 2017. Continuing introduction into routine immunization programmes and maintaining high coverage is critical to avoid the resurgence of epidemics.

Cases of meningitis and outbreaks due to other meningococcal serogroups, apart from serogroup B, continue to strike. The roll out of multivalent meningococcal conjugate vaccines is a public health priority to eliminate bacterial meningitis epidemics in the African Meningitis Belt.

Pneumococcus

The pneumococcus has over 97 serotypes, 23 causing most disease.

- Conjugate vaccines are effective from 6 weeks of age at preventing meningitis and other severe pneumococcal infections and are recommended for infants and children up to the age of 5 years, and in some countries for adults aged over 65 years, as well as individuals from certain risk groups. Two different conjugate vaccines are in use that protect against 10 and 13 serotypes. New conjugate vaccines designed to protect against more pneumococcal serotypes are either in development or have been approved for use in adults. Research continues into protein based vaccines.
- A polysaccharide vaccine against 23 serotypes is available but, as for other polysaccharide vaccines, this type of vaccine is considered less effective than conjugate vaccines. It is used mostly in those aged over 65 years to protect against pneumonia, as well as in individuals from certain risk groups. It is not used in children under 2 years of age and is less useful in protecting against meningitis.

Haemophilus influenzae

Haemophilus influenzae has 6 serotypes, serotype b causing most meningitis.

- Conjugate vaccines protect specifically against *Haemophilus influenzae* serotype b (Hib). They are highly effective in preventing Hib disease and are recommended for routine use in infant vaccine schedules.

Group B streptococcus

Group B streptococcus has 10 serotypes, 1a, 1b, II, III, IV and V causing most disease.

- Conjugate and protein vaccines designed to protect against group B streptococcal disease in mothers and babies are in clinical development.
  2. Antibiotics for prevention (chemoprophylaxis)

Meningococcus

Antibiotics for close contacts of those with meningococcal disease, when given promptly, decreases the risk of transmission. Outside the African meningitis belt, chemoprophylaxis is recommended for close contacts within the household. Within the meningitis belt, chemoprophylaxis for close contacts is recommended in non-epidemic situations. Ciprofloxacin is the antibiotic of choice, and ceftriaxone an alternative.

Group B streptococcus

Identifying mothers whose babies are at risk of getting Group B streptococcal disease is recommended in many countries. One way to do this is by universal screening for carriage of Group B streptococcus in pregnancy. Mothers at risk are offered intravenous penicillin during labour to prevent their babies developing Group B streptococcal infection.

Diagnosis

Initial diagnosis of meningitis can be made by clinical examination followed by a lumbar puncture. The bacteria can sometimes be seen in microscopic examinations of the spinal fluid. The diagnosis is supported or confirmed by growing the bacteria from specimens of cerebrospinal fluid or blood, by rapid diagnostic tests or by polymerase chain reaction (PCR). The identification of the serogroups and susceptibility to antibiotics are important to define control measures. Molecular typing and whole genome sequencing identify more differences between strains and inform public health responses.

Treatment

Meningitis is fatal in up to half of patients, when left untreated, and should always be viewed as a medical emergency. Admission to a hospital or health centre is necessary. Isolation of the patient is not usually advised after 24 hours of treatment.

Appropriate antibiotic treatment must be started as soon as possible in bacterial meningitis. Ideally, lumbar puncture should be done first as antibiotics can make it more difficult to grow bacteria from the spinal fluid. However, blood sampling can also help to identify the cause and the priority is to start treatment

without delay. A range of antibiotics is used to treat meningitis, including penicillin, ampicillin, and ceftriaxone. During epidemics of meningococcal and pneumococcal meningitis, ceftriaxone is the drug of choice.

Complications and sequelae

One in five people surviving an episode of bacterial meningitis may have long lasting after-effects. These after-effects include hearing loss, seizures, limb weakness, difficulties with vision, speech, language, memory, and communication, as well as scarring and limb amputations after sepsis.

Support and after-care

Meningitis sequelae can have an enormous impact on individuals, families and communities, both financially and emotionally. Sometimes, complications such as deafness, learning impairment or behavioural problems are not recognized by carers and healthcare workers and therefore go untreated.

Those who have lived through meningitis often have health-care needs requiring long-term medical treatments. The ongoing psychosocial impacts of disability from meningitis can have medical, educational, social and human rights-based implications. Despite the high burden of meningitis sequelae on people with meningitis, their families and the community, access to both services and support for these conditions is often insufficient, especially in low and middle income countries. Individuals and families with members disabled by meningitis should be encouraged to seek servies and guidance from local and national Organizations of Disabled People (ODPs) and other disability focused organizations, which can provide vital advice about legal rights, economic opportunities and social engagement to ensure people disabled by meningitis are able to live full and rewarding lives.

Surveillance

Surveillance, from case detection to investigation and laboratory confirmation is essential to the control of meningitis. Main objectives include:

- Detect and confirm outbreaks.
- Monitor the incidence trends, including the distribution and evolution of serogroups and serotypes.
- Estimate the disease burden.
- Monitor the antibiotic resistance profile.
- Monitor the circulation, distribution, and evolution of specific strains (clones).
- Estimate the impact of meningitis control strategies, particularly preventive vaccination programmes

WHO response

The global roadmap "Defeating Meningitis by 2030" was developed by WHO with the support of many partners. The strategy was approved in the first ever resolution on meningitis by the World Health Assembly in 2020 and endorsed unanimously by WHO member states.

The roadmap sets a comprehensive vision "Towards a world free of meningitis" and has three visionary goals:
- Elimination of bacterial meningitis epidemics.
- Reduction of cases of vaccine-preventable bacterial meningitis by 50% and deaths by 70%.
- Reduction of disability and improvement of quality of life after meningitis due to any cause.

It sets a path to achieve goals, through concerted actions across five interconnected pillars:
- Prevention and epidemic control focused on the development of new affordable vaccines, achievement of high immunization coverage, improvement of prevention strategies and response to epidemics.
- Diagnosis and treatment, focused on speedy confirmation of meningitis and optimal patient care.
- Disease surveillance to guide meningitis prevention and control.
- Care and support of those affected by meningitis, focusing on early recognition and improved access to care and support for complications from meningitis, and

- Advocacy and engagement, to ensure high awareness of meningitis, to promote country engagement and to affirm the right to prevention, care, and after-care services.

In a complementary initiative, the WHO is working on the I*ntersectoral global action plan on epilepsy and other neurological disorders* in consultation with Member States to address many challenges and gaps in providing care and services for people with epilepsy and other neurological disorders that exist worldwide. Human rights for people affected by disability are also recognised and addressed in the WHO Global Disability Action Plan in alignment with the Convention on the Rights of the Child and the Convention on the Rights of Persons with Disability (CRPD) and in a landmark resolution on attaining the highest standard of health for persons with disabilities adopted at the 74th World Health Assembly.

While the road map on defeating meningitis addresses all meningitis regardless of the cause, it primarily focuses on the main causes of acute bacterial meningitis (meningococcus, pneumococcus, *Haemophilus influenzae* and group B streptococcus). These bacteria were responsible for over 50% of the 250,000 deaths from all-cause meningitis in 2019. They also cause other severe diseases like sepsis and pneumonia. For each of these infections, vaccines are either available, or in the case of group B streptococcus, likely to become available in the next few years.

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