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EDITORIAL

REVIEW ARTICLE

ORIGINAL ARTICLES

CASE REPORTS

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C O N T E N T S

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Burrows B, Lebowitz MD. The β agonists dilemma (editorial). N Engl J Med 1992; 326:560-561.

Book

Roberts NK. The cardiac conducting system and His bundle electrogram. New York, Appleton-Century-Crofts, 1981; 49-56.

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Editorial

Ban on Gene Patenting-A Boon to Mankind

Belle M Hegde
The Journal of the Science of Healing Outcomes, State College, Pennsylvania, USA and Mangalore, India*
Manipal University, Manipal India**
The Middlesex Medical School, University of London, UK#
Northern Colorado University, USA##

Kuwait Medical Journal 2014; 46 (2): 99 - 100

"Sometimes the questions are complicated and the answers are simple."

Dr. Seuss

Recently, the United States Supreme Court ruled that gene patenting is illegal[1]. This is a sensational judgment and a step forward for mankind. I have not been a votary of the intellectual property rights and patenting business anyway. The recent judgement of the highest court in the USA is a greater step ahead for mankind compared to Neil Armstrong landing on the moon! The latter only speeded up the arms race between the two great rivals during the cold war.

"The Supreme Court's unanimous ruling, announced recently, that human genes may not be patented, generated extensive media coverage in the USA, with nearly nine minutes devoted to the ruling on network newscasts, and on the front pages of several major newspapers. The ruling was seen as a powerful statement by the Court on an important intellectual property issue that could have lasting effects on the biotech industry. Many sources also pointed out the ruling could mean that more patients will have access to genetic testing," writes the American College of Cardiology's web site on June 13th 2013[2]

The genetic testing and genetic engineering efforts in modern medicine today have been a boondoggle for the simple reason that the human meta-genome just has 25,000 odd human genes while there are nearly three trillion germ genes alongside[3]. How could one make any dent in the outcome when one is only able to manipulate a microscopic minority of the genome? But the genetic engineering is all about that. Medical science of reductionism, an inexact science at that, has been a great curse for mankind. A recent book, Biocentrism, by a thinking physician turned astrophysicist, Robert Lanza makes matters still clearer[4].

When one understands quantum physics and quantum biology everything becomes clear. Our dependence on randomised controlled trials looks foolish if one understands sub-atomic biology and 'quantum non-locality'. Human body works as a whole and not in bits and pieces as is being taught now. Trying to tamper with genes or trying to predict the future from gene studies is like the astrologer predicting the future of man. While we are quick at calling astrology a myth we foolishly believe that reductionist science of medicine is pure "science." When one applies a little ointment on the dorsum of the hand even the brain cells show changes in them in response, as documented by Fritz-Albert Popp's bio-photon camera[5].

Medicine since Hippocrates has been able to survive basically due to ignorance of the masses and the statistical trickery in manipulating the data which in themselves are unreliable anyway. We were bleeding, purging and giving emetics to our patients to get the bad humours out for nearly 2500 years without any one questioning that dogma[6]. We are in the same spot today with no one questioning this dogma of genetic engineering and RCTs. Some of us are ignorant of the scientific developments but the majority of us is happy the way we practise medicine as it nets billions of dollars in cash and also a status symbol for doctors and hospitals. Give you one example here would be compelling. While 89% the science of cancer and its management are found to be fraudulent there is so much claptrap about our cancer industry even today. No one dares to challenge this myth of cancer. Genetic engineering,

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**Editor in Chief; ** Cardiologist & Former Vice Chancellor (Retd); #Former Visiting Professor of Cardiology; ##Affiliate Professor of Human Health*

organ transplants, coronary revascularisations and many such quick fixes are just simple myths waiting to be demolished[7].

Energy runs this universe and hence energy runs the human system. Human body is but a bundle of energy and is the other face of the human mind. Naturally holistic disease management must also depend on energy. Good news is that there are pockets of awareness in the world. These oases in the vast desert of ignorance in modern medicine will have to be harnessed and developed for mankind's good. In that direction this week's US Supreme Court judgement should be a great boon to mankind.

All energy comes from the sun through electromagnetic rays. The first to take advantage of this energy was the plant kingdom by harnessing this energy to make chlorophyll from carbon dioxide and water thus making more oxygen available for the animal kingdom to breathe. Chlorophyll is our food base even if one eats meat! Then came the human species to take advantage of the sun's energy directly through the semi-conductors in the human skin. Professor Robert Becker, a famous orthopaedic surgeon in New York, was the first to show all these and use energy to heal infected and complicated fractures. He also devised simple tests to diagnose cancer using electric mapping of the skin areas. He was, of course, demonised and harassed as he questioned the medical dogmas. His book Body 'Electric' should be the Bible for every medical person, young and old alike[8]. The photon light emitted from each atom in our body could now be photographed to show the energy pattern of the body making a clear difference between healthy patterns where the human body cells are in synch while absence of this happy state denotes disease[9].

Naturally the next question would be to make energy treatment for healing be the next logical step ahead. We, at the World Academy of Authentic Healing Sciences, [www.waahs.com) a charitable body of scientists from all over the world, have been working with many kinds of energy healing methods to authenticate them scientifically. We are more than happy with our success. The beauty of it all is that these scientific treatments have no side effects. The demon of Adverse Drug Reactions (ADR) is not a bother for the patient either. The results are published elsewhere[10]. There are three known energies-electromagnetic, nuclear and gravitational, which together form just about 5% of the universal energy source while a vast majority of 95% energy remains occult to date as the inadequate science of reductionism does not know how to measure that huge sea of energy which could be made use of for human good.

The Supreme Court in the US gave a very sensible judgment for which the whole world will be grateful as the US laws affect all our lives with Angelina Jolie's example being followed by millions round the world as she is an icon. Making decisions based on a single gene in a holistic dynamic human system is the most ridiculous of all ideas in the name of science of genetic prediction. Professor William Firth writes in the 'British Medical Journal' that doctors have been predicting the unpredictable future of their patients[11]. In the bargain if they make money still better. Let sanity prevail and mankind be happy.

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Review Article

Painless Labor and Delivery

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ABSTRACT

Labor is one of the most painful situations. Unrelieved labor pain can have adverse effects both on the mother as well as the baby. In this review article we summarized the clinical application of various methods used for painless labor and delivery, especially, the new development in epidural anesthesia and analgesia (EA) technique. We discuss the best time to introduce the epidural catheter and when to start the local anesthetic drug through epidural catheter. In the light of newer studies, we compared the mode of epidural drug delivery, continuous infusion or intermittent boluses and different adjuncts to local anesthetic used in modern practice for this purpose. In various recent research studies it was found that the volume of local anesthetic drug is more important in relieving the labor pain and providing satisfaction to patient than the drug concentration. In the end, we discuss the various complications and their prevention.

KEY WORDS: epidural anesthesia, epidural anesthetic drugs, epidural catheter, painless labor

INTRODUCTION

The first recorded use of an epidural analgesia (EA) was in 1885, when New York neurologist J. Leonard Corning injected cocaine into the back of a patient suffering from "spinal weakness and seminal incontinence[1]. Labor is one of the most painful situations, more painful than cancer pain and more painful than cutting a finger without anesthesia. Unrelieved labor pain can have adverse effects on the course of labor as well as on the fetal well-being. Labor is characterized by painful uterine contractions that increase in frequency and are associated with progressive cervical effacement and dilatation. Labor has been divided into three stages. The first stage occurs from onset of cervical changes to 10 centimeter dilatation. The second stage occurs from full cervical dilatation (10 cm) to delivery of the baby. Normally the second stage lasts for 2 - 3 hours in primipara and 1 - 2 hours in multipara. The third stage occurs from delivery of the baby to separation and expulsion of placenta. Pain relief has no other purpose than patient satisfaction. Neuraxial labor analgesia is the most effective method of pain relief during childbirth, and the only method that provides complete analgesia without maternal or fetal sedation[2]. More than 150,000 women use EA for labor and delivery every year in the UK[3], which is approximately one-fifth of the population[4]. However, there are side- effects of conventional EA particularly motor blockade, increased rate of instrumental vaginal delivery, prolonged duration of labor (notably second stage) and increased augmentation with oxytocin.

All women in true labor should be managed with intravenous fluid (usually with lactated Ringer`s with dextrose) to prevent dehydration. An H_2 receptor blocker drug (ranitidine 100 - 150 mg PO) or metochlopramide 10 mg PO should be given. All patients should ideally have a fetal heart rate monitor. The supine position should be avoided unless a left uterine displacement device (> 15 degree wedge) is place under the right hip.

Review of Literature

EA has become the most popular method of analgesia in labor rooms. In 2002, almost two-thirds of laboring women (up to 60%) who had a vaginal birth, reported that they were administered an epidural. In Canada, in 2001 - 2002, around half of women who delivered vaginally used an epidural, and in the UK in 2003 - 2004, 21% of women had an epidural before or during delivery.

EA involves the injection of a local anesthetic drug into the epidural space. A conventional epidural will block both the sensory and motor nerve roots as they exit from the spinal cord, giving very effective

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pain relief for labor but the recipient will not be able to move the lower part of her body. In the last five to ten years, with the introduction of very potent opioids like fentanyl, remifentanil, sufentanil, we can use EA with lower concentrations of local anesthetic drugs and adding these short acting potent opiates to reduce the motor block. This technique results in the patient walking without pain and is known as 'walking epidural'. In addition, if we want to produce immediate pain relief during labor, epidurals could be co-administered with spinal anesthesia using a very small dose (0.5 ml 0.5 % bupivacaine), known as combined spinal epidural (CSE). Women who have experienced EA alone or CSE for labor pain relief rate these techniques very high in terms of satisfaction. However, satisfaction with pain relief does not equate with overall satisfaction of giving birth to a baby, and epidurals are associated with major disruptions to the processes of birth. These disruptions can interfere with a woman's ultimate satisfaction with her labor experience, and also compromise the safety of birth for the mother and baby[5].

In the absence of a medical contraindication, maternal request is a sufficient medical indication for pain relief during labor[6]. Although severe pain is not life-threatening in healthy parturient women, it can have neuropsychological consequences. Postnatal depression may be more common when analgesia is not used[7] and pain during labor has been correlated with the development of post-traumatic stress disorder[8]. In addition, one study suggested that the impairment of cognitive function in the postpartum period can be mitigated by the use of any form of intrapartum analgesia[9]. Men are also affected by severe labor pain. A survey of first-time fathers showed that the men whose partners received an epidural felt three times as helpful and involved during labor and delivery and had less anxiety and stress, as compared with men whose partners did not receive an epidural[10].

Pain Pathways during labor

The pain during the first stage of labor results from uterine contractions and cervical dilatation. In initial phase pain is confined to T11- T12 dermatomes, but when labor enters into the active phase, T10-L1 dermatomes are eventually involved. Much of the labor pain arises from dilatation of the cervix and lower uterine segment, but contraction of the myometrium against the resistance of the cervix and perineum also plays a major role. The onset of perineal pain at the end of first stage signals the beginning of fetal descent and second stage of labor. Stretching and compressing of pelvis and perineal structures intensifies the pain. Sensory innervation of the perineum is provided by the pudendal nerve (S2 - S4). That is why pain during second stage of labor involves the T10 - S4 dermatomes.

METHODS FOR LABOR ANALGESIA

Psychological & Non-pharmacological Techniques

These techniques are based on the idea that labor pain can be suppressed by reorganizing their own previous thoughts. The Lamaze technique involves training the parturient to take a deep breath at the beginning of each contraction followed by rapid shallow breathing for the duration of the contraction. The parturient can also concentrate on an object in room in attempts to focus her thoughts away from the pain.

Other techniques are hypnosis, transcutaneous electrical nerve stimulation (TENS) and acupuncture. But success of all these techniques varies from patient to patient. In trials where acupuncture was compared with conventional analgesia, women receiving acupuncture required less meperidine (pethidine) and other analgesic methods. No acupuncture related adverse events were reported[11].

Parenteral Agents

We know that all parenteral opioid analgesics cross the placental barrier and can cause fetal depression. Therefore, its use is limited. Remifentanil, a potent ultra-short-acting mu-opioid receptor agonist which is rapidly hydrolyzed in the maternal and fetal blood and which does not accumulate even after prolonged infusion may be ideal for labor analgesia. Remifentanil could be used successfully in patient-controlled analgesia (PCA), with a bolus dose of 0.25 - 0.5 microgram/kg with a lock-out interval of two minutes[12]. In a recent study, 12 randomized controlled trials (RCT) published between 2011 and 2012 were compared. Women treated with remifentanil-PCA had a lower risk of conversion to epidural analgesia and higher satisfaction compared with pethidine [13]. However, close monitoring is essential and supplementary oxygen with ready access to naloxone is mandatory[14].

Recently, dexmedetomidine (Precedex) a highly selective α_2-adrenoreceptor agonist, has been used as an analgesic, sedative without causing any hemodynamic instability and insignificant respiratory respiratory depression[15,16]. The placental transfer is minimal and therefore does not have any effect on fetus. The α_2-adrenoreceptor agonists have been shown to provide neuraxial analgesia *via* the central α_2-adrenoreceptors[17]. Dexmedetomidine modulates the release of catecholamine and is useful when given as an infusion to patients with eclampsia in controlling the blood pressure[18].

Regional Anesthetic Techniques

These are the most popular technique and are considered the gold standard for painless labor. More than 50% parturient are receiving intrapartum EA in

the United States. We use either epidural or combined spinal-epidural technique. Regional techniques do not cause any alteration in duration and outcome of the labor[19]. There is no need for labor augmentation with oxytocics[20]. There is no difference in the rates of normal vaginal delivery[21]. In a recent study it was observed that EA in the latent phase of labor at cervical dilation of 1.0 cm or more does not prolong the progress of labor and does not increase the rate of cesareans in nulliparous women compared with the delayed analgesia at the cervical dilation of 4.0 cm or more[22].

However, there is significant increase in maternal satisfaction in parturients administered EA. Regional analgesia should be provided whenever possible, within 30 minutes of woman's request for analgesia. There are no absolute ''early or late" limits for regional analgesia in labor. Each situation should be assessed individually. However, the anesthesiologist should discuss with the obstetrician, when an epidural is requested in a spontaneously-laboring woman whose cervix is less than 3 cm dilated. Neuraxial blockade used for labor analgesia should produce a perceptible sensory block from T10 - S5 with retention of sensation without pain and maintenance of motor power allowing mobility. Therefore we are keeping local anesthetic usage at minimum. This is could be achieved in four ways:

1. Use the initial dose intrathecally (CSE)
2. Combine local anesthetic with opioid (fentanyl)
3. Do not use a conventional "test dose"
4. Maintain analgesia using intermittent top-ups and not infusions

CSE analgesia produces more reliable symmetrical analgesia, requiring lower drug doses and is of faster onset than epidural analgesia[23].

Spinal Injection: For the initial intrathecal dose we are either using 25 mcg fentanyl alone or 25 mcg fentanyl with 2.5 mg of 0.5% bupivacaine.

Epidural top ups: For top-up epidural we prepare 0.1% bupivacaine with 2 mcg fentanyl per milli litre. For first epidural top-up 10 ml of such mixture is given then for subsequent top ups 8-10 ml of above mixture is given. There is no need to give a test dose through the epidural catheter. Epidural top-ups can be administered in the chair or sitting in the bed or standing.

Mobility of mother is always beneficial to the progress of labor and should be encouraged under proper assessment. The upright position has been shown to improve fetal condition. This may be because of reduced aorto-caval compression, which could occur to parturients who remain in bed. However, mobility could be limited in case of continuous cardiotocography (CTG) monitoring or if a syntocinon infusion is in progress. The mother should at least get out of bed and sit in a chair.

Anesthethesiologists should not allow low dose regional analgesia to wear off in the second stage and it is also important to preserve the ability to push if mother wants to do so. Inadequate analgesic blocks never occur because of the low concentration solutions of the local anesthetic used. It may exacerbate the pain in the unblocked area. Always consider re-positioning of the catheter or increase the volume rather than the dosage or additional opioids.

In the 2nd and 3rd stage of labor the anesthesiologist should be present to check on the mother before delivery to prevent hypotension, aorto-caval compression and ensure an adequate block for any procedure if needed and if cesarean section is required.

Top ups in 1st Stage: Epidural top ups to be administered in the delivery room, preferably with the mother sitting in a chair or standing, not in bed. After each top up allow mother to mobilize only if full motor power is present.

Top ups in the 2nd Stage: Each top up lasts approximately one hour to one and half hour. Therefore give a top up if required during the second stage before pushing starts. Analgesia should not be allowed to wear off in the second stage.

Conventional EA has been successfully used to provide labor analgesia and even anesthesia for cesarean delivery in patients with Guilain-Barre syndrome[24].

In cases like Guilain-Barre syndrome, patient could present with severe labor pain. The CSE technique provides rapid onset, less motor block, and high overall patient satisfaction with analgesia. Furthermore, the incidence of uterine hyperactivity, fetal bradycardia, or conversion to cesarean delivery has not increased[25].

One case of Guillain-Barre Syndrome with labour pain management was reported by Dmitri and Elisabeth, in which they used 3 mg of ropivacaine with six microgram fentanyl intrathecally and continuous epidural infusion of 0.1% ropivacaine with two microgram fentanyl at the rate of 13 ml per hour. Pain control was satisfactory without a higher than usual level of neuraxial block[26].

Patient-controlled epidural analgesia (PCEA)

The first use of PCEA in labor was described by Gambling in 1988, and the technique has since become increasingly popular. Then there were a lot of subsequent studies to examine bolus dose and lock out intervals and the place of background infusion. In a recent study, using patient-controlled epidural

analgesia, lower concentrations of bupivacaine, ropivacaine and levobupivacaine with sufentanil produce similar analgesia and motor block and safety for labor analgesia. The analgesic efficacy mainly depends on the concentration rather than the type of anesthetics[27].

PCEA allow patient to receive the analgesic dose according to their need with progress of labor and provide more maternal satisfaction. Clinical top-ups, amount of local anesthetic and opioid requirement are less with this technique and there are reduced incidents of motor block along with more extensive spread than with the mechanical pump[28]. However, the PCA infusion pump is expensive and women require instruction on utilization of PCEA.

Non-opioid adjuncts

The α-adrenergic receptor agonist epinephrine (1:200,000 - 1:600,000)[29] or adrenergic receptor agonist clonidine and anti-cholinestrase neostigmine[30] have been used as epidural and spinal adjuncts. However, these drugs have still not gained widespread acceptance for labor analgesia.

Complications of Regional Analgesia in Labor

Fentanyl is responsible for up to 10% pruritis, when given as a spinal injection, especially in mothers who had previous itching during pregnancy or mothers with psoriasis or eczema. In such mothers, one should omit fentanyl from the spinal injection. If itching does occur after the spinal injection, reassure the mother that it will settle in 30 - 60 minutes and will not occur after epidural top-ups. If itching persists administer 50 micrograms of naloxone intravenously every 10 minutes up to a maximum dose of 500 microgram. There is no need to omit fentanyl from the epidural top-up mixture. There are no increased risks of chronic back pain in women who have used EA for painless labor and delivery[31].

A woman in labor who is receiving EA is more likely to experience hyperthermia and overt clinical fever. Maternal fever could be associated with neonatal brain injury and can manifest as cerebral palsy, encephalopathy, and learning deficits in later childhood. At present there are no safe and effective means to inhibit epidural-associated fever. Further research is required to know the etiology of this fever and effective interventions to prevent it and its complications[32].

Some immediate serious complications of obstetric EA include misplaced epidural catheter that may be intravascular or intrathecal, a high level of the block or total spinal block and hypotension. Then there might be delayed complications like postdural puncture headache (PDPH), urinary retention, epidural hematoma, abscess or meningitis. Fortunately these are rare complications. Diastasis pubis is an infrequently unrecognized complication of labor and delivery[33]. EA has been reported as part of analgesic management of prepartum diastasis pubis[34]. If diastasis pubis is suspected at the time of delivery, anesthetist should keep epidural catheter in place for analgesic management of this condition[35].

CONCLUSION

In a recent finding, the prevalence rate of chronic pain after cesarean section is in between 6 -18% and after vaginal delivery it is about 4 - 10%. As labor pain is rated as one of the most serious kind of acute pain, we can predict about chronic pain after labor and delivery. We can effectively control the chronic pain, if we treat labor pain with EA[36]

Most of the neurological injuries or complications are not because of neuraxial analgesia but due to labor and delivery. However, anesthetist should perform his technique carefully to further limit the rare injuries directly related to anesthesia.

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Original Article

Investigating the Effects of Negative Calorie Diet Compared with Low-Calorie Diet on Weight Loss and Lipid Profile in Sedentary Overweight / Obese Middle-Aged and Older Men

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ABSTRACT

Objectives: Negative-calorie diet (NCD) is among the popular dieting guides for weight loss; however, there is still little knowledge about this method. The aim of this study was to determine the effects of negative-calorie diet on weight loss and lipid profile, and to compare its efficiency with low-calorie diet (LCD).
Design and Setting: Randomized study (the CONSORT statement) at the Ukrainian Center for Sports Medicine, Kiev, Ukraine
Subjects: Sedentary men (aged 45 - 75 years) who were overweight or obese (n = 104)
Interventions: The patients were randomly divided into two groups: NCD, and LCD. Out of 104 participants, 90 persons completed the treatment.
Main Outcome Measures: The weight assessment parameters including change in body weight, total cholesterol (TC), high density lipoprotein –cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were performed pre- and post-intervention for all subjects and compared to each other. A p-value of < 0.05 was considered statistically significant.
Results: Our results showed significant differences with respect to all parameters between pre- and post-intervention in both the groups. Reduction in TC ($p < 0.05$) and HDL-C level ($p < 0.003$) were different between the two groups. There was no change in the TC to HDL-C ratio in weight loss of the NCD but weight loss performed by the LCD typically reduces the TC to HDL-C ratio.
Conclusions: Contrary to expectations, both experimental groups showed a similar pattern of weight loss. The influence of NCD on lipid profile had no advantage as compared to the low-calorie diet.

KEYWORDS: dietary formulations, lifestyle, obesity, overweight, weight loss

INTRODUCTION

The prevalence of abnormal weight gain is increasing world-wide. Obesity is associated with an increased prevalence of chronic diseases, including type 2 diabetes, hypertension and cardiovascular disease (CVD)[1]. About 70% of the middle-aged and older populations (age ≥ 45 years) have an abnormal weight gain. This population is under an increased risk for obesity[2]. Physical dysfunction, higher health care costs, as well as increased morbidity and mortality are some consequences of obesity among elderly adults[3,4].

Today, weight-loss medications and diets are widely used for weight loss. These things are associated with several side effects influencing the health of the person[5]. Previous studies have shown that there is a direct relation between nutrition and body condition. Therefore, health experts stress on the low-calorie diet (LCD) consumption. However, recently negative-calorie diet (NCD) has gained a great deal of research and popular attention[6,7].

The origin of the NCD idea is still unclear. This notion first appeared in the website www.negativecaloriediet.com as an 80-page downloadable e-book in 2007. The negative calorie diet is a kind of very-low-fat diet (details of diet: 15% protein, 75% carbohydrate, 10% fat) that have a very high carbohydrate and fibre content[8]. It is believed that negative calorie foods help to lose weight by burning

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more calories than what has been eaten. For example, for digesting a piece of dessert containing 400 calories, the body needs 150 calories of energy. The remaining 250 calories add to the body fat. However, based on the idea of NCD, eating a 100 calorie dessert needs 150 calories for digestion. Actually, the body should burn 50 extra calories simply by eating the food. This idea for weight loss is appealing. This gives such foods, a natural fat-burning property. They are called catabolic foods. According to the claims of NCD's supporters, this kind of diet will contribute more than other diets to weight loss. In other words, more eating reduces more weight.

The LCD (details of diet: 15% protein, 55% carbohydrate, 30% fat) help to lose weight by reducing total calorie intake, but they do not help to burn calories. In general, LCD is high in carbohydrates and low in fat[6,9]. A large part of NCD includes low-calorie fruits and vegetables that are high in fiber[10-12].

Nowadays, there are lots of advertisements about the NCD. Notwithstanding the popularity of NCD idea among dietary plans, there has been no scientific evidence about the efficacy and effects of this diet on weight loss. The study of diet is important in different age groups.

The present study was undertaken to evaluate the effects of NCD on weight loss and compare its efficacy with LCD in sedentary overweight / obese middle-aged and older males. Changes in lipid profile of the subjects, body weight, body mass index (BMI), HDL-C level and TC to HDL-C ratio were assessed prior to and post intervention to assess weight-loss trend.

SUBJECTS AND METHODS

This was a randomized clinical trial (RCT). Participants of the study (n = 104) were randomly selected among overweight or obese men aged 45 - 75 years who had attended (during two months prior to the study) the Ukrainian Center of Sports Medicine (Kiev) to receive weight loss consulting programs. The research team consisted of three physicians (two sport physicians and one resident) and two nurses. Enrollment, classification and assignment of subjects to experiments were done under the supervision of a sports medicine physician. Participants were non-smokers and weight-stable (± 2 kg, for more than one year) with no history of regular exercise in at least three months before the study. They had no history of cardiovascular disease (CVD) and other disorders such as diabetes, depression, eating disorders, chronic medications, kidney disease, cancer, food allergies or intolerances to items used in meals. Subjects with abnormality in thyroid or the ECG, any history of anti-obesity medication or weight loss drugs or dietary supplementations for weight control were excluded from the study. Throughout the study, participants were requested not to use any type of alcohol, sugar, honey, sugar substitutes, or any of the commercial dressings (high-fat and sugar content).

The participants were assessed for vital signs and their blood pressure was measured after a 10-min rest period on a seat from right arm (twice at five-minute interval) with a manual mercury sphygmomanometer. The averaged value of three measurements (SD = ± 6 mmHg) was used for further analysis. Those subjects with blood pressures lower than 140/90 mmHg were recruited into the study.

Weight loss assessment parameters included body weight, body mass index (BMI) and blood lipid parameters which were measured prior to and post-intervention.

BMI calculated as body weight (kg) / height (m^2), equal to or higher than 25.0 kg/m^2 was defined as overweight and obesity condition. Height was measured to the nearest 0.1 cm by a wall-mounted stadiometer. Body weight was measured to the nearest 0.1 kg on a digital scale (Scale-Tronix model 5002, Wheaton, IL, USA). All physical measurements were performed with light street clothing and no shoes.

The study design was simple randomization. Following initial assessments, the participants were divided randomly into two parallel groups. There were no other restrictions such as blocking and block size (There was a balance between study groups in size or baseline characteristics)[13]. Study group I (53 participants) received the NCD. Study group II (51 participants) received the LCD or healthy weight maintenance diet. Both groups had 15% caloric restriction from their maintenance energy requirements. Table 1 shows daily calorie needs estimated by multiplying basal metabolic rate (BMR) and physical activity level (PAL) of the subject. For a more accurate estimate of BMR in men, Eq. (1) and Eq. (2) were used for the 31 - 60 years and more than 60 years participants, respectively (Table 1).

Eq. (1) - (weight in kg × 11.6) + 879

Eq. (2) - (weight in kg × 13.5) + 487

Table 1: Diets and daily energy intake

Group	Type of diet	BMR × PAL	Daily energy intake* (kcal)
I (NCD)	Negative calorie diet	2302 ± 166	1956 ± 141
II (LCD)	Low-calorie diet	2289 ± 156	1941 ± 136

*Daily energy intake = (BMR x PAL) – 15%

PAL is the ratio of total daily energy expenditure to BMR. At baseline, for selecting participants with a sedentary lifestyle, the PAL values were determined using a customized self-report questionnaire. The questionnaire consisted of a seven point Likert-

type scale ranging from 'not at all' (1) to 'every day' (7). The PAL value was classified into one of seven categories as follows: Not at all, less than once a month, 1 - 2 times a month, about once a week, 2 - 3 times a week, 4 - 5 times a week and every day. The exercise frequency of 1 - 2 times a month or less was considered as continuously inactive[14,15]. Before and during the study, the participants had an inactive lifestyle (exercise session less than 1 - 2 times a month), and PAL was considered to be 1.2. To assess weight loss at a healthy and effective rate, we considered 15% reduction in the maintenance calorie needs[16].

Blood samples were taken from the antecubital vein. TC, triglycerides (TG) and HDL-C were measured by spectrophotometry at 500 nm using enzymatic kit (Elitech Diagnostics, Sees, France). LDL-C was calculated using the Friedewald formula defined as LDL-C = TC – HDL-C – triglycerides / 5[17]. The participants did not eat or drink, except water, for 9 - 12 hours prior to the blood test. Blood sample assessments for further analyses were as follows: the plasma concentration of TC below 200 mg/dl, LDL-C below 130 mg/dl, TG below 150 mg/dl, and finally the plasma HDL-C levels below 40-60 mg/dl.

Participants used a suitable method for identifying their diet and beverage habits. They recorded food and beverage consumption (including water) for four days (three days a week + a weekend day). They did it at the start of study (baseline) and every month during the study. They used a diary that was previously approved based on household measures. Diaries were checked for completeness and energy, and macronutrient compositions were calculated using the Diets in Details software. The NCD included more than 100 different foods containing high level of proteins, vitamins, carbohydrates, dietary fiber and minerals needed for a healthy condition. It consisted of lean protein such as poultry, red meat, fish, and eggs, vegetables, and fruits. Fruits consisted of apple, blueberry, cranberry, grapefruit, honeydew, lemon / lime, mango, orange, papaya, pineapple, strawberry, tangerine, watermelon. Poultry sources included chicken and turkey breast. Red meat consisted of top round, extra lean sirloin, game meats. Fish sources included all varieties such as buffalo fish, catfish, clams or cooked, cod steaks, crab, crayfish, flounder, mussels, oysters or half shell, shrimp, trout and tuna. Eggs included egg whites and whole eggs in moderate quantities but at least about one yolk a day. Vegetables consisted of asparagus, bean sprouts, beetroot, broccoli, cabbage, carrot, cauliflower, celery, cucumber, green beans, kale, leeks, lettuce, radish, spinach, tomato and turnip. Traditional methods of weight loss include low-calorie diets. Low-calorie foods list, as described above, include low-fiber fruits and vegetables as well as other foods that were not in the NCD foods list. All groups received special recipe developed with food guide pyramid and Dietary Guidelines (United States Department of Agriculture), The UK Food Standards Agency (FSA), and the NCD plan[15,16,18].

At the start of the experiments, the participants had a weekly meeting. They learned how to stick to their dietary plans to increase their motivation and moral commitment to the program. Variations in activity levels or diets are associated with potential confounding effects over the study. Therefore, we recommend them to preserve their current PALs and diets throughout the intervention. They were instructed to report any problems that could affect their involvement in the study.

Weight loss assessment parameters and laboratory tests were performed pre- and post-intervention for all subjects and compared to each other. The method did not change during the study. We explained all procedures and requirements for subjects. They voluntarily signed a consent form before enrolling in the study. The study was according to the declaration of Helsinki and was approved by the local Medical Ethical Committee of the National Medical Academy of Postgraduate Education named after P.L. Shupyk (NMAPE, 04112, Dorogozhytska, 9).

Statistical Analysis

All statistical data of the study were expressed as means ± SD (standard deviation). The normal distribution of the collected data was evaluated using the Kolmogorov-Smirnov test. The data was normally distributed. The pre- and post-intervention results for the groups were compared using the paired t-test, and the differences between the groups were evaluated by the independent t-test. The linear regression analysis (R) was used to examine a relation between all significant values (dependent) and weight change (independent).

Statistical analysis was performed using SPSS (version 19.0 for windows). A p-value less than 0.05 was considered as statistically significant.

RESULTS

All those who participated in the program, changed their diet and reduced energy intake. Fourteen participants did not follow their individual PALs and/or diets, and they were excluded from

Table 2: Subjects' morphological characteristics in each group before intervention

Morphological characteristics	Group I (n = 53)	Group II (n = 51)
Sex: women / men,	0/53	0/51
Race: white / non-white	51/2	48/3
Age (years)	59.2 ± 10	58.4 ± 9.3
Height (cm)	174.4 ± 5.6	173.7 ± 6.8

Table 3: Subjects' demographic characteristics in each group at pre-test and post-test plus the p-value of comparing mean within groups

Morphological characteristics	Age groups	Group I (NCD)		Group II (LCD)	
		Before treatment Mean (±SD)	3 months Mean (±SD)	Before treatment Mean (±SD)	3 months Mean (±SD)
Weight (kg)	45-54	90.8 ± 6.2	83.7 ± 6.4	95.9 ± 11.9	87.9 ± 11.5
	55-64	102 ± 9.8	94.5 ± 9.8	91.6 ± 9.9	84.3 ± 9.8
	65-75	90.4 ± 12.3	82.4 ± 12.1	89 ± 4.8	81.6 ± 4.9
	Total population	92.1 ± 10.13	84.6 ± 10.15*	92.3 ± 9.83	84.8 ± 9.62*
BMI (kg/m^2)	45-54	30.3 ± 2.8	27.9 ± 2.7	30.7 ± 3.4	28.1 ± 3.3
	55-64	32.6 ± 3.9	30.2 ± 3.8	30.3 ± 2.1	27.8 ± 2.1
	65-75	30.05 ± 3.4	27.3 ± 3.4	31.5 ± 3.07	28.9 ± 2.98
	Total population	30.5 ± 3.25	28 ± 3.23	30.78 ± 2.86	28.2 ± 2.8
Total-C (mg/dl)	45-54	194 ± 4.2	167.3 ± 5.1	190.2 ± 4.1	171 ± 4.5
	55-64	193 ± 4.3	163.5 ± 3.8	190.1 ± 4.9	174 ± 5.8
	65-75	189.8 ± 9.1	159 ± 6.9	193.7 ± 4.2	176.7 ± 2.8
	Total population	192.2 ± 6.85	163.4 ± 6.9* ‡	191.1 ± 4.66	173.7 ± 5.2*
HDL-C (mg/dl)	45-54	52 ± 3.2	42 ± 3.4	51.6 ± 2.8	50.6 ± 2.8
	55-64	51 ± 0.0	43 ± 0.0	53.1 ± 2.8	52.3 ± 2.6
	65-75	51.1 ± 2.3	44.3 ± 1.9	48.5 ± 3.1	47.5 ± 3.1
	Total population	51.5 ± 2.68	43 ± 2.84* ‡	51.4 ± 3.4	50.4 ± 3.4*
LDL-C (mg/dl)	45-54	121.8 ± 4.5	98.8 ± 2.9	122.8 ± 4.1	103.3 ± 3.2
	55-64	124.5 ± 0.5	105.5 ± 0.5	125.3 ± 3.4	106.8 ± 2.9
	65-75	126.6 ± 3.6	110.5 ± 4.9	122.5 ± 4.9	104.2 ± 4.4
	Total population	124.1 ± 4.42	104.4 ± 6.6*	123.7 ± 4.21	104.8 ± 3.8*
Total-C / HDL-C ratio	45-54	3.7 ± 0.3	3.9 ± 0.3	3.6 ± 0.1	3.3 ± 0.2
	55-64	3.7 ± 0.05	3.7 ± 0.05	3.5 ± 0.2	3.2 ± 0.2
	65-75	3.6 ± 0.3	3.5 ± 0.2	3.9 ± 0.2	3.7 ± 0.2
	Total population	3.7 ± 0.29	3.7 ± 0.35	3.6 ± 0.29	3.4 ± 0.28

further assessments. The final sample size was 90 men in good health (45 participants in each group). The morphological characteristics for individuals who completed the three-month intervention are summarized in Table 2. Most participants were white (~ 94%), and the remaining were African (n = 3), and "other" (n = 2) (Table 2).

There were no significant differences in values of body weight, TC, HDL-C and LDL-C, in both groups before the treatment. However, results of the assessments showed significant differences with respect to all parameters of values between pre- and post-intervention of both groups (Table 3).

There were no significant differences in the body weight between the NCD (M = 84.6, SD = 10.15) and LCD (M = 84.8, SD = 9.62) after intervention. There was no significant difference with respect to LDL-C values between groups post intervention ($p > 0.05$). TC values were significantly different between NCD (M = 163.4, SD= 6.9) and LCD (M = 173.7, SD = 5.2) groups. Additionally, there were significant differences between HDL-C of the NCD (M = 43, SD= 2.8) and in the LCD (M = 50.4, SD = 3.4) ($p < 0.001$).

Linear regression analysis was implemented to find a relation between all significant values and weight change. The relation of TC, LDL-C and HDL-C with weight change were analyzed and this revealed no significant effect (data are not shown).

Subjects' demographic characteristics in each group at pre-test and post-test plus the p-value of comparing mean within groups are shown in Table 3.

DISCUSSION

Aging is associated with degeneration, loss of functional ability and obesity[19]. Although age-related changes have a strong genetic component, it is also influenced by diet. Thus, middle-aged and older men were chosen for the study. During the study, we did not face any trial limitations such as potential bias, multiplicity of analyses etc., except the lack of commitment of some participants to their individual PALs or/and diets. Such volunteers were excluded from the study. Reasons for dropping out of subjects included: change in PAL and / or diet, drinking alcohol, sugar, honey, sugar substitutes, or any of commercial dressings (high-fat and sugar content) throughout the intervention. On the basis of previous studies, weight loss and lipid profile changes with diet takes at least 12-weeks and many investigators have used it[20, 21]. Therefore, the study was stopped after three months. Blood levels of triglycerides are related to eating, and LCDs reduce its value. Therefore, we removed the triglyceride variable from this study. To our knowledge, this was the first RCT investigating the influence of NCD on weight loss and lipid profile in sedentary overweight / obese middle-aged and older men.

All parameters including weight, TC, HDL-C, LDL-C declined in post-intervention compared to pre-intervention in both groups which shows effectiveness of both treatments. Both diets are low in fat. Accordingly, it seems the cholesterol level in each diet has an important influence on the results. Our findings are consistent with the findings of the Franz *et al* study evaluating weight-loss efficacy of dietary interventions and meal replacements[22].

High blood lipids are risk factors for CVD that gets worse with age[23-25]. Reductions in the level of TC induced by the NCD were not similar in the LCD group; however, effectiveness of both diets was similar with respect to weight loss. Some other researchers have reported similar results for other diets[26-28].

The NCD-induced decrease of HDL-C value was significantly greater than the LCD. This finding confirmed previous studies by other investigators that were believed to be diet-induced changes in HDL levels[29]. Its change is of utmost importance in healthcare, because one benchmark to estimate the risk of CVD is the ratio of TC to HDL-C[30-32]. As shown in Table 3, the TC / HDL-C ratio changed in both experimental groups. The TC / HDL-C ratio between pre- and post- intervention, did not change in NCD but decreased in LCD; there was a disadvantage in the NCD compared to LCD in this field. This finding may have clinical applications for weight loss with diet.

Furthermore, results of the simple linear regression demonstrated that the efficacy of both diets on lipid profile in prediction of CVD was not the same.

Finally, it should be noted that fat is involved in building the membranes of all body cells. In addition, it provides essential fatty acids, vitamins A, D and E. Fats in the NCD are less than the recommended amount in food standards. Such diets, because of their high carbohydrate content, can contain over twice (40 - 70 g/d) the recommended amount of fiber[7]. High fiber intakes can decrease the absorption of zinc, calcium and iron[33]. Complaints of flatus and abdominal fullness have also been reported[7]. Therefore, it appears that this diet is unsuitable for long-term use and probably will lead to some problems.

In spite of many advertisements regarding the NCD, this diet has the same effect on weight-loss as the LCD. It seems that the concept of negative calorie have no external meaning and application. In addition, it may be possible that some diets cause weight-loss in a short time but it leads to remarkable side-effects on the physical health of patients.

CONCLUSIONS

Unlike the growing body of advertisements and their claim, both experimental groups showed a similar pattern of weight loss. Weight loss obtained by NCD had no advantage over LCD as regards the lipid profile and preventing the occurrence or development of cardiovascular dysfunctions in sedentary overweight / obese middle-aged and older men. Further study in this area is recommended.

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Original Article

Relationship between Interferon Regulatory Factor Expression and Coronary Artery Disease

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ABSTRACT

Objectives: To investigate the relationship between expression levels of interferon regulatory factors (IRF) in patients with coronary artery disease.
Design: Prospective case-control study
Setting: Department of Cardiology, Puren Hospital, Wuhan University of Science and Technology, Wuhan, China (PRC)
Subjects and Methods: Between October 2010 and July 2011, 106 patients (aged 37 - 71 years) were enrolled. Patients were assigned to four groups: acute myocardial infarction (AMI) (n = 28), stable angina (SA) (n = 26), unstable angina (UA) (n = 26), and control group (without CAD, n = 26).
Interventions: Blood samples were drawn from all the patients *via* the antecubital vein following a 12 h fasting period
Main Outcome Measures: Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and high sensitivity C-reactive protein (hs-CRP) were measured. The mRNA and protein expression levels of IRF-1, IRF-2, IRF-4 and IRF-8 in each patient were measured using PCR and western blotting. Serum levels of IL-12 and IFN-γ were detected by ELISA.
Results: Within both the AMI and UA groups, IL-12 and IFN-γ were significantly increased compared with SA and control patients. Both the mRNA and protein expression levels of IRF-1, IRF-2 and IRF-8 in the AMI and UA groups were significantly higher than those in the SA and control groups. There was no significant difference between the SA and control groups.
Conclusions: The increase in expression levels of IRF-1, IRF-2 and IRF-8 may be associated with atherosclerotic plaque formation and rupture, which are seen in AMI and UA.

KEY WORDS: atherosclerosis, coronary artery disease, interferon regulatory factors

INTRODUCTION

Coronary artery disease (CAD), is one of the major causes of death and morbidity worldwide and is classified as a clinical manifestation of atherosclerosis. The inflammatory response is an underlying mechanism of the atherosclerotic process[1]. Specifically, T lymphocytes have been found to exert pro-atherogenic plaque destabilizing influences and are present in human atheroma[2,3]. Recent studies[4,5] have shown that T helper cell (Th1 / Th2) functional imbalance exists in both coronary arterial inflammation and myocardial inflammation. Increased Th1 activity has been demonstrated in patients with CAD[6,7]. However, the mechanisms remain unclear.

The interferon regulatory factors (IRF) family, a group of transcription factors regulating the expression of interferons and interferon-stimulated genes, participates in the early host response to pathogens in immunomodulation and hematopoietic differentiation[8]. IRF plays a crucial role in the Th cell differentiation[9]. We speculate that IRF may participate in the atherosclerotic process *via* regulation of Th cell differentiation and enhancement of Th1 activity. In the present study, we investigated the expression levels of the IRF family in Th cells in patients with or without CAD.

SUBJECTS AND METHODS

Patients

Between October 2010 and July 2011, 106 consecutive patients (68 men, 38 women, aged between 37 - 71 years) were enrolled from the Department of Cardiology, Puren Hospital, Wuhan University of Science and Technology, China. The study protocol conformed to the ethical guidelines of the 1975 Helsinki Declaration and was approved by the institutional

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medical ethics committee of the hospital. Patients were assigned to one of the following four groups: acute myocardial infarction (AMI, n = 28), stable angina (SA, n = 26), unstable angina (UA, n = 26), and a control group (without CAD, n = 26). The diagnostic criteria were in accordance with the American College of Cardiology / American Heart Association guidelines for angina and myocardial infarction[10-12]. All patients underwent coronary angiography to confirm the diagnosis of CAD. Patients with other confounding diseases associated with inflammatory response such as myocarditis, cardiomyopathy, peripheral vascular disease, valvular heart disease, fever and liver or renal dysfunction, autoimmune disease and cancer were excluded.

Blood sample collection and biochemical investigation

Blood samples were drawn from all the patients *via* the ante-cubital vein following a 12 h fasting period. Each of the blood samples were drawn into sodium heparin vacutainers (Becton Dickinson, San Jose, CA) and centrifuged for 15 min at 1,000 g. We then applied standard techniques using a Hitachi 912 Analyzer (Roche Diagnostics, Germany) and obtained plasma samples. The samples were stored at −70 °C to measure serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and high sensitivity C reactive protein (hs-CRP) as patients' baseline characteristics. Density gradient centrifugation was used to isolate peripheral blood monocyte cells. A human CD4+ T cell kit using an AutoMacs isolator (Miltenyi Biotec, Bergisch Gladbach, Germany) was used to enrich CD4+ T cells, and cell purity was confirmed using flow cytometry (90 - 95%).

Serum interleukin (IL)-12 and interferon gamma (IFN-γ) detection

Th1 cells are characterized by secretion of IFN-γ, and IL-12 plays a central role in controlling the development of Th1 cells. Thus, serum levels of IL-12 and IFN-γ were measured to reflect Th1 response[6,7,13]. Serum IL-12 and IFN-γ levels were determined with commercially available human enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences, San Jose, CA USA), according to the manufacturer's protocols. The minimum detectable dose of IL-12 and IFN-γ was 1 pg/ml and 7.8 pg/ml, respectively.

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

RNA was extracted from CD4+ T cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. We assessed quantitative gene expression with the ABI Prism 7500 sequence detection system (PE Applied Biosystems) using the SYBR Green RT-PCR master mix-plus (TOYOBO). To normalize the data, GAPDH and the 2-ΔΔCt method were used for analysis[14]. The primers utilized in this study are shown in Table 1.

Table 1: Primers used for IRFs and GAPDH

IRF-1	
Forward primer	5′-CCTGATACCTTCTCTGATGGACTCA-3′
Reverse primer	5′- TGCATGTAGCCTGGAACTGTGT-3′
IRF-2	
Forward primer	5′-TCCTACTCCATGCTTGATGACC-3′
Reverse primer	5′-AAGATGGGCTGGATGTTG-3′
IRF-4	
Forward primer	5′ -GACCCTCCCACCTGGAAGAC-3′
Reverse primer	5′- CGCTCAACCAGTTCCTCAAAG-3′
IRF-8	
Forward primer	5′- GCCGTGGTGTGCAAAGG-3′
Reverse primer	5′-GAACTGGCTGGTGTCGAAGAC-3′
GAPDH	
Forward primer	5′-AAGGTCGGAGTCAACGGATTTGG-3′
Reverse primer	5′-AGGCATTGCTGATGATCTTGAGGC-3′

$p < 0.05$, compared to control patients; $^{\dagger}p < 0.05$, compared to SA patients. SA: stable angina; UA: unstable angina; AMI: acute myocardial infarction

Western Blot Analysis

IRF protein expression was determined using Western blot analysis. Nuclear extract (500 μg protein) from enriched CD4+ T cells was incubated with anti-IRF-1, anti-IRF-2, anti-IRF-4 and anti-IRF-8 (Santa Cruz, Heidelberg, Germany) antibodies overnight at 4 °C. Following incubation, we washed the cells in triplicate in Tris-Buffered Saline Tween-20 (TBST). The protein bands were then incubated with horseradish peroxidase-conjugated secondary antibodies for two hours at room temperature and processed with ECL (Pierce) according to the manufacturer's instructions. A BCA protein assay (Pierce) was used to measure the protein concentration, which was expressed as a ratio to levels of GAPDH.

Statistical Analyses

Data were expressed as mean ± SD. We used SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA) for statistical analyses. A one-way ANOVA test followed by a post-hoc Bonferroni / Dunn test was used to compare between groups. A p-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Table 2 summarizes the baseline characteristics of AMI, SA, UA and control patients. There were no significant differences in age, sex, risk factor and medications among the four groups. However, the levels of hs-CRP in the UA and AMI groups were significantly higher than those of the SA and control groups ($p < 0.05$).

Table 2: Characteristics of AMI, SA, UA and control patients

	Control (n=26)	SA (n=26)	UA (n=26)	AMI (n=28)
Age (mean ± SD)	55.7 ± 7.2	56.2 ± 8.3	59.7±12.4	58.6 ± 8.8
Sex (male/female)	17/9	19/7	18/8	20/8
Risk factor (%)				
Hypertension	11 (42.3)	12 (46.2)	10(38.5)	13 (46.4)
Diabetes	5 (19.2)	6 (23.1)	7 (26.9)	9 (32.1)
Tobacco use	10 (38.5)	12 (46.2)	11 (42.3)	14 (50)
Laboratory profile				
TC (mmol/l)	4.62 ± 0.86	4.53 ± 1.12	4.76±1.35	4.92 ± 1.56
TG (mmol/l)	1.38 ± 0.62	1.47 ± 0.73	1.83±1.62	1.96 ± 1.52
HDL-C (mmol/l)	1.23 ± 0.47	1.14 ± 0.35	1.19±0.44	1.15 ± 0.39
LDL-C (mmol/l)	2.52 ± 0.87	2.69 ± 0.82	2.97±1.12	3.05 ± 1.18
hs-CRP (mg/l)	1.24 ± 0.68	3.31 ± 2.12*	5.95±2.88*†	6.26 ± 3.12*†
Mediations (%)				
ACE inhibitors	5 (19.2)	15 (57.7)	14 (53.8)	17 (60.7)
β-Blocker	3 (11.5)	14 (53.8)	12 (46.2)	15 (53.6)
Calcium blocker	8 (30.8)	11 (42.3)	15 (57.7)	12 (42.9)
Nitrate	—	13 (50)	17 (65.4)	18 (64.3)
Statins	—	14 (53.8)	12 (46.2)	15 (53.6)
Aspirin	—	22 (84.6)	19 (73.1)	21 (75)

*p < 0.05, compared to control patients; †p < 0.05, compared to SA patients. SA: stable angina; UA: unstable angina; AMI: acute myocardial infarction

Serum levels of IL-12 and IFN-γ

The serum levels of IL-12 and IFN-γ are shown in Table 3. Both IL-12 and IFN-γ in the AMI and UA groups were significantly higher compared to patients in the SA and control groups ($p < 0.05$). No significant difference between SA and control groups was identified ($p > 0.05$).

The mRNA expression of IRFs

The mRNA expression of IRF-1, IRF-2, IRF-4 and IRF-8 in isolated CD4+ T cells from patients with AMI, SA, UA, and control is shown in Fig. 1. mRNA expression levels of IRF 1, 2 and 8 in the AMI group (2.75 ± 0.36, 1.58 ± 0.16 and 1.78 ± 0.21, respectively) and the UA group (2.62 ± 0.30, 1.62 ± 0.15 and 1.63 ± 0.19, respectively) were significantly increased compared with patients in the SA group (0.74 ± 0.12, 0.56 ± 0.08 and 0.52 ± 0.05, respectively) and the control group (0.58 ± 0.07, 0.50 ± 0.05 and 0.48 ± 0.04, respectively) (all $p < 0.05$). No significant difference between the SA and control groups was observed ($p > 0.05$). In addition, the relative IRF-4 mRNA expression levels were similar among the four groups (AMI 0.84 ± 0.08; UA 0.75 ± 0.07; SA 0.52 ± 0.05; control 0.51 ± 0.04; $p > 0.05$).

The protein expression of IRFs

The protein expression of IRF-1, IRF-2, IRF-4 and IRF-8 in isolated CD4+ T cells from patients with AMI, SA, UA and control is shown in Fig. 2. Protein expressions of IRF 1, 2 and 8 in the AMI group (4.96 ± 0.42%, 3.12 ± 0.33% and 3.88 ± 0.38%, respectively) and the UA group (4.45 ± 0.41%, 3.32 ± 0.35% and 3.18 ± 0.32%, respectively) were significantly increased compared with patients in the SA group (1.56 ± 0.18%, 1.48 ± 0.16% and 1.32 ± 0.14%, respectively) and the control group (1.27 ± 0.15%, 1.34 ± 0.17% and 1.28 ± 0.16%, respectively). No significant difference was found between the SA and control groups ($p > 0.05$). Similar to mRNA expression, IRF-4 protein expression was not significant between the four groups (AMI 1.85 ± 0.14%; UA 1.78 ± 0.15%; SA 1.12 ± 0.13%; control 1.23 ± 0.14%; $p > 0.05$).

DISCUSSION

In this study, IRF-1, IRF-2 and IRF-8 mRNA and protein expression in isolated CD4+ T cells from patients with AMI and UA were significantly higher than that in SA and control patients. These results were also accompanied with an increased Th1 response (higher serum levels of IL-12 and IFN-γ) in AMI and UA patients, suggesting that heightened IRF-1, IRF-2 and IRF-8 expression may be associated with increased Th1 activity. Meanwhile, IRF-4 mRNA and protein expression were similar in CAD and control patients, indicating that IRF-4 may not contribute to an increased Th1 response. In fact, IRF-4 may play a

Table 3: Serum levels of IL-12 and IFN-γ

	Control (n =26)	SA (n=26)	UA (n=26)	AMI (n=28)
IL-12 (pg/ml)	84.2±18.2	94.7±21.3	285.6±42.4*†	266.7±38.8*†
IFN-γ (pg/ml)	8.2±0.6	9.7±0.7	16.3±1.2*†	19.6±1.4*†

*p < 0.05, compared to control patients; †p < 0.05, compared to SA patients. SA: stable angina; UA: unstable angina; AMI: acute myocardial infarction

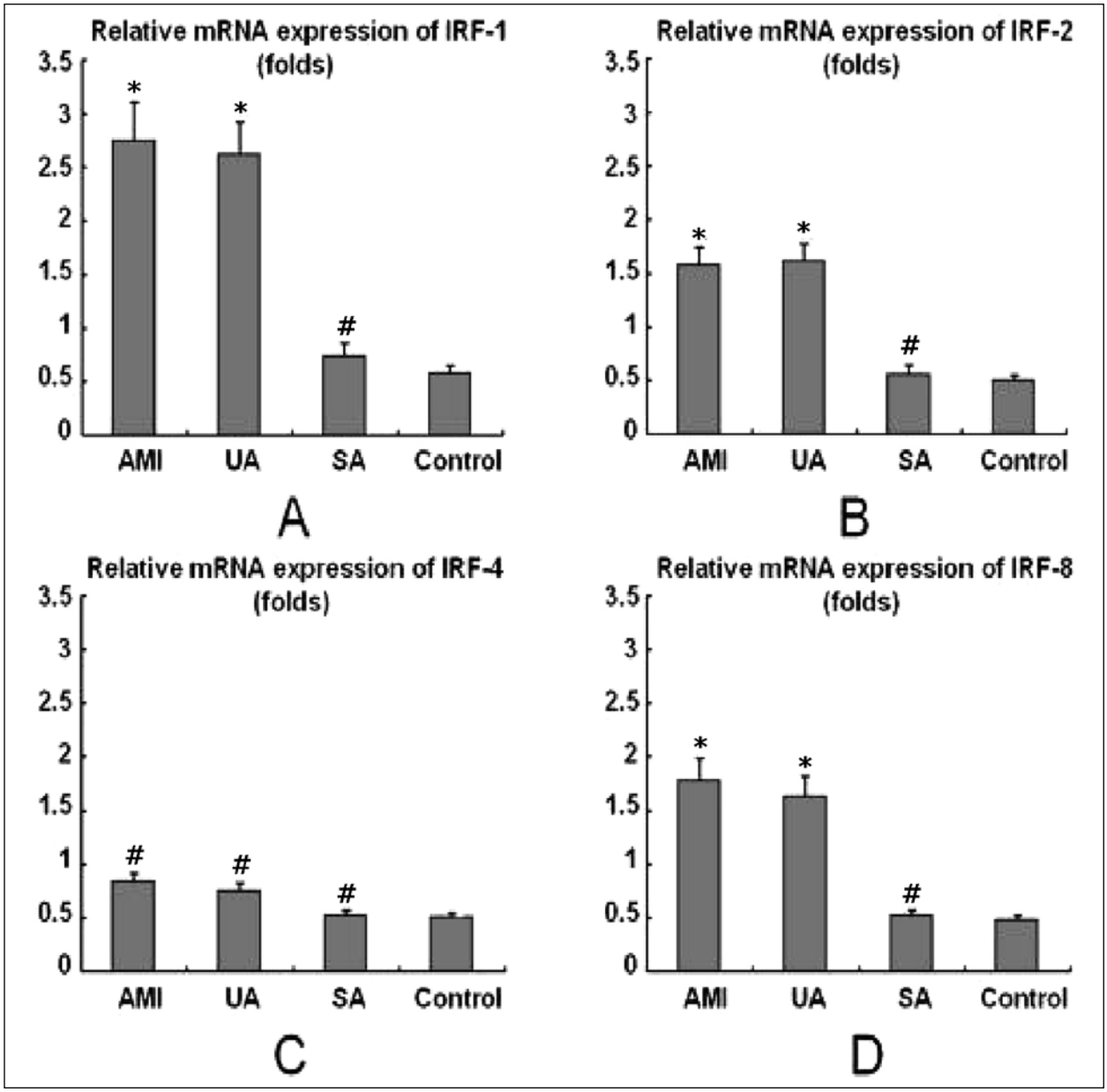

Fig. 1: Relative mRNA expression of IRF-1 (A), IRF-2 (B), IRF-4 (C) and IRF-8 (D) assayed using RT-PCR in CD4+ T cells. RT-PCR amplification was normalized to GAPDH. *p < 0.05 compared with the SA and control group, #p > 0.05 compared with control group.

role in the inflammation process by facilitating the differentiation of Th2 cells[15].

It has been well documented that atherosclerosis is a chronic inflammatory disease and the immune response plays an important role in its progression[1]. In particular, CD4+ T cells have been strongly associated with the development of atherosclerosis. Previous studies[16-18] have demonstrated that either pharmacologic blockade of the Th1 pathway or a knockout targeted to IFN-γ, the distinct cytokine secreted by Th1 cells, can inhibit the development of atherosclerosis. This indicates that Th1 cells play a vital role in atherosclerosis; however, Th2 cells are thought to also play an auxiliary role[19]. Recent clinical studies have shown significant, functional changes of a subset of CD4+T cells in patients with acute coronary syndrome[4-7,20], especially with an increase of Th1 cell activity and enhancement. Consistent with a previous study[7], we also showed an increased Th1 response in AMI and UA patients. The elevated activity and function of Th1 cells may contribute to the increasing instability of atherosclerotic plaque due to T-cell-mediated endothelial cell injury[21]. The positive feedback association between the Th1 response and the pro-inflammatory mediators produced by Th1 cells such as IFN-γ may play a critical role in the atherosclerotic process and plaque destabilization[22-24]. The Th1 response promotes the secretion of IFN-γ, which prevents vascular smooth muscle cell proliferation and collagen synthesis, damaging the protective fibrous cap of the plaque. Meanwhile, IFN-γ further

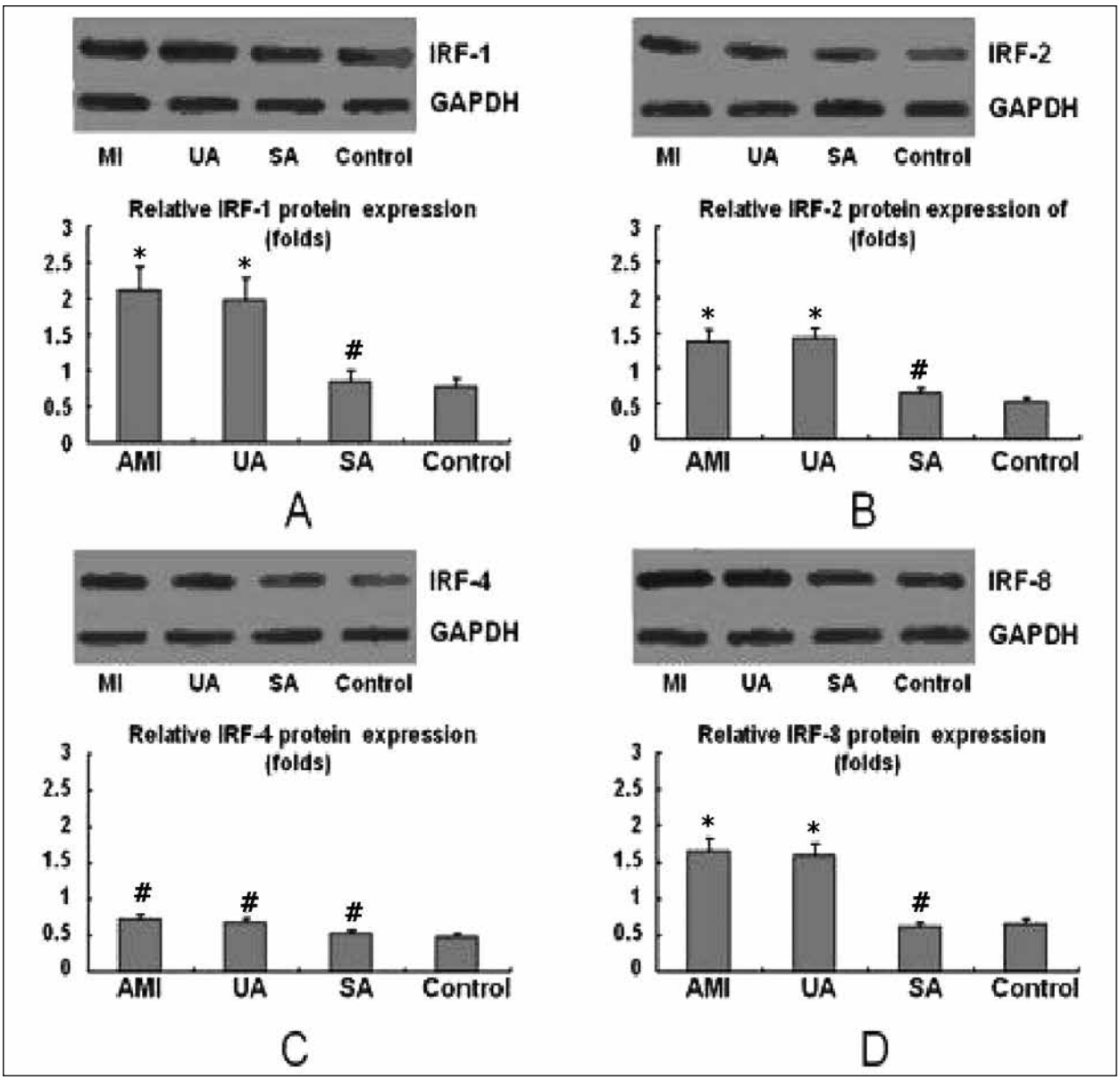

Fig. 2: Relative protein expression of IRF-1 (A), IRF-2 (B), IRF-4 (C) and IRF-8 (D) assayed using western blot for CD4+ T cells. The protein levels of IRF-1, IRF-2 and IRF-8 are significantly increased in AMI and UA patients compared with SA and control patients. *$p < 0.05$ compared with the SA and control group, #$p > 0.05$ compared with control group.

activates monocytes / macrophages and dendritic cells, perpetuating the pathogenic Th1 response. Although IL-10 is considered as an anti-inflammatory cytokine, IL-10-producing Th1 cells can have disease promoting effects in response to pathogens[25]. However, whether this effect is also involved in the atherosclerotic process remains unclear.

Abundant evidence has shown that the members of the IRF family of transcription factors are important regulators of both immunity and other physiological processes[26]. All IRFs promote a Th1 response and exert a role in Th1 differentiation[27-30], while only IRF-4 promotes a Th2 response and plays an important role in Th2 differentiation[31,32]. Our study demonstrated that both mRNA and protein expression of IRF-1 in AMI and UA patients were significantly higher than that of SA and control patients[33]. Moreover, IRF-2 and IRF-8 had a similar mRNA and protein expression as IRF-1. On the other hand, IRF-4 expression was similar among the four groups. However, it is reasonable to presume that different IRFs may play various roles in the inflammation process of atherosclerosis. These results may provide a mechanistic explanation for the increase of Th1 cell activity and enhancement of function in patients with acute coronary syndrome.

The mechanisms by which IRFs regulate the development and function of CD4+ T cells are complex.

IRF-1, IRF-2 and IRF-8 promote Th1 cell differentiation or Th1 response. IRF-1 has been shown to promote Th1 differentiation *via* the IFN-γ-IL-12 signaling axis[28]. If IRF-1 is deficient, both IL-12 production by antigen-presenting cells and the development of IFN-γ-producing natural killer cells will be defective[34]. IL-12 receptor β1 subunit up-regulation in response to IFN-γ *via* IRF-1 will also be defective, causing T cell-intrinsic hyporesponsiveness to IL-12[28]. In addition, IRF-1 may directly induce the expression of inducible nitric oxide synthase in antigen-presenting cells, promoting IFN-γ production by Th1 cells[37]. IRF-2 also promotes a Th1 response. If IRF-2 is deficient or defective Th1 immunity will present due to the impaired development of natural killer cells and the reduced IL-12 p40 expression by antigen-presenting cells[38]. IRF-8 may promote Th1 cell differentiation by activating the transcription of the IL-12 p40 gene[39] and synergizing with the transcription factor PU.1 to induce IL-18 expression in macrophages[40]. IRF-8 may also promote the proliferation of plasmacytoid dendritic cells that produce IFN-γ and IL-12[41] and the differentiation and maturation of macrophages that secrete IL-12[42]. IRF-4 inhibits Th1 cell differentiation, but promotes Th2 cell differentiation. In addition, IRF-4 induces the expression of the Th2 cytokine IL-4, promoting Th2 cell proliferation[43, 44].

This study has some limitations. First, this study provided observational data in a small group of CAD patients, and thus a large cohort study is required for further investigation. Moreover, we focused only on a small subset of the IRF family. Further investigation is warranted regarding whether other members of the IRF family may have a role in CAD. Second, the mechanisms by which IRFs regulate the function and activity of T cells remain unknown. Future studies might be performed *in vitro* to elucidate the cellular and molecular mechanism.

CONCLUSION

The present study demonstrates increased Th1 response and expression levels of IRF-1, IRF-2 and IRF-8 in patients with AMI and UA. This further indicates that increased expression levels of IRF-1, IRF-2 and IRF-8 may be associated with atherosclerotic plaque formation and rupture.

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Conflict of interest: The authors declare that there are no conflicts of interest.

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Original Article

Comparison of the Applicability and Safety of the Cobra Perilaryngeal Airway and the Laryngeal Mask Airway Classic in Anesthetized and Paralyzed Patients

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ABSTRACT

Objectives: To compare two supraglottic airway devices (SAD), laryngeal mask airway classic (LMA-Classic) and cobra perilaryngeal airway (Cobra-PLA), in terms of insertion characteristics, sealing pressure, hemodynamic effects and post-operative complications
Design: Prospective study
Setting: Okmeydani Teaching and Research Hospital, Istanbul, Turkey
Subjects: A total of sixty patients were included in our study. Patients were randomly divided into two groups as Group Cobra-PLA and Group LMA-Classic.
Interventions: Upon successful insertion, the cuff was inflated with air to a pressure of 60 cm H_2O by using a manometer. A maximum of two attempts were made to insert the SAD. In case of failure, orotracheal intubation was performed.
Main Outcome Measures: The success rate of inserting the SAD devices, the duration of insertion, the number of attempts, the insertion complications, ventilation parameters, hemodynamic parameters and postoperative adverse effects were recorded for each group.
Results: In Group Cobra-PLA and Group LMA-Classic, the SAD were successfully inserted respectively in 26 (86.7%) and 28 (93.3%) patients. The duration of insertion was shorter in group LMA-Classic ($p < 0.05$). The seal pressure was significantly higher in group Cobra-PLA ($p < 0.001$). Bleeding was less frequently observed in patients with LMA-Classic group ($p < 0.05$).
Conclusions: Both SAD can safely be used as an alternative to endotracheal intubation. According to the results of our study, LMA-Classic seems to be a more practical device regarding the insertion characteristics and the postoperative complications; however, it should be noted that sealing pressure is higher in Cobra-PLA.

KEY WORDS: airway management, anesthesiology, ventilation

INTRODUCTION

The laryngeal mask airway-classic (LMA-Classic; North America, Inc., San Diego, CA, USA) was the first supraglottic airway device (SAD) to be available as an alternative to an endotracheal tube[1]. Recently, the LMA-Classic has been successfully and safely used in general anesthesia practice[2,3]. A variety of other SAD, have been introduced as an alternative to the LMA-Classic, for clinical use within the past decade. In general, the SGA devices have similar structures and characteristics as the LMA-Classic. The cobra perilaryngeal airway (Cobra-PLA; Engineered Medical Systems, Indianapolis, IN, USA) differs from the LMA-Classic in terms of structure and anatomic location[4]. The Cobra-PLA is a cuffed, disposable, sterile, and latex-free SAD[5,6]. The device is made of polyvinyl chloride and consists of a head, a circumferential pharyngeal cuff and a breathing tube. The Cobra-PLA is available in eight different sizes. Patients can be intubated by passing an endotracheal tube of appropriate size through the Cobra-PLA. The main differences between the Cobra-PLA and LMA-Classic are the shape of the cuff and the area of insertion. In the present study, we compared these two airway devices in terms of insertion characteristics (the success rate, the number of insertion attempts, and the duration of insertion), sealing pressure, hemodynamic effects, and post-operative complications.

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SUBJECTS AND METHODS

Approval from the local ethics committee and written consent from all patients who participated in the present study was obtained. Sixty consecutive patients weighing between 50 and 100 kg, with the American Society of Anesthesiologists (ASA) physical status I and II class who underwent general anesthesia for varicose vein stripping or unilateral inguinal herniorrhaphy in supine position, were included in the study. The patients were randomly assigned to the Cobra-PLA (group Cobra-PLA, n = 30) or LMA-Classic device (group LMA-Classic, n = 30), for airway management (Fig. 1). Patients with respiratory tract pathology, known airway problems, or any condition that might increase the risk of gastroesophageal regurgitation were excluded. The patients were randomly assigned to group Cobra-PLA or group LMA-Classic using the sealed envelope method. In the present study, the sizes of the airway devices were selected based on the patient's weight according to the manufacturer's recommendations. The insertion of the SAD was done by the same anesthesia resident experienced in the use of both devices (LMA-Classic ≥ 1000 uses; and Cobra-PLA ≥ 100 uses). Demographic characteristics (gender, age, height, and weight), Mallampati score, and the duration of surgery were recorded. The patients were monitored with respect to heart rate (HR; three-channel electrocardiography), non-invasive blood pressure, peripheral oxygen saturation (SpO_2), end-tidal CO_2 ($ETCO_2$; Datex-Ohmeda S/5TM Compact Critical Care Monitor), bispectral index (BIS, A-2000 BIS Monitoring System; Aspect Medical Systems, BIS XP, Framingham, MA, USA), and neuromuscular transmission (TOF-T1, TOF-Watch SX®; Organon Instruments, Boxlet, Netherlands). For anesthesia induction, 1 mcg/kg of fentanyl and 0.6 mg/kg of rocuronium bromide were administered, and 1% propofol was used until the BIS value was < 60. In order to standardize the procedure, SGA devices were inserted while the BIS was between 50 - 60 and T1 was 0, in all patients. Upon successful insertion, the cuff was inflated with air to a pressure of 60 cm H_2O by using a manometer (Cuff Pressure Gauge; VBM Medizintechnik, Sulz, Germany). Fresh gas flow was adjusted to 6 l/min (O_2/air mix 50 : 50%) during surgery. Sevoflurane was used as the inhalation agent for maintenance of anesthesia. The tidal volume was adjusted to 8 ml/kg with a ventilatory frequency of 12 breaths/min by volume-controlled mechanical ventilation (Datex-Ohmeda S/5 Avance). A maximum of two attempts were made to insert the SAD. In case of failure, orotracheal intubation was performed. The success rate of inserting SAD devices, the duration of insertion, the number of attempts, and the insertion complications were recorded for each group. The heart rate, mean arterial pressure (MAP), and peripheral oxygen saturations of the patients were recorded before and after SGA device insertion, during surgery (30^{th} min), and before and after extubation. During surgery, the percentage of tidal volume leakage, peak airway pressure (Ppeak), mean airway pressure (Pmean), $ETCO_2$, and seal pressure was measured three times in all patients and mean values were recorded. Leakage percentage was expressed as the ratio of the difference between the expiratory tidal volume and the inspiratory tidal volume over the inspiratory tidal volume. The seal pressure was determined, as previously described previously by Keller and Shimbori[7,8], by closing the expiratory valve of the circle system at a fixed gas flow of 3 l/min and noting the airway pressure at which equilibrium was reached. Fresh gas flow was adjusted to 3 l/min without air mix during each measurement. Considering adverse effects, such as blood staining of the device, sore throat, hoarseness, aphasia, nausea, vomiting, and agitation after extubation, the patients were monitored for at least one hour in the recovery room. Patients with a modified Aldrete score > 10 were transferred to the ward. A pilot study was conducted on 10 patients and the sample size was calculated, based on a difference of 20% between the groups for duration of insertion, for a type 1 error of 0.05 and a power of 0.8. The results are expressed as the mean ±

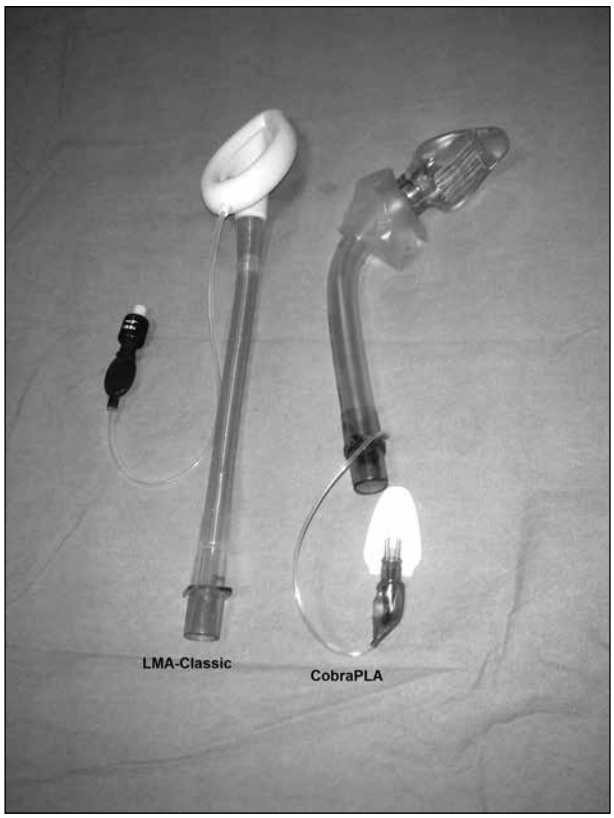

Fig. 1: CobraPLA and LMA-Classic device for airway management

SD. All statistical analyses were carried out using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). A t-test was used for comparison of quantitative variables. Qualitative variables were compared using a chi-square test. A p-value < 0.05 was considered statistically significant.

RESULTS

There was no difference between the groups with respect to demographic characteristics and the Mallampati scores (Table 1). The mean duration of surgery was 45.8 ± 10.0 and 44.2 ± 11.1 min in group LMA-Classic and group Cobra-PLA, respectively (p = 0.570).

Table 1: Patient demographic parameters and Mallampati score

Demographic Parameters	LMA-Classic Group	Cobra-PLA Group
Sex (M / F)	15 / 15	18 / 12
Age (years)	45.4 ± 7.8	43.2 ± 8.0
Height (cm)	169.8 ± 6.3	168.5 ± 4.9
Weight (kg)	75.2 ± 6.9	73.0 ± 5.4
Mallampati score (I/ II/ III)	7 / 19 / 4	10 / 15 / 5

Data are mean ±SD or number of patients, F: female, M: male, LMA: laryngeal mask airway, PLA: Perilaryngeal airway

Parameters of Laryngeal Mask Insertion and Ventilation: In group LMA-Classic, the SAD was successfully inserted in 25 patients (83.3%) on the first attempt and in 28 patients (93.3%) on the second attempt. In group Cobra-PLA, the SAD was successfully inserted in 23 patients (76.7%) on the first attempt and in 26 patients (86.7%) on the second attempt (Table 2). The success rate of insertion was similar in both the groups. The Mallampati scores of four out of six patients in whom the SAD could not be inserted were determined to be grade III. The duration of insertion was shorter in group LMA-Classic and there was a significant difference between the groups (p < 0.05; Table 2). No complications were observed during insertion in both groups. There was no difference between the two groups with respect to Ppeak, Pmean, $ETCO_2$, and tidal volume leakage percentage values measured by ventilator. The seal pressure was significantly higher in group Cobra-PLA as compared to the group LMA-Classic (p < 0.001; Table 3).

Table 2: Insertion features between different groups

Insertion Features	LMA-Classic Group	Cobra-PLA Group
Number of attemps (1 / 2)	25 (83.3 %) / 5 (16.7 %)	23 (76.7 %) / 7 (23.3 %)
Insertion time (s)	18.1 ± 3.0*	20.1 ± 3.2

Data are mean ± SD or number of patients and percentage, LMA: laryngeal mask airway, PLA: Perilaryngeal airway, *p-value < 0.05

Table 3: Ventilation parameters

Demographic Parameters	LMA-Classic Group	Cobra-PLA Group
Airway Sealing Pressure (cmH_2O)	23.4 ± 3.3*	27.6 ± 4.2
Leakage Percentage	4.9 ± 1.3	4.3 ± 1.4
Ppeak (cm H_2O)	16.4 ±2.0	17.1 ± 2.5
Pmean (cm H_2O)	6.7 ± 1.5	7.3 ± 1.8
$ETCO_2$ (mmHg)	33.7 ± 1.5	33.2 ± 1.6

Data are mean ± SD, $ETCO_2$: end-tidal CO_2, LMA: laryngeal mask airway, PLA: Perilaryngeal airway, *p-value < 0.001

Hemodynamic Parameters

There was no difference between the groups in terms of hemodynamic parameters including mean arterial pressure (MAP) and heart rate (HR) (Tables 4 and 5).

Table 4: Mean arterial pressure[a] between different groups

Mean Arterial Pressure	LMA-Classic Group	Cobra-PLA Group
Before Insertion of SAD	90.9 ± 11.2	93.1 ± 11.3
After Insertion of SDA	96.2 ± 10.1	97.8 ± 11.2
During Operation (30^{th} min.)	91.6 ± 5.3	93.2 ± 8.2
Before Extubation	91.2 ± 7.4	93.7 ± 6.4
After Extubation	97.3 ± 7.4	98.8 ± 5.3

Data are mean ± SD, SAD: Supraglottic airway device/s, LMA: Laryngeal mask airway, PLA: Perilaryngeal airway, [a] : in mmHg

Table 5: Heart rate[a] between different groups

Heart Rate	LMA-Classic Group	Cobra-PLA Group
Before Insertion of SAD	72.0 ± 8.5	72.4 ± 8.3
After Insertion of SDA	79.2 ± 7.9	81.5 ± 7.0
During Operation (30^{th} min.)	75.0 ± 8.9	78.2 ± 8.8
Before Extubation	71.7 ± 5.6	73.1 ± 4.7
After Extubation	78.5 ± 5.1	79.9 ± 7.2

Data are mean ± SD, SAD: Supraglottic airway device/s, LMA: Laryngeal mask airway, PLA:Perilaryngeal airway, [a]: in beats $^{min-1}$

Post-operative Complications

None of the patients had hoarseness or difficulty in swallowing. The most common complications encountered in both groups were agitation, nausea-

Table 6: Adverse effects between different groups

Adverse Effects	LMA-Classic Group n (%)	Cobra-PLA Group n (%)
Blood Staining	4 (13.3)*	12 (40)
Sore Throat	3 (10)	6 (20)
Agitation	3 (10)	5 (16.7)
Nausea and Vomiting	3 (10)	5 (16.7)

Data are number of patients and percentage, LMA: laryngeal mask airway, PLA: Perilaryngeal airway, *p-value: < 0.05

vomiting, sore throat, and bleeding (Table 6). Bleeding was less frequently observed in patients using the LMA-Classic device ($p < 0.05$).

DISCUSSION

A variety of alternative SAD such as the LMA-Classic and CobraPLA, are available for clinical use. These two alternatives have been used successfully and safely in general anesthesia practice[9-11]. An available airway device is one that is easily and rapidly applicable for use in general practice. The insertion success rate of the LMA-Classic device, which has been used for years, has been proven to be considerably high and can easily be used even by less-experienced physicians[12-15]. Numerous studies in the literature report high rates of successful insertion of the Cobra-PLA device, which has recently become available[16,17]. In the present study, a high rate of success was obtained with both SAD. Although the insertion success rate of insertion of the LMA-Classic device (93.3%) was higher when compared with that of the Cobra-PLA device (86.7%), no significant difference was observed as determined between the groups. In a study conducted by Gaitini *et al*[18] on 80 patients, the insertion success rate was similar for both SAD. In addition, the time required for insertion in the LMA-Classic and Cobra-PLA groups was found to be approximately 23.7 ± 2.47 s and 26.6 ± 7.1 s, respectively, with a significant difference between the groups. Other studies have reported that the time required for insertion is similar for both the Cobra-PLA and LMA-Classic devices[9,16].

Van Zundert *et al*[19] concluded that the time required for insertion of the LMA-unique, which is similar to the LMA-classic but in a single-use form, was significantly shorter than that of the Cobra-PLA device. In our study, we found that time required for insertion of the LMA-Classic (18.1 ± 3.0 s) was shorter than that required for the Cobra-PLA (20.1 ± 3.2 s). The depth of anesthesia and the level of muscle relaxation during SAD insertion might affect the success rate and time required for insertion. Furthermore, more objective outcomes were obtained in the present study because the anesthetic depth and muscle relaxation were monitored using BIS and TOF during device insertion, unlike in other studies. Another noteworthy point was the fact that the Mallampati scores of the four out of six patients in whom the SGA device could not be inserted was three. Although sufficient data are not available, we also suggest that the Mallampati score, which is the criteria for possible difficult intubation, might influence the insertion success rate of the LMA-Classic and Cobra-PLA devices.

Moreover, McCrory *et al*[20] demonstrated that the Mallampati score influenced the insertion success rate of the LMA. The sealing pressure of the SAD is of importance in terms of airway safety and its appropriateness for different types of surgeries. Recently, SAD has been used safely, even in laparoscopic surgeries that require ventilation with a high peak airway pressure. In a study conducted by Akca *et al*[9] the LMA-Classic and Cobra-PLA were compared in terms of sealing pressure, and it was found that the Cobra-PLA had a significantly higher sealing pressure. Similar results have been obtained in numerous studies in which the Cobra-PLA was compared with the LMA-Classic or the LMA-Unique[17-19,21].

The present study also showed that the sealing pressure was significantly higher in the Cobra-PLA group (27.6 ± 4.2 cm H2O) than in the LMA-Classic group. We suggest that the high sealing pressure of the CobraPLA confers an advantage over the LMA-Classic when high-pressure ventilation is required either because of the patient's medical condition (*e.g.*, COPD and obesity) or the type of surgery (*e.g.*, laparoscopic procedures). In the present study, no significant difference in the ventilation parameters was noted between the groups, including Ppeak, Pmean, and $ETCO_2$. The Ppeak values were also similar between patients using the LMA-Classic and Cobra-PLA devices in the study conducted by Nam *et al*[21]. In a number of studies, LMA have been shown to cause fewer hemodynamic alterations compared to endotracheal intubation[22-24]. Since the Cobra-PLA has a larger cuff compared to other types of SAD, the hemodynamic response would be higher during device insertion. However, in the present study, no differences were noted in MAP and HR between the LMA-Classic and Cobra-PLA groups either after insertion or at any other time. Variations in the anesthetic depth levels of the patients during intubation affect the severity of the hemodynamic response to intubation. In the present study, using BIS monitoring during SAD insertion provided a better comparison of the two groups in terms of hemodynamic response.Bleeding and sore throat are the most frequently observed postoperative complications after the use of SAD. In our study, sore throat was observed at a rate of 10% in the LMA-Classic group and at a rate of 20% in the Cobra-PLA group. In previous studies, the frequency of sore throat was reported to be between 10% and 50% after the use of the Cobra-PLA[9,16,18]. In a study conducted by Turan *et al*,[16] the LMA, laryngeal tube, and Cobra-PLA were compared, and bleeding was more frequently observed in patients who used the Cobra-PLA device. In the present study, the respective frequency of bleeding after the use of the Cobra-PLA and LMA-Classic was 40% and 13.3%, with a significant difference between the groups. This difference in the frequency of bleeding can be attributed to the larger and different cuff structure of the Cobra-PLA device. Therefore, we recommend that unnecessary inflation of the cuff to a

high pressure should be avoided and the cuff pressure should be monitored *via* a manometer during the use of the Cobra-PLA, in which the complications of sore throat and bleeding are more frequently observed.

CONCLUSION

Both SAD can safely be used as an alternative to an endotracheal tube. The results of the present study suggest that the LMA-Classic is a more practical device in terms of the insertion characteristics and postoperative complications; however, the mean sealing pressure is higher in the Cobra-PLA device. The Cobra-PLA should be considered as an alternative to the LMA-Classic in patients who require high-pressure ventilation.

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Original Article

The Efficacy of Kasmitad Gel in the Management of Recurrent Minor Aphthous Ulceration

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ABSTRACT

Objective: To evaluate the efficacy and safety of Kasmitad™ gel for the treatment of recurrent minor aphthous ulceration, particularly when applied at the onset of prodromal symptoms
Design: Self-controlled before – after study conducted during a 26-month period (October 2007 - September 2009)
Setting: Outpatient clinic at the School of Stomatology, Tongji University, Shanghai, China
Subjects and Methods: Seventy patients with recurrent minor aphthous ulceration were randomized into the prodromal or ulceration group
Interventions: Four parameters (pain score, ulcer incidence, size, duration) were recorded before and after treatment.
Main Outcome Measures: Incidence of ulcer occurrence after treatment at different stages; changes in ulcer size, duration, and pain score.
Results: Only 75.8% of subjects in the prodromal group developed an ulcer. Compared with no treatment, the maximum pain score, ulcer size, and ulcer duration were clearly reduced for subjects in both groups ($p < 0.01$). The two groups showed significant differences in the reduction of ulcer size and duration ($p < 0.01$), but no significant difference in pain scores ($p = 0.236$).
Conclusion: In the treatment of recurrent aphthous ulceration, the use of Kasmitad™ gel at the onset of prodromal symptoms can prevent progression to ulcer development and significantly reduce symptoms if ulcers do develop.

KEYWORDS: aphthous ulcer, Kasmitad gel, recurrent, treatment

INTRODUCTION

Recurrent aphthous ulceration (RAU) is one of the most prevalent oral mucosal diseases, affecting an estimated 5 - 50% of the general population[1]. RAUs have been classified into three subtypes: minor aphthous ulcers, major aphthous ulcers, and herpetiform ulcers. Minor RAU (diameter < 1 cm) is the most common manifestation of the disorder, occurring in 75 - 80% of patients with RAU[2]. It is characterized by a prodromal localized burning or pricking sensation, usually lasting 24 - 48 h, followed by the classical clinical appearance of ulceration presenting as self-limited, painful, clearly defined, shallow round or oval ulcers with gray-white centers and erythematous halos. Minor RAU usually affects the non-keratinized mucosa, such as the buccal and labial mucosa and the lateral border of the tongue[1-3]. Ulcers may occur singly or in multiple locations at intervals ranging from a few months to a few days, with some subjects experiencing almost continuous recurrence with no ulcer-free days[3].

RAU lesions can be extremely painful and may lead to difficulty in speaking or eating, greatly reducing the quality of daily life[2, 3]. Due to the often-uncertain etiology of RAU and the unpredictable course of the disease, the primary goals of therapy are to control the pain caused by the ulcer, ensure adequate food and fluid intake, promote ulcer healing, and prevent recurrence[4]. Several medical approaches have been adopted for the symptomatic management of RAU. In general, topical therapies are recommended as a first-line approach, particularly in otherwise healthy patients, although such therapies do not prevent ulcer recurrence[5]. A multitude of topical agents, including corticosteroids, antibiotics, local anesthetics, antihistamines, non-steroidal anti-

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inflammatory drugs, enzymatic preparations, and immunosuppressants, are available for symptomatic relief[1,4,5].Topical corticosteroids are commonly used to alleviate symptoms in the treatment of RAU, but their extended use may be associated with oral Candida overgrowth[4]. Topical antibiotics have some clinical efficacy for the treatment of RAU, but adverse effects such as candidiasis, allergic reactions, bitter taste, and tooth discoloration may reduce patient compliance[2]. Local analgesics, such as 3% viscous lidocaine, are often prescribed for the topical treatment of RAU, but the long-term analgesic action is poor and these medications cannot enhance ulcer healing[4]. Thus, a proper combination of currently used topical agents may be more effective than the use of a single agent, providing higher efficacy and fewer side effects.

Kasmitad™ gel is a topical compound preparation developed by the STADA pharmaceutical company (STADA Arzneimittel AG, Bad Vibel, Germany) and approved in 1978 for production in Germany. In 2000, the State Food and Drug Administration of China approved the application of the gel (symbol XL2000030) for the treatment of recurrent oral ulcers[6]. One gram of Kasmitad™ gel contains 200 mg chamomile flower tincture (1:5.5), 20 mg lidocaine, 1 mg thymol, and 1 mg benzalkonium chloride as a preservative. The efficacy and safety of Kasmitad™ gel in the management of RAU have been investigated[6,7], but researchers have noted that this gel might be of further benefit if treatment is commenced during the prodromal stage of ulceration. To this end, the aims of the present study were twofold. First, the efficacy of Kasmitad™ gel application in the prodromal stage of ulceration in preventing progression to ulcer development was determined. Second, the maximum pain score, ulcer size, and ulcer duration were evaluated in subjects treated with Kasmitad™ gel either at the onset of prodromal symptoms or at the onset of ulceration and compared with those of subjects receiving no treatment.

MATERIAL AND METHODS

Subjects

All subjects were recruited by advertising among staff and students at the study sites or from clinical patients of the Department of Oral Medicine, College of Stomatology, Tongji University, between October 2007 and September 2009. Minor RAU was characterized as recurring ulcers < 5 mm in diameter that were confined to the oral mucosa, with no other sign of disease[8]. Prodromal symptoms were identified as burning or pricking sensations with red spots, and ulceration was classified as painful, clearly defined, shallow round or oval ulcers with gray-white centers and erythematous halos[2,3]. The inclusion and exclusion criteria are shown in Table 1. Based on these criteria, 70 subjects were enrolled in this study.

Study design

Given the individual variations in pain sensation, ulcer size, and ulcer duration, we performed a self-controlled before – after trial. The incidence of ulcer occurrence after treatment at different stages, and changes in ulcer size, pain score, and ulcer duration were considered the main outcomes of interest.

The research was designed and carried out independently in the School of Stomatology, Tongji University. Ethical approval for the study was obtained from the Research Ethics Committee of Tongji University, and all subjects provided written informed consent. During the study, one investigator was responsible for the diagnosis, randomization of patients and instruction in the use of the gel, and two other investigators who were blinded to the randomization and had been trained in standardized data collection served as evaluators responsible for measuring pain scores, ulcer sizes and durations, and adverse events. Ulcer size, pain score, and ulcer duration values are the averages of the two investigators' measurements.

Table 1: Selection criteria for participants with recurrent minor aphthous ulceration

Inclusion criteria

1. Man or woman aged 18 – 65 years
2. Willingness to participate and provide written informed consent
3. Presenting with 1 - 5 aphthous ulcers ≤ 5 mm in diameter
4. Expected ulcer duration ≥ 5 days without treatment

Exclusion criteria

1. Known history of serious drug hypersensitivity
2. Pregnancy or lactation
3. Concurrent clinical condition(s) that could pose a health risk, including serious liver, kidney, and/or heart dysfunctions
4. History of an immunological problem
5. Ulceration as a manifestation of a systemic disease process, such as ulcerative colitis, Crohn's disease, Behçet's syndrome, or severe anemia
6. Treatment with systemic steroids or other immunomodulatory agents within one month of study entry
7. Use of non-steroidal anti-inflammatory drugs or oral antihistamines within one month of study entry
8. Treatment with systemic antibiotics within two weeks of study entry
9. Participation in any other clinical trial within three months of study entry

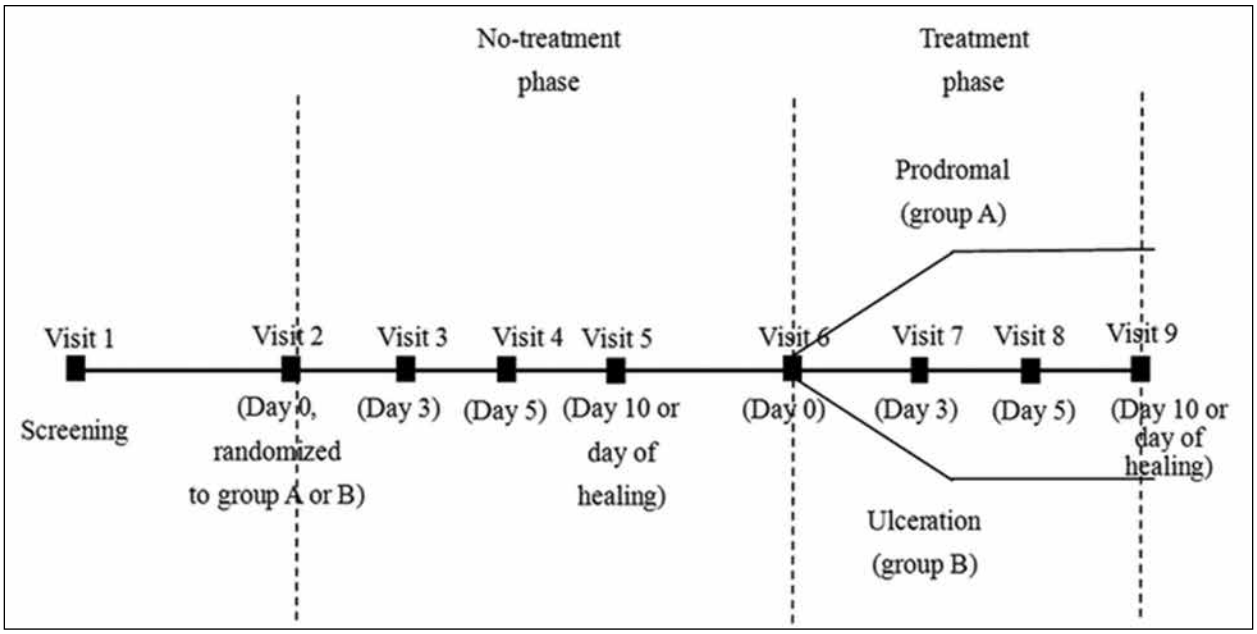

Fig. 1: Summary of the study protocol

Protocol

Fig. 1 shows a summary of the study protocol. After investigators screened subjects to confirm that they had RAU (visit 1), subjects returned at their next ulcer occurrence for randomization into one of two treatment groups (group A, treatment at prodromal symptom onset; group B, treatment at ulceration onset) according to a computer-generated randomization list (visit 2). Subjects then entered a no-treatment run-in period to enable subsequent comparisons between treatment and no treatment.

During the first ulcer episode in the no-treatment period, subjects recorded pain score and ulcer size twice daily on diary cards. On days 0, 3, 5, and 10 or the day of healing (visits 2 - 5), investigators assessed the severity of pain and ulcer diameter.

At the second ulcer episode, subjects were required to visit the clinic within 12 h of prodromal stage or ulceration onset to receive Kasmitad™ gel treatment (visit 6). For subjects with more than one ulcer, only the most recently occurring ulcer was evaluated. All subjects were supervised for the first application of Kasmitad™ gel and provided with written instructions for application. Subjects were instructed to squeeze 0.2 cm of Kasmitad™ gel onto a wet fingertip and to dab the paste onto the identified ulcer four times a day (after three meals and before bedtime) until ulcer healing. The subjects assessed ulcer size and pain score before each application of the drug, and recorded all assessments and treatment applications, with the date and time, in subject diaries. On treatment days 0, 3, 5, and 10 or the day of healing (visits 6 - 9), investigators assessed the severity of pain and ulcer size. Each subject was interviewed at each visit concerning the emergence of any adverse events.

All subjects were monitored at study entry (visit 1) and the end of the study (visit 9) for the occurrence of abnormalities in laboratory test results for the following indices: complete blood cell count with differential and platelet count; serum chemistry levels, including blood urea nitrogen, creatinine, aspartate transaminase, and alanine transaminase; and urinalysis of protein, glucose, and urine blood cell count. Treatment compliance was monitored by evaluating the recorded date and time of gel applications in subject diaries and by weighing each tube of Kasmitad™ gel at the time of dispensing and at the end of the study.

Measurements

Subjects scored the severity of pain using a self-administered visual analogue scale. They were instructed to bisect a 10 cm line from 0 (no pain) to 10 (extreme pain) at an appropriate point to rank pain at the moment when their ulcerative lesion was brushed gently with a tampon. Maximum ulcer diameter was calculated by measuring the distance between two opposing external edges of the white lesion border with a periodontal probe, and a second measurement was taken approximately perpendicular to the maximum diameter. These two measurements were then multiplied to determine the cross-sectional area of the ulcer. Ulcer healing was identified as a pain score of 0, whether the mucosa appeared normal or had white striae. Ulcer duration was calculated from the day of prodromal symptom onset to the day of healing.

Table 2a: Changes in the mean maximum ulcer size, pain score, and ulcer duration after treatment with Kasmitad™ gel at the onset of prodromal symptoms compared with scores recorded during the no-treatment run-in period

Characteristics	No treatment N = 33	Treatment at prodromal symptom onset N = 33	95% confidence interval	p-value[a]
Maximum ulcer size (mm^2)	7.18 ± 3.03	3.10 ± 2.17	3.08 - 4.81	< 0.01
Maximum pain score (VAS)	5.82 ± 1.60	2.72 ± 0.84	3.23 - 4.09	< 0.01
Ulcer duration (d)	9.06 ± 1.14	4.51 ± 2.75	3.76 - 4.54	< 0.01

[a]Treatment at prodromal symptom onset Vs. no treatment, paired-sample t-test, VAS = visual analogue scale

Table 2b: Changes in the mean maximum ulcer size, pain score, and ulcer duration after treatment with Kasmitad™ gel at the onset of ulceration compared with scores recorded during the no-treatment run-in period

Characteristics	No treatment N = 33	Treatment at prodromal symptom onset N = 33	95% confidence interval	p-value[a]
Maximum ulcer size (mm^2)	6.62 ± 2.66	4.84 ± 2.37	1.48 - 2.02	< 0.01
Maximum pain score (VAS)	6.25 ± 1.38	3.55 ± 1.34	2.01 - 2.69	< 0.01
Ulcer duration (d)	8.97 ± 1.27	7.94 ± 1.33	1.23 - 1.80	< 0.01

[a] Treatment at ulceration onset Vs no treatment, paired-sample t-test. VAS = visual analogue scale

Statistical analyses

Background and demographic data were summarized with descriptive statistics. Baseline ulcer parameter values were compared between the two groups with the Mann–Whitney U-test. The proportion of subjects developing ulcers was compared between groups using Fisher's exact probability test. Ulcer size, pain score, and ulcer duration were compared between the two treatment groups and within each subject between no-treatment and treatment periods using independent-sample and paired-sample t-tests. Safety was analyzed with descriptive statistics for the measurement of adverse events at each study visit. A p-value < 0.05 was considered statistically significant. All statistical tests were carried out using the SPSS software (ver. 12.0; SPSS Inc., Chicago, IL, USA).

Results

A total of 70 subjects were enrolled in this study. Two subjects in the prodromal symptom group dropped out because they had no second ulcer recurrence before the end of the trial. Thus, 33 subjects in the prodromal symptom group and 35 subjects in the ulceration group completed the study. Given the low discontinuation rate (2.86%), our evaluation of demographic and efficacy data without these two subjects had no influence on the interpretations or conclusions. The randomization procedures ensured that demographic variables, including age (p = 0.45), sex (p = 0.52), and subject number (p = 0.09), were well matched between groups.

Efficacy

Only 75.8% (25 / 33) of subjects treated with Kasmitad™ gel at the onset of prodromal symptoms developed an ulcer between days 0 and 3, whereas all subjects (100%, 35 / 35) treated at the onset of ulceration developed an ulcer (p = 0.02). Significant reductions in mean maximum ulcer size and ulcer duration were observed within (p < 0.01, Tables 2a, b) and between (p < 0.01, Table 3) treatment groups. The reduction in mean maximum pain scores was significant within each group (p < 0.01, Table 2), but did not differ between the two treatment groups (p = 0.236, Table 3).

Safety

Five subjects experienced a tingling sensation at the application site, which investigators considered to be a mild effect that was possibly related to the studied medication. None of these five subjects discontinued the trial due to this adverse event. No difference in laboratory findings was observed between treatment groups at baseline or at the end of the study.

Table 3: Efficacy of Kasmitad™ gel treatment at the onset of prodromal symptoms (group A) or ulceration (group B)

Characteristics	Estimated difference between run-in and treatment periods		p-value[a]
	Group A	Group B	
Maximum ulcer size (mm^2)	- 4.08 ± 1.99	-1.78 ± 0.89	< 0.01
Maximum pain score (VAS)	- 3.10 ± 1.49	-2.70 ± 1.20	0.236
Ulcer duration (d)	- 4.55 ± 2.22	-1.03 ± 0.66	< 0.01

[a]Group A Vs group B, independent-sample t-test , VAS = visual analogue scale

DISCUSSION

For the treatment of minor RAU, topical medication has played a crucial role in reducing ulcer pain and accelerating ulcer healing. Given the individual variation in pain sensation and ulcer duration, we carried out a self-controlled, before - after trial to investigate the efficacy and safety of Kasmitad™ gel application in the treatment of this disorder. A randomized, double-blind, controlled clinical trial proved the efficacy of Kasmitad™ gel in the management of minor RAU[6], and the present study evaluated the efficacy and safety of this treatment, particularly when applied at the onset of prodromal symptoms.

Most previous studies of RAU treatments have begun observation within 24 - 48 h after the appearance of an ulcer. In this study, we observed the whole cycle of RAU, from the onset of prodromal symptoms to the healing of the ulcer, and recorded ulcer size and pain score four times a day, which allowed us to recognize small changes in an ulcer. Thus, this study more accurately reflected the real situation of the ulcer cycle, enabling us to calculate ulcer duration accurately. However, the study lacked a placebo because Kasmitad™ gel contains natural plant extracts, for which we were unable to obtain a placebo.

The results of the present study demonstrated that Kasmitad™ gel effectively promoted ulcer healing when applied at the onset of prodromal symptoms or ulceration. Compared with no treatment, Kasmitad™ gel not only reduced the mean maximum ulcer size and pain score, but also hastened ulcer healing with no systemic side effect and only minor adverse events. These results are consistent with those of previous studies[6,7], and demonstrate the efficacy and safety of Kasmitad™ gel application in the treatment of RAU.

To our knowledge, this study was the first to determine the efficacy of applying Kasmitad™ gel in the prodromal stage. Only 75.8% of subjects randomized to treatment at prodromal symptom onset developed an ulcer, whereas all subjects treated at the onset of ulceration developed ulcers ($p = 0.02$). Furthermore, treatment in the prodromal stage hastened healing when ulcers did occur. The mean maximum ulcer size and ulcer duration were greatly reduced in subjects treated at prodromal symptom onset compared with those treated at the onset of ulceration ($p < 0.01$, Table 3). These results indicate that Kasmitad™ gel application in the prodromal stage can prevent ulcer development, and that early application enhances the therapeutic effects of this medication.

Chamomile is a widely recognized herb in Western culture. Research has shown that chamomile flower tincture has anti-inflammatory, antimicrobial, and antioxidant effects and can potentially accelerate burn wound healing[9-11]. Both lidocaine and chamomile flower tincture have analgesic effects. Previous studies have demonstrated that Kasmitad™ gel provides immediate (within 5 - 10 min) pain relief [6,7]. This gel can be used before eating or during severe pain episodes to control ulcer pain and ensure adequate food and fluid intake, thereby improving the quality of life. In this study, subjects recorded pain scores before, rather than after, each application of the drug, at least 3 - 4 h after the last gel application. Thus, the significant pain-relieving effects we observed in both treatment groups may have been due to the promotion of healing by ingredients such as chamomile flower tincture, instead of the analgesic effect.

CONCLUSION

The results of this study provide evidence that Kasmitad™ gel is a well-tolerated, effective treatment modality for minor RAU. The early application of this treatment is particularly effective in preventing ulcer development and providing symptomatic relief of ulcers that do occur. Studies in larger series of subjects are needed to further confirm the results.

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Conflict of Interest: The authors declare no conflict of interest.

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Original Article

Prevalence and Determinants of Smoking in Iranian Adolescents

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ABSTRACT

Objectives: To examine smoking habits among male adolescent students in Jahrom, Iran, assess the prevalence of smoking in this group and examine the determinants of smoking behavior among male adolescents
Design: Prospective cross-sectional study conducted in spring 2009.
Setting: Jahrom office of education, Iran
Subjects and Methods: Data were obtained through self-administered questionnaires that were completed by all students present on the day of the study. A total of 949 male students responded to the questionnaires. Binary logistic regression was used to examine the determinants of smoking behavior.
Intervention: None
Main Outcome Measures: Prevalence of smoking and factors associated with smoking behavior
Results: The prevalence of smoking increased from 18.8% to 32.5% with advancement of school level (p = 0.005). This result was similar for cigarette smoking (5.4% among middle school students and 12.8% among pre-college students; p < 0.001). Having parents who smoked increased the risk of smoking for middle school students by 4.18 times (95%CI = 1.35-12.91).
Conclusion: For high school students, smoking parents and siblings were two important factors for being a smoker. The prevalence of smoking is relatively high among male adolescents in Jahrom, Iran. Effective smoking prevention programs should take into account the dominant influence of parents and siblings on the smoking behavior.

KEYWORDS: adolescents, school level, smoking predictors, smoking prevalence

INTRODUCTION

Tobacco use is the foremost preventable cause of death, cancers, hypertension and chronic obstructive airway diseases in the world[1-3]. From each ten adult deaths occurring in the world, one is caused by tobacco-related disorders[4]. The World Health Organization (WHO) appraises that roughly more than four million deaths take place per year due tobacco, a figure that is forecasted to increase to 10 million deaths a year by 2020[1]. Most smokers live in developing countries and most are male[5]. If smoking is started at a younger age, the risk of becoming a heavy smoker and suffering from cigarette-related diseases increases very highly compared to older ages of onset[6]. Moreover, teenage smoking increases both the numbers of smokers and the numbers of teenagers who smoke regularly. Peer pressure and family members who smoke are two important factors influencing teenagers to smoke[7].

The Global Youth Tobacco Survey (GYTS) in 25 European countries found that 22% of boys were cigarette smokers[1]. In 2003, the prevalence of cigarette use in Alaska was 26.9% for middle and 42% for male high-school students[8].

Tobacco use is frequent in developing countries and may be considered an epidemic condition. In Syria, cigarette smoking is increasing by about 3.4% per year. The prevalence of current smoking was 15.9% among male Syrian high-school students[9], 22.7% for male Korean high- school students[10] and 29.3% among Saudi Arabia secondary school students[11].

Numerous studies have reported the prevalence of smoking among Iranian school students[6]. The findings of the Global Youth Tobacco Survey (GYTS) in the Islamic Republic of Iran in 2003 showed that 19.1% of male students had smoked cigarettes[12]. One research reported the prevalence of occasional smoking as 31% and that of daily smoking as 6% among male high school students in Tehran[6].

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The aim of the present study was to describe the patterns of smoking among adolescents. In addition, we shall examine the association of some factors with the smoking behavior.

SUBJECTS AND METHODS

This cross-sectional study was done during the year 2009. For this study, we used data from Jahrom office of education. Jahrom, the site of the present study, is a city in the southern region of Fars province, Iran, with a population of 230,000 people. The target population of this survey consisted of male adolescent students. A multistage cluster random sampling was performed for student selection. Due to differences expected in school (middle school, high school and pre-college center) and residence area (urban or rural), these variables were stratified. There were 107 male schools (59 middle schools, 38 high schools and 10 pre-college centers) with a total of 12,684 students. The schools were then stratified by school level and by urban or rural district. Subsequently, the numbers of schools were selected randomly, and then from each selected school, the numbers of students were selected randomly. The total number in the sample was 1500 students. Respondent rate was 80% (1200 students, with 315 students from 31 middle schools, 690 students from 20 high schools and 145 students from 10 pre-college centers).

Data on smoking behaviors were collected using a self-administered questionnaire with 0.75% reliability (Cronbach's alpha). The questionnaire included questions about smoking status, presence of smokers at home, first tobacco provider and school level. Verbal consent was obtained from every respondent. This study was ethically approved by the institutional ethics committee at Jahrom University of Medical Sciences. All present participants were asked to complete the questionnaire. Students smoking at least one cigarette or hookah per month were considered smokers. Any respondent who had used any tobacco products during his lifetime, even for once, was deemed as ever-smoker. School level was categorized into four levels according to the Iranian schooling system: 1- primary school (first to fifth grade), 2- middle school (sixth to eighth grade), 3- high school (ninth to eleventh grade) and 4- pre-college center (twelfth grade).

The qualitative variables were reported as frequency and percent. Binary logistic regression (backward) analyses were performed for investigating the relationship between study variables (place of stay, mother's education, father's education, family smoked, parents smoked and siblings smoked) and smoking. Odds ratios (OR) were calculated and the values were expressed with 95% confidence interval (CI). P-values < 0.05 were considered statistically significant. All statistical analyses were carried out using SPSS software, version 11.5.

RESULTS

A total of 949 male subjects participated in our study. Among them, 27.4% went to middle school, 60.3% went to high school and 12.3% attended a pre-college center. About 70% were urban students. The participants' characteristics are summarized in Table 1. The percentage of tobacco use in family was 36.9% for middle school, 29.5% for high school and 33.3% for pre-college students. Furthermore, 29.6% of middle school students had a father who smoked; the rate was 23.4% for high school and 22.2% for pre-college students.

Table 1: Characteristics of male students by school level, Iran, 2007

Characteristics	Middle School n(%)	High School n(%)	Pre-college n(%)
Residence area			
Urban	165 (63.5)	419 (73.3)	78 (66.7)
Rural	95 (36.5)	153 (26.7)	39 (33.3)
Father's Education			
Illiterate	47 (18.1)	90 (15.8)	18 (15.4)
Elementary or Middle	163 (62.7)	249 (43.6)	55 (47.0)
Diploma	33 (12.7)	125 (21.9)	29 (24.8)
Bachelor and upper	17 (6.5)	107 (18.7)	15 (12.8)
Mother's Education			
Illiterate	42 (16.2)	83 (14.5)	23 (19.7)
Elementary or Middle	164 (63.1)	303 (57.1)	65 (55.6)
Diploma	40 (15.4)	101 (17.7)	24 (20.5)
Bachelor and upper	14 (5.3)	84 (14.7)	5 (4.2)
Tobacco use in family	96 (36.9)	169 (29.5)	32 (27.4)
Number of family smokers			
None	164 (63.1)	403 (70.5)	85 (72.6)
One	86 (33.1)	158 (27.6)	30 (25.6)
More than one	10 (3.8)	11 (1.9)	2 (1.8)
Family member smokes			
Father	77 (29.6)	134 (23.4)	26 (22.2)
Mother	12 (4.6)	5 (0.9)	2 (1.8)
Siblings	17 (6.5)	41 (7.16)	6 (5.1)

Forty nine (18.8%) middle school students were ever smokers (Table 2) which increased with advancement of school level ($p = 0.005$). The prevalence of smoking (defined as smoking cigarettes or hookah at least once a month) increased dramatically from 5.4% to 17.6% with advancing school levels ($p < 0.001$). The prevalence of smoking for other variables (residence area, father and mother education, smoking family members and smoking father, mother or siblings) is presented in Table 2.

The onset age of first smoking was 11.25 ± 2.66 years for middle school, 12.92 ± 3.0 years for high school and 13.9 ± 2.57 years for pre-college students, indicating statistical significance ($p = 0.047$).

The smoking rate was not significantly associated with father and mother education for school levels. In addition, the difference in percentage of smoking family members was not statistically significant among students of the three school levels ($p > 0.05$). The

Table 2: Prevalence of smoking in students by school level

Smoking criteria	Middle School n(%)	High School n(%)	Pre-college n(%)
Ever tobacco consumption	49 (18.8)	161 (28.13)	38 (32.5)
Smoking*			
Overall	14 (5.4)	81 (14.2)	21 (17.9)
Residence area			
Urban	6 (3.6)	63 (15)	14 (17.9)
Rural	8 (8.4)	18 (11.8)	7 (17.9)
Father's Education			
Illiterate	1 (2.1)	14 (15.7)	2 (11.1)
Middle school	12 (7.4)	32 (12.9)	8 (14.8)
Highschool and Diploma	1 (3)	16 (12.8)	8 (27.6)
Bachelor and upper	0 (0.)	19 (17.8)	3 (20.0)
Mother's Education			
Illiterate	3 (7.1)	14 (16.9)	5 (21.7)
Middle school	10 (6.1)	37 (12.3)	12 (18.5)
Highschool and Diploma	0 (0)	18 (17.8)	3 (13)
Bachelor and upper	1 (7.1)	12 (14.3)	1 (20)
Number of family smokers			
None	5 (3)	42 (10.08)	14 (16.5)
One	4 (4.7)	34 (21.5)	7 (24.1)
More than one	5 (50)	5 (45.5)	0 (0)
Father smokes			
No	5 (2.7)	52 (11.9)	16 (11.9)
Yes	9 (11.7)	29 (21.6)	5 (19.2)
Mother smokes			
No	10 (4)	80 (14.1)	20 (17.4)
Yes	4 (33.3)	1 (20.0)	1 (50)
Siblings smoke			
No	13 (5.3)	66 (12.4)	20 (18.0)
Yes	1 (5.9)	15 (36.6)	1 (16.7)
Mean age of onset (smoking)	11.25 ± 2.66	12.92 ± 3.0	13.9 ± 2.57

* Smoking was defined as cigarette and water pipe smoking at least once a month

percentage of smoking mothers was significantly different between students of different school levels ($p = 0.002$). However, the percentage of smoking fathers and siblings were not significantly different ($p > 0.05$). Only two high school students had sisters who smoked.

Students were asked about who first induced their smoking (Table 3). Among ever smokers, 64.6% of middle school, 59.3% of high school and 73.6% of pre-college students reported that friends were their

Table 3: First provider for smoking and ever smoking by school level

Smoking Indicator	Middle school n (%)	High school n (%)	pre-college n (%)
Ever smoking			
One of parents	0 (0)	8 (5.1)	2 (5.3)
One of second relatives	13 (27.1)	33 (21)	5 (13.2)
One of friends	31 (64.6)	93 (59.3)	28 (73.6)
Others	4 (8.3)	23 (14.6)	3 (7.9)
Smokers			
One of parents	0 (0)	6 (7.6)	1 (4.8)
One of second relatives	5 (38.5)	18 (22.8)	2 (9.5)
One of friends	7 (53.8)	43 (54.4)	17 (81.1)
Others	1 (7.7)	12 (15.2)	1 (4.8)

first providers compared with 0.0%, 5.1% and 5.3% who reported one of their parents. In summary, 27.1% of middle school, 26.1% of high school and 18.5% of pre-college ever smokers reported one of the relatives (parents and second degree relatives) as first providers. Among cigarette smokers, one of the relatives was responsible as the first provider of cigarette for middle school students in 38.5% of cases. However, it was 30.4% and 14.3% for high school and pre-college students. Still, more than half of first providers for all three school levels were reportedly friends.

We analyzed the relationship between tobacco use in school levels and some factors such as place of residence, smoking family members, smoking parents or siblings smoking, as well as mother and father's education (two groups: middle school education or lower, and high school education or higher). Among middle school students, only smoking by parents was associated with a higher prevalence of smoking behavior. Middle school students whose parents smoked were nearly four times more likely to smoke themselves than those whose parents did not smoke (Table 4). Two out of six indicators of smoking in high school students (smoking parents and siblings) were statistically significant. However, the strongest factor was smoking siblings (OR = 4.71; CI, 2.31 - 9.62). For pre-college students, none of the six variables entered in the regression model contributed significantly to the prediction of smoking behavior.

Table 4: Factors associated with smoking by school level

Associated factors	Middle school (n = 260)			High school (n = 971)			Pre-college (n = 174)		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Place of stay	0.51	0.16 - 1.54	0.23	1.75	0.96 - 3.17	0.12	1.02	0.34 - 3.04	0.96
Mother's education	0.42	0.05 - 3.63	0.43	1.26	0.74 - 2.17	0.39	0.45	0.12 - 1.61	0.22
Father's education	0.60	0.06 - 5.60	0.65	1.03	0.53 - 2.01	0.93	2.06	0.79 - 5.37	0.13
Family smoked	0.00	— —	— —	0.89	0.18 - 4.26	0.88	1.33	0.45 - 3.94	0.61
Parents smoked	4.18	1.35 - 12.91	0.013	2.14	1.27 - 3.59	0.004	0.47	0.03 - 6.04	0.56
Siblings	1.08	0.12 - 9.37	0.94	4.71	2.31 - 9.62	0.000	0.00	— —	— —

"Smoking" is defined as cigarette and water pipe smoking at least once a month. Logistic regression data are odds ratios (OR) and 95% confidence intervals (CI). p-value (p).

DISCUSSION

The prevalence of tobacco smoking among male students increased with advancement of school level. Most previous studies confirm that the prevalence of smoking is positively related with increasing age or grade[13-18]. Also, Wen *et al*[19] and Thomas *et al*[8] reported that smoking behavior increased stepwise through grades or school levels. On the other hand, Zulu *et al*[3] reported as significant, negative association between age and smoking.

In our study, the prevalence of tobacco smoking among male students (at each school level) is lower than the figure reported by other studies[8, 20-23]. This prevalence is lower than the 25.1% reported by Syziya *et al*[24], 11.3% by Kyrlesi *et al*[25] and 17.5% by Mpabulungi *et al*[26] among male students of secondary school. One research reported the prevalence of current tobacco use to be 47.9% among middle school and 56.9% among high school students in Alaska[8]. In Turkey, 23.26% of male high school students of 9th - 11th grades were smokers[15]. This was more than reported in our study. In an Iranian study, the prevalence of smoking was 12.9% among male students aged 11 - 18 years[16]. Another study demonstrated that the prevalence of smoking among male students aged 14 - 18 years was 31%[6]. Also, Kelishadi *et al* reported the prevalence of smoking as 18.5% among male students aged 11-18 years[17].

The prevalence of tobacco use was lower than our result in Ethiopia (4.5%) among students aged 13 - 15 years[27]; in Korea (4.9%) among middle school students[18] and in Syria (13.5%) for high school students[9].

About one third of students had at least one parent who smoked. This finding is lower than that of Baska *et al* (mean: 61.6%)[1] and Kuznar-Kaminska *et al* (65.2%) [28], but higher than the figure reported by Heydari *et al* (26%)[6]. In a Portuguese study, 57.2% of high school students had parents with smoking habits[29].

Among middle school students, the parental smoking was indicated to affect smoking strongly. Similar finding has been reported by Siziya *et al* in Iraq, where students who had smoker friends and parents were more likely to be smokers[24]. O'Loughlin *et al* reported that daily smoking was significantly related to smoking parents and siblings and completion of college by parents among students in grade seven[30]. Others' studies suggested the effects of parents, siblings, family members and friends on smoking behavior[11, 31].

The most important factors for being smokers among high school students were smoking parents and siblings. Nevertheless, none of these factors affected smoking behavior among pre-college students. The findings of a study conducted by Lee and Tak[32], Maziak and Mzayek[9] and Tyc *et al*[33] indicated that parents, siblings and peer smoking were most strongly associated with male smoking behavior. Others' studies suggest the effects of smoking parents, brother, family members, friends and peers on smoking behavior among male students[13, 15, 19, 22, 34-37]. Some Iranian studies suggest that presence of smokers in family, smoking parents, smoking peers and smoking siblings were the most important factors for adolescents smoking[6, 17, 38]. Parental smoking affects the availability and access to tobacco by offspring and influences the parents' tolerance toward smoking. Children of parents who are intolerant toward smoking are less likely to smoke themselves.

Our study had several limitations. First, the sample students that participated in the survey were limited to adolescents enrolled in school and present on the day of the survey. Secondly, our study was based on self reports. Third, our study was a cross-sectional survey. Fourth, while we investigated the impact of some factors on smoking behavior, there are other factors, such as psychological disorders, that require evaluation, as well.

CONCLUSIONS

The prevalence of smoking increases with advancing school level. Preventive measures targeting younger students must become available to them before they start smoking. We believe that a well-planned integrated anti-smoking campaign, especially among students and parents, is immediately required.

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Original Article

Sero-Prevalence of Hepatitis-A Virus among Children Aged 1 - 18 Years in Eastern Anatolia, Turkey

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ABSTRACT

Objectives: To determine the seroprevalence of hepatitis A virus among children aged 1-18 years in Elazıg before national hepatitis A virus immunization program in Turkey
Design: Prospective study
Setting: Elazig Education and Research Hospital and Elazig Harput State Hospital in Eastern Anatolia, Turkey
Subject and Methods: 1258 patients admitted to pediatric polyclinic with suspicion of hepatitis A virus infection between January 2011 and December 2012 were included in the study.
Interventions: ELISA blood test
Main Outcome Measures: Serological markers of hepatitis A, anti-HAV IgM and anti-HAV IgG, were tested using the ELISA method.
Results: Two hundred and forty-eight patients who were Anti-HAV IgM positive were excluded from study. The mean age of the remaining 1010 pediatric patients was 10.7 ± 5.1 years (range: 1-18 years). Out of these, 558 (55.2%) were male and 452 (44.8%) female. Overall, 575 (56.9%) cases were seronegative against hepatitis A virus. Anti-HAV IgG was detected in 435 patients (43.1%). Out of these, 236 (54.3%) were male and 199 (45.7%) female. The mean age of seropositive cases was 11.7 ± 5.2 years.
Conclusions: Hepatitis A is an important public health problem in our region. This study has revealed an advanced age of exposure to hepatitis A virus infection. We argue that the application of routine hepatitis A vaccine to children in our region will reduce the potential severe complications of the infection.

KEY WORDS: complication, hepatitis A virus, immunization seroprevalence

INTRODUCTION

Viral hepatitis is a major public health problem in developing countries worldwide[1]. Hepatitis A virus (HAV) infection occurs throughout the world but is most prevalent in developing countries. HAV is a highly contagious virus, and its infection spreads predominantly by the fecal-oral route. The prevalence rate of HAV infection is about 100% among children by five years of age in these countries[2].

The clinical spectrum of HAV infection ranges from asymptomatic infection to fulminant hepatitis. Clinical manifestations depend on the age of host; less than 30% of infected young children are symptomatic, while approximately 80% of adults manifest severe hepatitis with remarkably elevated serum aminotransferases[3]. Acute liver failure that occurs in less than 1% of cases is rare. However, the case-fatality rate was 0.3% in children younger than 14 years, 0.1% in adults (40 - 59 years), and 1.7% in people older than 60 years[4]. It may lead to severe clinical manifestations, including fulminant hepatitis, in about 10 - 15% of adults[5]. Similarly, HAV is the most common detected cause of fulminant hepatitis among children in our country as well as worldwide[6, 7].

This study aims to determine the seroprevalence of HAV infection among children aged 1 - 18 years and to observe the seroprevalence changes in Elazig in Eastern Anatolia, Turkey.

SUBJECTS AND METHODS

This prospective study was conducted in two secondary referral hospitals, Elazig Education and Research Hospital and Elazig Harput State Hospital, between January 2011 and December 2012. The patients admitted to the pediatric clinics due to any reason were included in the study. Patients aged 1 - 18

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years, who do not have any chronic liver disease, were screened, and those who had IgM seropositivity were excluded from the study. The serological markers of HAV were evaluated. Anti-HAV IgM and anti-HAV IgG markers were tested using the ELISA method (Abbott Architect I 2000 SR).

RESULTS

Overall, 1258 patients were included in the study. Out of them, 248 were excluded because of anti-HAV IgM seropositivity. The mean age of 1010 pediatric patients was 10.7 ± 5.1 years; Out of these, 558 (55.2%) were male and 452 (44.8%) were female. A total of 575 (56.9%) cases were seronegative against HAV. Anti-HAV IgG was positive in 435 patients (43.1%); Out of these 236 (54.3%) were male and 199 (45.7%) were female. The mean age of seropositive cases was 11.7 ± 5.2 years (Table 1). Anti-HAV IgM was detected in 248 patients; Out of these 141 (56.9%) were male and 107 (43.1%) female. The mean age of Anti-HAV IgM positive patients was 9.9 ± 3.9 years.

Table 1: Demographic characteristics of 1010 patients

	Patient n (%)	Mean Age (yrs) ± SD	Male n (%)	Female n (%)
All cases (N)	1010 (100)	10.7 ± 5.1	558 (55.2)	452 (44.8)
Seropositive cases	435 (43.1)	11.7 ± 5.2	236 (54.3)	199 (45.7)
Seronegative cases	575 (56.9)	9.1 ± 4.5	322 (56)	253 (44)

SD: Standard deviation

DISCUSSION

The seroprevalence rates of HAV in previous studies conducted in Turkey are presented in Table 2. According to these results, the seroprevalence rate of HAV in our study was lower than the other studies[8-10]. This discordance has been considered probably due to the high socio-economic level, and improved sanitary and hygienic condition of the population included in the study. Also, since the present government has increased the investment for sewerage, sanitary and hygienic condition in our region in the last ten years, the prevalence of HAV has decreased. In Turkey, improvements in socio-economic conditions and quality of drinking water have been followed by a decrease in HAV infection[11].

Our relatively higher rate of HAV seropositivity among children indicates the requirement for vaccination against hepatitis A virus because hepatitis A leads to severe complications as the child gets older. Nowadays, age of exposure to HAV infection is increasing towards puberty worldwide. This is probably because of the epidemiological changes of HAV[3]. Similarly, HAV is the most commonly detected cause of fulminant hepatitis among children in our country as well as worldwide[6].

In this study, an increased mean age of HAV-infected patients may be due to the exposure to HAV during their time in nursery school and primary school. Improved sanitary conditions and hygienic practices have reduced the incidence of HAV infection, especially in developed countries. Reduction in the number of new cases is generally accompanied by a shift in the age of first contact with HAV towards older age groups. As a consequence, both the severity of the reported cases and the risk of outbreaks of disease would increase[11-16]. In our country, Topal *et al* have reported that the seropositivity rate of HAV among children aged between 1 - 6 years is 9.4% in western region (Istanbul)[17]. Ceran *et al* have reported that the seropositivity rate of HAV among children aged between 5 - 24 years is 40% in western region[18]. It is noticed that Turkey has intermediate endemicity of HAV infections, and endemicity may be changed by the geographical and socio-economic conditions[11]. The results of the present study have confirmed that this condition has been similar to that reported in previous studies.

The lower results from this study have shown that the age of exposure to HAV has increased toward puberty. Thus, vaccination is necessary for

Table 2: Hepatitis A seroprevalence studies conducted in Turkey

Researcher	Location in Turkey	Date	Age (years)	No. of Cases	Sero-positivity rate (%)
Kanra *et al*[8]	General	2002	1 - 4	727	42.7
Alabaz *et al*[9]	Southern	2005	1	147	36.1
Ozen *et al*[10]	Eastern	2006	3 - 6	286	17.5
Ceyhan *et al*[11]	Southeastern	2006	0 -14	701	90
Aslan *et al*[12]	Southeastern	1999	2 - 64	400	66.5
Tekay F[13]	Eastern	2004	0 -14	416	63
Deveci *et al*[14]	Eastern	2010	1 - 16	351	13.1
Arabacı *et al*[15]	West	2009	0 - 6	77	49.3
Okur *et al*[16]	Eastern	2008	0 - 18	3409	69.9
Topal *et al*[17]	West	2008	1 - 6	319	9.4
Ceran *et al*[18]	West	2010	5 - 24	630	40

children older than one year in order to prevent the severe complications of the disease among adults. Universal vaccination of young children in Israel, United States and Catalonia has resulted in significant reductions in the incidence of hepatitis A disease in these countries[19-21]. It is good that the universal HAV immunization program has begun in Turkey since October 2012. We hope that this vaccination program will be successfully applied, and thus, the prevalence of the infection will decrease in the near future.

CONCLUSION

HAV infection is an important public health problem in our region. This study has revealed an increased age of exposure to HAV. The application of a routine HAV vaccine among children will reduce the potential for the development of severe complications. Therefore, we believe that complications of HAV infection will reduce in our country in the future.

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Case Report

Rare Presentation of Infertility: Recurrent Hydatid Cysts in Pelvic Cavity: A Case Report

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ABSTRACT

Hydatid cyst of pelvic cavity leading to secondary infertility is extremely rare. We present the case of a 32-year-old lady with recurrent hydatid cysts of bilateral ovarian fossae responsible for her secondary infertility.

KEYWORDS: hydatidosis, infertility, parasitic disease, pelvic cavity

INTRODUCTION

Human hydatid disease is caused by *Echinococcus granulosus*. Its distribution is world-wide, affecting mainly the liver and lungs[1, 2]. However, other organs could be involved. Primary involvement of the pelvic cavity is very rare. We present a rare case of recurrent hydatid cyst in ovarian fossa leading to secondary infertility.

CASE HISTORY

A 32-year-old lady presented for treatment of secondary infertility in April 2009. She was a known case of hypothyroidism with history of still born female child at 8.5 months amenorrhea before 3.5 years. She had past history of two laparotomies for a mass in right ovarian fossa 2.5 years ago and bilateral multiple cystic masses in pelvis two years back which were diagnosed as hydatid cysts. She was treated for the same with albendazole, 400 mg OD x 3 months for the first time and for one year for the second time. She was keeping well for the last 1.5 years and had no symptoms or signs of hydatid cyst on clinical examination as well as on investigations.

On examination in our outpatient clinic, her physical examination including vitals and systemic examination were unremarkable. On investigations, her hemoglobin was 10.8 gm/dl, total leucocyte count: 7.8×10^3 / µl, differential counts were polymorphs, 66%, lymphocytes, 29%, eosinophils, 4% and monocytes, 1% and random blood sugar, 91 mg/dl. Her urine routine and microscopy, renal and liver function tests, and thyroid function tests were unremarkable on maintenance dose of Eltroxin. She was non-reactive for HIV / HbsAg and HCV (by ELISA). Her X-ray chest was normal. Ultrasonography of the abdomen revealed that both, the kidneys, liver, spleen, pancreas, bladder, pelvic cavity and uterus to be normal with endometrial thickness of 5 mm. No mass was noted anywhere. Both ovaries were normal and no adnexal mass was seen. Hysterosalpingography (HSG) showed left side spill present and right side spill absent.

After nine months of treatment for infertility, she was posted for hysteroscopy and laparoscopy. Her endometrium, cervix, endometrial cavity and bilateral ostia were normal. The uterus was convex with adhesions, left fallopian tube and ovary was normal, right fallopian tube was normal but a terminal block was found which was repaired with fimbrioplasty. A small cystic mass, 3 x 1.5 cm in size was present in left ovarian fossa, separate from uterus and adnexae. The mass was sent for histopathology examination. On gross examination, a partially cut open cystic mass weighing 10 grams, measuring 3 x 2 x 1 cm, with smooth external shining white surface was received. Cut section revealed multiple grape-like transparent small variable sized cysts filling the cystic cavity. Examination of the fluid inside the cavities revealed scolices and brood capsules of hydatid cyst.

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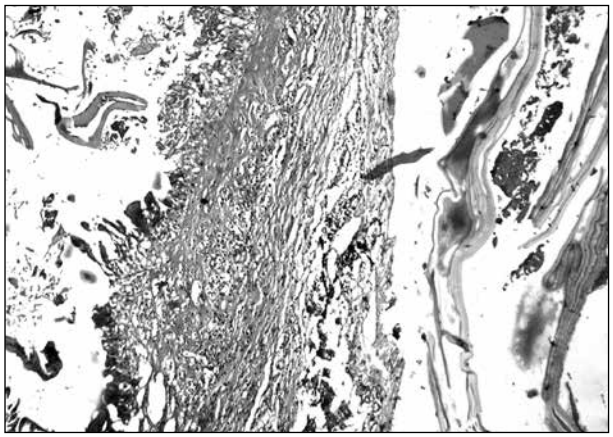

Fig. 1a: Microphotographs from cyst wall showing outer laminated and inner germinal layer with chronic inflammatory cells and giant cell reaction. (H&E x100)

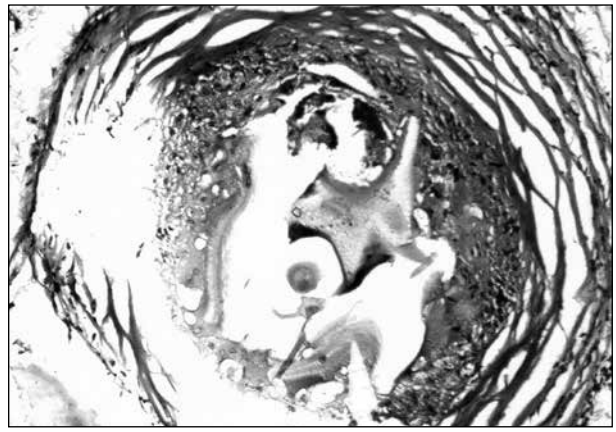

Fig. 1b: Hydatid cyst showing outer laminated and inner germinal layer with brood capsules containing scolices. (H&E x100)

Histopathological examination showed multiple cysts of variable size lined by inner nucleated germinative layer and outer opaque, laminated non-nucleated layer, filled with gelatinous fluid and occasional hooklet-bearing degenerated scolices. The outer layer was surrounded by severe host inflammatory reaction of foreign body giant cells, fibroblasts, histiocytes, lymphocytes, rare eosinophils and cell debris. Ovarian stroma containing part of cyst wall was also present, adjacent to the tissue (Fig. 1a, 1b). It was diagnosed as hydatid cyst of the ovarian fossa.

She was treated with albendazole, 400 mg twice a day for six months. On follow-up for one year she did not conceive. There was no recurrence over the follow-up of one year.

DISCUSSION

Hydatid cyst is a parasitic disease caused by larval stage of the tape worm *Echinococcus granulosus*. While involvement of the liver (in 60%) and lungs (in 50%) are the most common sites of affection in adults it may develop in almost any part of the body[1]. Primary hydatidosis of the pelvic cavity / ovary and broad ligament is rare but well-documented in endemic areas like Mediterranean countries, South America, Middle East and Australia[2-3]. The involvement of female pelvic organs by hydatid disease is extremely rare and may be missed until operated upon[4-5].

In our case, this young female patient was found to have recurrent hydatid cysts in ovarian fossae and this was suspected on laparotomy only to be confirmed on histopathological examination. She came in for treatment of secondary infertility and while evaluating for the cause with investigations including HSG and diagnostic laparoscopy, it was only on the operation table that a cystic mass in left ovarian fossa was found which was diagnosed as hyaditid cyst.

Parasitic cysts are uncommon in female reproductive system and comprise 0.5 to 3.1% of total hydatid cysts of the body[6]. The common sites are pouch of Douglas, pelvis, broad ligament, *etc*., and may present as lump in the lower abdomen, sterility, obstructed labor, menorrhagia, retention of urine *etc*. Mebendazole was used previously. However, nowadays albendazole, is the drug of choice given in the dosage of 400 mg once a day for 12 weeks to six months and results in cure in 30% of cases. Praziquental, 50 mg/kg BW daily for two weeks may be added in resistant cases. The patient should be treated with albendazole, 15 mg/kg/day in two divided doses for four days prior to surgery for prophylaxis from spillage[7]. Our patient who had been managed outside was not offered this treatment and perhaps this led to recurrence of hydatidosis eventually leading to secondary infertility.

CONCLUSION

The involvement of female genital tract in Echinococcal disease leading to infertility is extremely rare requiring early diagnosis and adequate management.

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Case Report

Metastatic Squamous Cell Carcinoma Masquerading as Liver Abscesses

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ABSTRACT

We report a case of multiple liver metastases mimicking liver abscesses which was diagnosed finally as a metastatic squamous cell carcinoma. A 37-year-old male patient presented with upper abdominal pain and fever. The imaging findings, combined with the patient's clinical presentation, were most suggestive of an infectious process. Core biopsies revealed metastatic squamous cell carcinoma whose primary site could not be identified despite thorough physical examination and extensive investigations. They were labeled as metastasis of an unknown primary. Liver squamous cell carcinoma metastases may be cystic and represent a differential diagnosis with liver abscesses.

KEYWORDS: abscess, liver, metastasis, unknown

INTRODUCTION

The liver accounts for 25% of all metastases to solid organs, which is the most common site after the lymph nodes. Liver metastases are 20 times more common than primary liver tumors[1]. Liver metastases represent 20 - 30% of unknown primary cancer (UPC). In 70 - 90% of cases of UPC, the site of primary origin is never found during the lifetime of the patient after thorough clinical examination and complementary investigations[2,3]. We report a case of multiple liver metastases mimicking liver abscesses which was finally diagnosed as metastatic squamous cell carcinoma (SCC).

CASE HISTORY

A 37-year-old man was admitted to the Jahra hospital, Kuwait with persistent fever (up to 39.5 °C), upper right-sided abdominal pain, and vomiting of five days duration. He denied smoking, drinking alcohol, or intravenous drug abuse and had not travelled outside Kuwait recently. On examination, the patient was febrile (39.2 ºC) with a pulse rate of 100 / minute and a blood pressure of 130/80 mmHg. The rest of the clinical examination was unremarkable.

Abdominal examination revealed tender hepatomegaly (2 fingers below the right costal margin) with epigastric tenderness. Initial laboratory results showed a white blood cell count of 17,200/mm^3. The eosinophil count was normal and C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were elevated. Liver function tests (LFTs) showed elevated liver enzymes, alkaline phosphatase, bilirubin level, and hypoalbuminemia

Abdominal ultrasonography and contrast computed tomography (CT) scans of the abdomen revealed multiple variable-sized cavitary lesions with density ranging from 15 - 20 Hounsfield units (HU) in the liver scattered in both lobes, a picture consistent with multiple liver abscesses (Fig. 1 a, b, c). Serological test for *E. histolytica* antibody and *E. granulosis* antibody were negative. Hepatitis viral markers were negative.

These findings, combined with the patient's clinical presentation, were most suggestive of an infectious process. Broad spectrum antibiotics were started with intravenous fluid support. Blood cultures isolated *Klebsiella pneumonia*.

Ultrasound guided needle aspirate (20 ml) from the largest lesion was sent for bacteriologic, histological,

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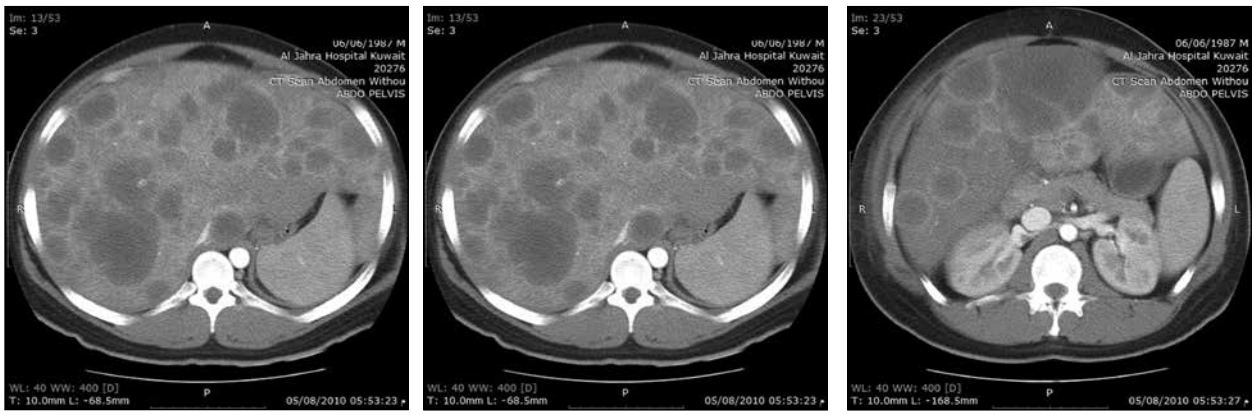

Fig. 1(a, b, c): CT of the abdomen showing variable sized, hypodense cystic lesions scattered in both lobes of the liver 1a: Non enhanced CT abdomen that shows low attenuation and a CT value ranging from 15-20 HU, 1b: Arterial phase showing a surrounding thick irregular-appearing rim, and 1c: Portal- venous phase showing peripheral enhancement and the edges that become more clearer.

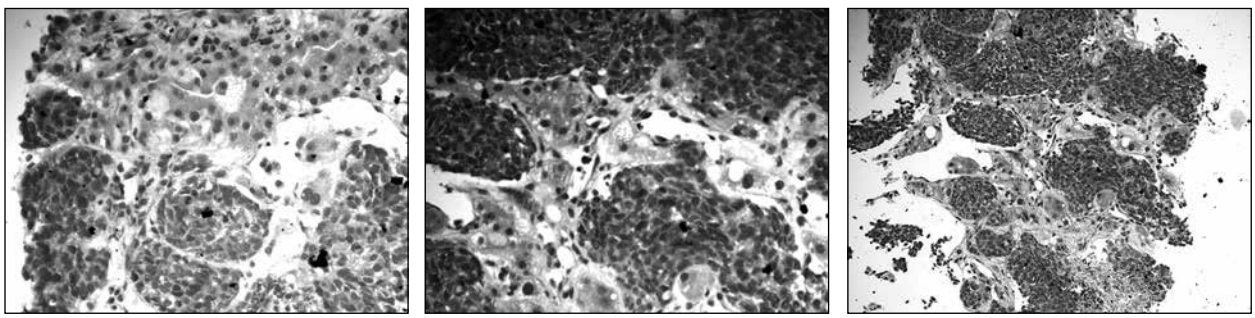

Fig. 2 (a, b, c): Solid sheets of poorly differentiated non-keratinized squamous cell carcinoma with dysmoplastic stroma and normal liver tissue compressed and squeezed out by the tumors.

for *E. histolytica*, cytologic examination and culture sensitivity. All of them came negative.

The patient gave an initial satisfactory response regarding his abdominal symptoms and fever, and his white cell count declined to 8,400/mm^3. On day four, he again became febrile (38.3 ºC) and he started to have massive ascites, bilateral pedal edema and respiratory embarrassment which necessitated ICU admission and intubation. Duplex study of the abdomen showed totally occluded suprahepatic portion of the inferior vena cava and the hepatic veins. To definitely rule out malignancy we decided to take ultrasound guided core biopsies which on examination were reported as metastatic poorly differentiated non-keratinized SCC of the liver (Fig. 2). This was confirmed by using immunohistochemical staining with antibodies to cytokeratin 8 (CK8). The primary site remained unknown after thorough physical examination, computed tomography (CT) scans of head and neck, thorax, abdomen, and pelvis, endoscopy for sinuses and upper respiratory tract and upper and lower gastrointestinal endoscopy, and tumor markers (CEA, CA19_9 and alpha fetoprotein).

Medical oncologists wanted to start salvage chemotherapy hoping to alleviate hepatic venous obstruction but the patient general condition was incompatible with the chemotherapy and his relatives declined further intervention. He died a few days later.

DISCUSSION

The imagining appearance of liver metastases depends on primary tumors and stage of treatment. Most of them are solid, but some have a complete or partially cystic appearance for variety of reasons. Hypervascular metastases like metastases from neuroendocrine tumors or sarcoma can appear cystic. Metastases from mucinous tumors such as tumors of colorectal area or ovary can undergo necrosis in response to chemotherapy and appear cystic (*e.g.*, metastases from a testicular teratoma or a gastrointestinal stromal tumor[4].

In 80% of cases of unknown primary, metastases are adenocarcinomas[5]. Metastatic SCC of the liver is uncommon and generally comes from lungs, esophagus, head and neck, genital primaries, or anorectal primaries. Metastases from SCC are generally solid on imaging but can be cystic because of the central necrosis[6].

Patients with metastatic SCC should undergo a thorough physical examination, upper and lower,

gastrointestinal endoscopy, computed tomography (CT) scans of the thorax, abdomen, and pelvis, head and neck, endoscopy for sinuses and upper airway and tumor markers. If these are inconclusive, magnetic resonance imaging (MRI), and positron emission tomography (PET), can be of help in localizing the primary site[7].

Cystic liver metastasis and liver abscess can present with fever with or without right upper abdominal pain. Typically the two can be differentiated by the solid nature of a metastasis and the cystic nature of an abscess on imaging. Distinguishing the two is not always easy[8].

Currently, no good therapeutic options are available for metastases of SCCs. Most treatments are purely palliative in nature. Surgical resection is not always feasible because of the number and co-morbid conditions. Other treatment modalities are available like radiofrequency ablation (RFA), systemic chemotherapy, local lesion therapy, conformal radiation therapy or hepatic arterial infusion chemotherapy. A tissue diagnosis needs to be established before treatment. This can be accomplished by image guided fine needle aspiration or core biopsy[9].

Although primary SCC is frequently sensitive to multimodality treatment with chemoradiotherapy, metastatic SCC is generally not as sensitive to systemic chemotherapy and chemotherapy does not offer the possibility of cure for metastatic disease. With current medical treatment complete responses are rare, duration of responses is short, and survival is usually in the range of 6 - 8 months[10].

CONCLUSION

SCC less commonly gives metastases to the liver and in most cases the primary neoplasm remains unknown even after an extensive study. In some cases of SCC, metastases are cystic with clinical picture of fever and leucocytosis which give an impression of infective hepatic disease and masquerades as liver abscesses. It should be borne in mind that no form of imaging is totally accurate and even cytology studies can be misleading. When there is doubt, a core biopsy under imaging may yield the most definitive results. The prognosis in such cases is very poor.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Case Report

A Rare Bronchopulmonary Malformation in Adulthood: Bronchial Atresia

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ABSTRACT

Bronchial atresia is a congenital anomaly characterized by focal obliteration of a part of the bronchus, which causes an accumulation of secretions and a bronchocele with absence of ventilation of the corresponding distal pulmonary area which is seen radiologically as hyperlucent . Approximately 50% of the patients are asymptomatic at the time of diagnosis, and this condition is more frequent in men than in women (ratio 2:1), and usually affects young people. Chest computed tomography is diagnostic, and makes it possible to differentiate between congenital anomalies, bronchiectasias and other types of bronchial obstruction. Treatment of asymptomatic patients is conservative, and surgery is only necessary if there should be symptoms such as recurrent respiratory infections, chronic cough or dyspnea. Herein we report a case of asymptomatic bronchial atresia with radiological features and differential diagnosis.

KEY WORDS: bronchocele, congenital anomaly, focal emphysema

INTRODUCTION

Bronchial atresia is a congenital anomaly where the lumen of a bronchus is interrupted at or near its origin. The mucus-filled, blind-terminating bronchus gives a variety of radiographic images. It is a rare anomaly that is characterized by the presence of a bronchial mucocele. The mucocele is usually seen as a result of a blindly terminating segmental or subsegmental bronchus, and hyperinflation of the obstructed segment and, sometimes, neighboring segments. Although this anomaly is exclusively congenital, the exact etiology of bronchial atresia still remains unknown[1,2].

Bronchial atresia resulting from a localized defect in normal bronchopulmonary embryogenesis is a rare disease. It can produce emphysematous changes in the affected pulmonary segment or lobe with or without dyspnea and / or episodic pulmonary infection.

Herein, we report a case of asymptomatic bronchial atresia with radiological features and its differential diagnosis.

CASE REPORT

A 23-year-old man was referred to our department because of an abnormal chest X-ray that was performed in the context of a routine examination. A routine chest radiograph showed branching tubular area of increased opacity and subtle focal hyperlucency in right lower lobe (Fig. 1). Contrast enhanced thorax CT showed a non-enhancing branching opacity with segmental emphysematous change in the superior segment of the right lower lobe. Right lower lobe anterobasal, laterobasal and posterobasal segmental bronchi were demonstrated whereas superior segment bronchus could not be identified (Fig. 2).

The patient was a non-smoker, had no symptoms and denied any chronic disease. He only mentioned two incidents of pneumonia previously at the age of 6 and 15 years. The physical examination was normal. The routine laboratory tests were also within normal limits.

PPD skin test was negative. Further work-up of the patient, including serum complement analysis, rheumatoid factor, antinuclear antibodies, antineutrophilic cytoplasmic antibodies, immunoglobulin levels, serologic tests for hydatid cyst, human immunodeficiency virus as well as for common viruses and atypical infectious agents did not reveal any abnormal findings.

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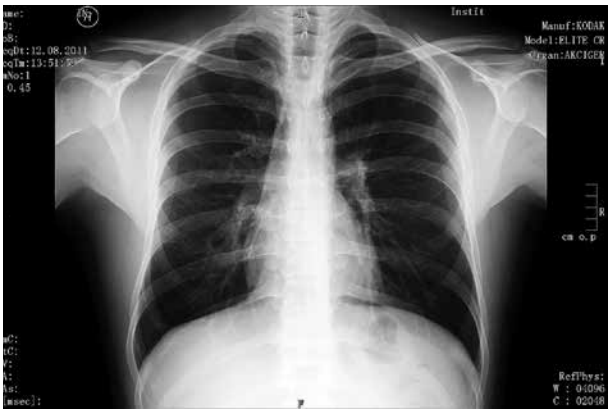

Fig. 1: Postero-anterior chest radiograph reveals branching tubular area of increased opacity which represents a bronchocele and extends from the right hilum into the right upper lobe. There is overinflated lung parenchyma and sparse vasculature in the lower half of the right lung.

Bronchoscopy revealed a narrowed bronchial orifice of superior segment of right lower lobe with a fish mouth appearence. A standard bronchoscope could not pass through this bronchial narrowing. Microbiologic and cytologic examinations of the washing specimens from the area were negative.

DISCUSSION

Congenital bronchial atresia is a rare disorder that develops due to a blind terminating segmental or lobar bronchus, in which mucus accumulates to form a bronchocele, and this causes hypoventilation of the lung[2].

The lung parenchyma that is to be supplied by the affected bronchus is usually emphysematous, non-compressible, non-inflamed, and minimally anthracotic, because it does not communicate directly with the environment. The air that exists in the affected parenchyma is the result of the collateral ventilation through the pores of Kohn, the bronchoalveolar channels of Lambert or *via* interbronchiolar channels. The process of hyperinflation may ocur shortly after birth with the start of respiration, since the proposed pathways for collateral ventilation favor the movement of air into the obstructed segment by a checkvalve type mechanism. At the root of the involved tissue,

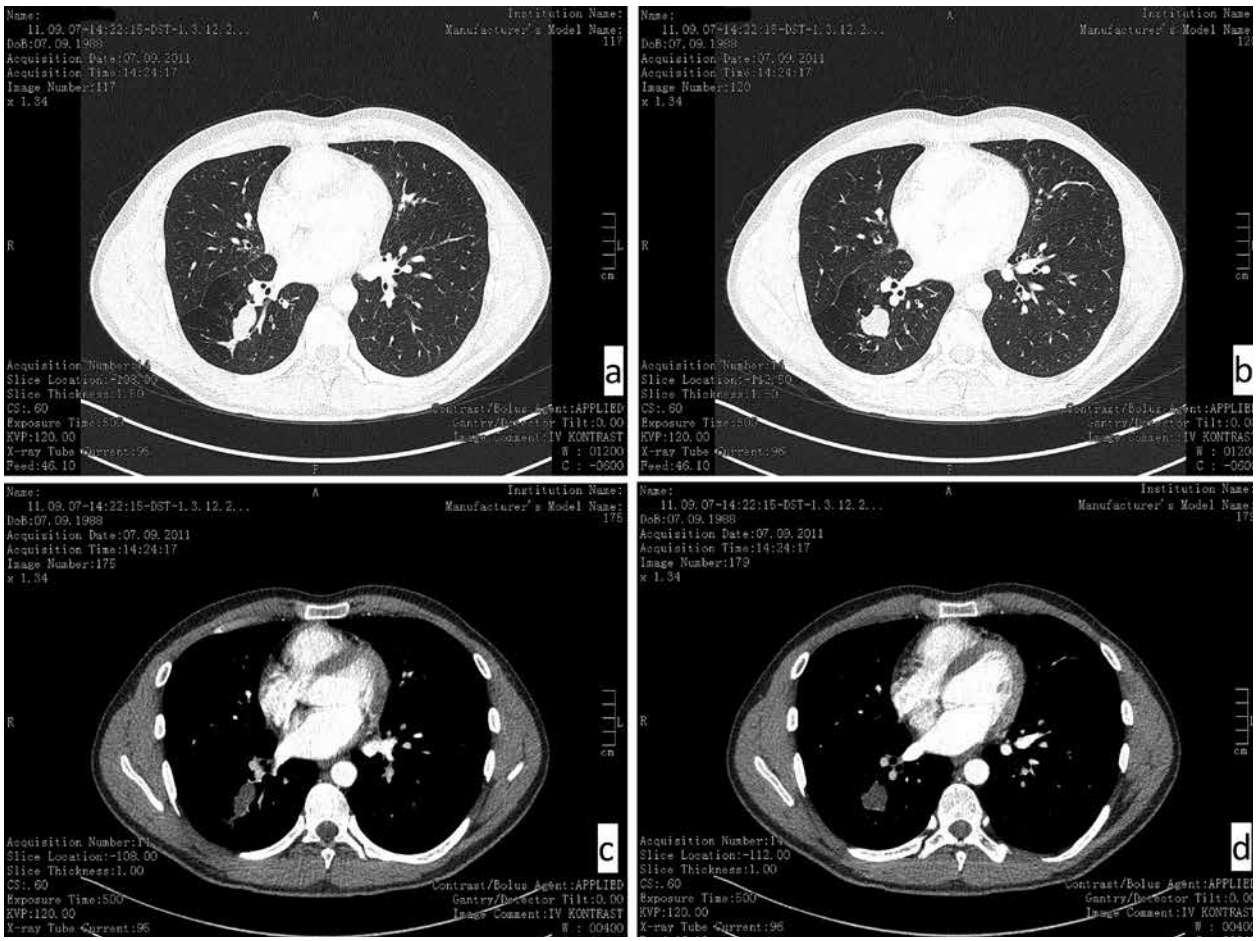

Fig. 2: Axial parenchyma window (a, b), axial mediastinal window (c, d), contrast enhanced thorax CT images showing a tubular non-enhancing lesion, a finding consistent with mucus impaction of the superior segment of right lower lobe bronchus (bronchocele). Decreased attenuation which is a finding indicative of associated air trapping is seen throughout the superior segment of the right lower lobe (a, b). These CT features are diagnostic of bronchial atresia.

a mucus filled cystic structure (mucocele) with fingerlike projections represents the atretic bronchus, which is isolated from the proximal bronchial tree and is dilated by the accumulated mucus. The bronchial pattern distal to the mucocele is usually normal[1,3].

Bronchial atresia is usually diagnosed in the second or third decade of life. It seems that the disorder has a male preponderance, with an estimated prevalence of 1.2 cases per 100,000 males[2]. The insidious course of the disorder explains its late detection in some patients. About half to two thirds of the reported patients are asymptomatic before diagnosis. Recurrent pneumonias, dyspnea, cough or hemoptysis have been reported less frequently[1,4].

A classic radiographic finding of bronchial atresia is a branching tubular or nodular area of increased opacity that extends from the hilum with surrounding hyperlucent lung parenchyma. Images acquired during both inspiration and expiration may help to confirm that a lung is hyperinflated. However, CT is the most sensitive imaging modality, and when findings are typical, they may be considered diagnostic in most cases. CT allows characterization of the lack of communication between the mucocele and hilum, can show smaller mucoceles not seen at conventional radiography, and is more sensitive in demonstrating segmental hyperinflation, associated mass effect, and possible calcification[5]. CT and magnetic resonance imaging are useful in depicting the absence of vascularity and enhancement within the lesion and may help exclude a vascular cause[6].

In a review of 86 patients, 49 (58%) patients were asymptomatic and were discovered incidentally when a routine chest radiograph was taken[3]. CT has greatly contributed to our ability to diagnose and localize mucoceles non-invasively, and surgery is no longer required to make the diagnosis in asymptomatic patients. The current indications for surgery are preservation of lung function in young patients and prevention of recurrent infections[3,6].

CONCLUSION

Congenital bronchial atresia is a rare and benign entity, which might occasionally resemble serious underlying diseases on radiographic examination. A CT scan of the chest is the procedure of choice for the diagnosis, but in suspicious cases bronchoscopy may be useful to exclude other conditions.

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Case Report

Thoracoscopic Resection of Bronchopulmonary Carcinoid Related to Cushing's Syndrome

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ABSTRACT

Bronchopulmonary carcinoids are rare neuroendocrine neoplasms and those occurring peripherally produce corticotropin, occasionally resulting in Cushing's syndrome (CS). We report two cases of bronchial carcinoids in a 68-year-old woman with poor glycemic control and hypokalemia and in a 33-year-old woman with irritable behavior and hypokalemia. The diagnosis was based on the cortisol and adrenocorticotropic hormone levels, chest computed tomography (CT) results, and histopathology of the wedge-resected nodule. Diagnosis of CS caused by ectopic adrenocorticotropic hormone secretion by using various endocrine and imaging tests is challenging; moreover, positron emission tomography (PET) failed to locate the tumor. Thus, thoracoscopic resection is an excellent diagnostic and therapeutic procedure for CS.

KEYWORDS: carcinoid tumor, neuroendocrine neoplasms, PET, thoracoscopic surgery

INTRODUCTION

Bronchopulmonary carcinoids (BPCs) are rare and account for 1 - 5% of all primary lung tumors[1]. These carcinoids are often associated with the endocrine disorder Cushing's syndrome (CS), which is caused by ectopic adrenocorticotropic hormone (ACTH) secretion (EAHS); approximately 10% of CS cases are secondary to non-pituitary neoplasms[1]. BPCs and small-cell carcinomas are the most common causes of EAHS. The chief problems encountered in such cases are the diagnosis and confirmation of the etiological factors of CS. Complementary laboratory tests and imaging studies can aid in this regard.

CASE REPORT

Case 1

A 68-year-old woman with a three-year history of diabetes and hypertension was admitted to our hospital because of muscular weakness and poor glycemic control. Her blood pressure was controlled using a calcium channel blocker and an angiotensin receptor blocker. On admission, moon face and high blood pressure (155/82 mmHg) were observed. Laboratory tests revealed high levels of plasma sugar (483 mg/dl) and sodium (Na^+: 148 mmol/l), a low level of potassium (K^+: 1.8 mmol/l), and plasma cortisol (55.4 μg/dl). The urinary cortisol level was 1,517 μg/day (normal: 55 - 286 μg/day). Physical and endocrine data were consistent with the diagnosis of CS. Various imaging modalities were used to differentiate CS from EAHS. Magnetic resonance imaging (MRI) of the brain and ultrasonography of the abdomen revealed no focal lesion in the pituitary gland or kidneys. A chest computed tomography (CT) identified one nodule measuring 0.7 cm in the left lower pulmonary lobe (Fig. 1A). Fluorine-18 fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) revealed no significant FDG uptake in the lesion (Fig. 1B). The patient was explained the surgical treatment, and she granted informed consent after which she underwent thoracoscopic partial resection of the left inferior pulmonary lobe. The tumor resection was consistent with the diagnosis of carcinoid tumor with positive immunostaining for ACTH and the neuroendocrine tumor markers. The postoperative course was uneventful, and her postoperative plasma ACTH and cortisol levels were normalized. Moreover, her blood pressure

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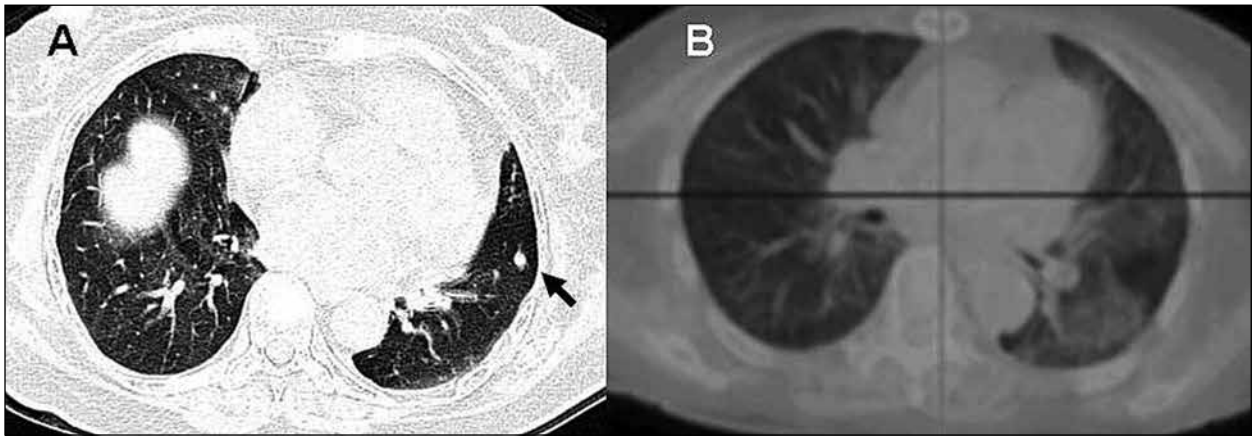

Fig. 1 (A): Chest CT scan: nodular lesion 0.7 centimeter (cm) in the left lower lobe of lung (black arrow), **(B)**: ^{18}F-FDG PET: no abnormality in FDG uptake area.

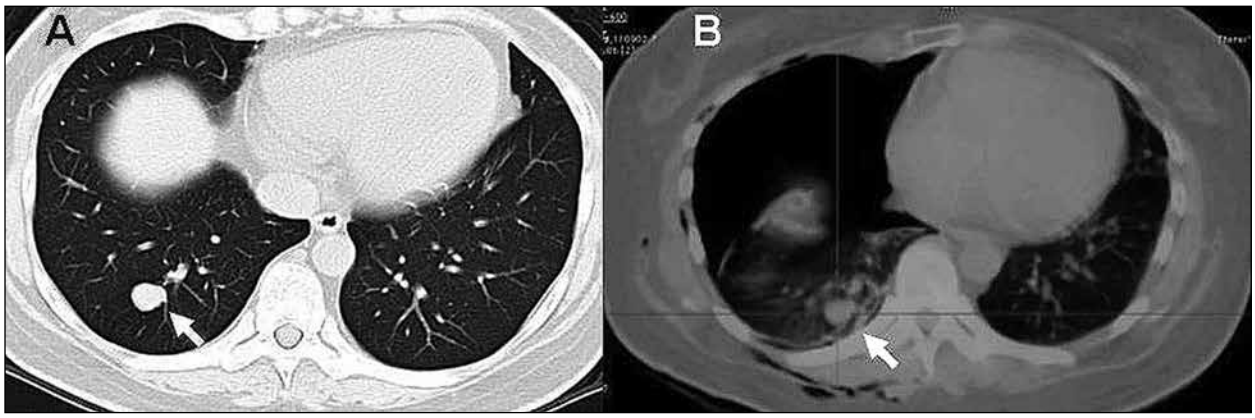

Fig. 2 (A): Chest CT scan: nodular lesion 1.5 centimeter (cm) in the right lower lobe of lung (white arrow), **(B)**: ^{18}F-FDG PET: FDG uptake within the primary tumor (white arrow) (maximum standardized uptake value, 2.4)

was normalized without any medication, and her glycemia was well under control. After two years, her outpatient follow-up revealed that the patient was free from tumor recurrence and hypercortisolism.

Case 2

A 33-year-old woman with no medical history was referred to our hospital because of irritable behavior and hypokalemia (K^+: 2.9 mmol/l). A moon- face, skin hyperpigmentation, and acne were seen. Laboratory tests revealed elevated levels of urinary cortisol (9,051 μg/day), plasma ACTH (270 pg/ml), and serum cortisol (46.4 μg/dl). The result of the dexamethasone suppression test was abnormal. A tentative diagnosis of CS secondary to EAHS of ACTH was established after imaging examinations. MRI of the brain and ultrasonography of abdomen showed no focal lesions in the pituitary gland or kidneys. Chest CT identified one circumscribed nodule measuring approximately 1.5 cm on the periphery of the right lower pulmonary lobe without any other enlarged mediastinal lymph nodes (Fig. 2A).

^{18}F-FDG PET in transaxial view revealed minimally increased FDG uptake in the lesion (maximum standardized uptake value [SUVmax] 2.4). There was no abnormal mediastinal FDG uptake (Fig. 2B). The patient underwent a wedge resection of the nodule. Histopathologic findings revealed strong expression of chromogranin and ACTH. Neuroendocrine and ectopic secretion of ACTH was confirmed. After one year, her follow-up clinical and imaging results showed no CS or tumor recurrence.

DISCUSSION

CS caused by EAHS is clinically rare and challenging to diagnose[2]. EAHS has been described in cases of many neoplasms, such as non-small-cell lung carcinoma, pancreatic islet tumor, and pheocromocytoma; however, it is highly prevalent in cases of BPCs[1,3,4]. Pulmonary manifestations are uncommon and the most common symptoms are arterial hypertension and hypokalemia[1]. Management strategies of EAS include early localization, confirmation, and surgical removal of the

tumor; moreover, imaging studies are the cornerstone in this regard[5]. Chest CT should be considered for all patients with corticotrophin-dependent CS[6]. However, the tumors are often too small to be detected by conventional imaging modalities. The ^{18}F-FDG uptake of carcinoid tumors is less than that of malignant tumors[7]. Wartski *et al*[8] found that both typical and atypical carcinoids show increased FDG uptake. Chong *et al*[9] reported that FDG uptake in carcinoids depends on the extent of their proliferation, and the value of SUVmax for carcinoids differs from that for neuroendocrine tumors. Zemskova *et al*[10] showed that FDG-PET could detect an ectopic ACTH source with a sensitivity of 64% and positive predictive value of 53%. In our patients, the lesions were identified using CT, but not with chest PET. FDG uptake had minimally increased in the lesion in the 33-year-old patient, and was absent in the 68-year-old patient. Surgical specimens confirmed the diagnosis of BPCs in both patients. Limited pulmonary resection is usually performed by wedge resection, when possible by video-assisted thoracic surgery, and the prognosis after pulmonary resection is excellent[1].

CONCLUSION

Surgical resection is the treatment of choice for BPCs. In our patients, we performed wedge resection of the lung with a clear margin, which resulted in complete remission. Video-assisted thoracoscopy may be ideal for the resection of peripheral pulmonary carcinoids, especially in patients with no obvious FDG uptake and no evidence of metastasis.

ACKNOWLEDGMENT

Disclosure: The authors declare that they have no conflict of interest.

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Case Report

Simple - Complex Vaginoplasty: Our Experience

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ABSTRACT

Congenital anomalies of female genitourinary tract may include vaginal atresia or agenesis, which occur either as isolated developmental defect or within a complex of more extensive anomalies. Several techniques have been used to create a neovagina as a part of the treatment for vaginal atresia. Mc Indoe vaginoplasty is one of the most commonly performed surgical interventions in Mullerian agenesis. Our aim was to evaluate a female undergoing simple vaginoplasty, when only mullerian agenesis is present with normal urinary tract or undergoing complex vaginoplasty, when there has been complex pelvic surgery or with previous unsuccessful surgery or when the patient is unsuitable for conservative treatment (*e.g.*, dilator treatment or minor surgical revision). Vaginal agenesis is a rare condition and surgical decision making is highly complex. These women should be only managed in specialist centers by a multidisciplinary team with psychological input.

KEY WORDS: hematocolpos, hematometra, Mayer Rokitansky Kuster Hauser syndrome

INTRODUCTION

Vaginal agenesis is a rare condition with devastating repercussions on fertility and sexual function[1]. It usually occurs in conjunction with an absent uterus along with normal ovaries and external genitalia, as in Mayer Rokitansky Kuster Hauser (MRKH) syndrome or with genital tract anomalies associated with cloacal and anorectal anomalies[1]. The condition first becomes apparent in adolescence when despite normal pubertal development, menstruation does not develop. Vaginal length can vary from a dimple to a pouch of several centimetres. Patients requiring reconstruction are as complex and varied in their circumstances as the treatments on the offer. Satisfactory result depends upon the underlying condition, previous pelvic surgery, surgeon's skill and the technique chosen.

The correction of vaginal agenesis requires the creation of a neovaginal cavity that is dissected between the bladder and the rectum[2]. The technique needs to use the split-thickness skin graft or full-thickness skin graft. In order to prevent a possible contraction of the reconstructed vagina, a long-term vaginal stent use is required to maintain vaginal width and depth. The vacuum assisted closure-system (VAC) has recently been introduced to improve the uptake of skin graft in vaginal reconstruction and it has been reported to exclude the need for vaginal stent[3]. Although new techniques which do not necessitate a prolonged dilatation are developed, McIndoe's method is still one of the very popular methods of vaginoplasty and it has been shown to be effective in creation of a neovagina for patients with mullerian agenesis[4]. Several vaginal stents have been described for postoperative maintenance after Mc Indoe vaginoplasty[5-8]. Good vaginal function was reported at four months follow-up with no stenosis or dryness.

CASE PRESENTATIONS

We present our experience in three patients of mullerian agenesis, who had Mc Indoe vaginoplasty. Split thickness skin grafts were used in all cases.

Case 1

A 14-year-old school girl presented with the chief complaint of primary amenorrhoea and abdominal pain for six months. Puberty had started at the age of 11 years. Her secondary sex characters were well developed. On local examination external genitalia

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was normal looking except that no identifiable vaginal opening was seen. On per rectal examination a big mass was felt anteriorly. On trans-abdominal sonography (Fig. 1) the uterus was seen with minimum endometrial collection along with hematocolpos. The case involved upper two-thirds of the vagina. Both the ovaries were normal along with normal renal system. Her MRI showed agenesis of the lower one-third of vagina with haematometra and hematocolpos.The option for definitive vaginal reconstructive surgery was discussed with the patient and her parents. After full investigation the patient was taken for examination under anesthesia (EUA) and definitive surgery.

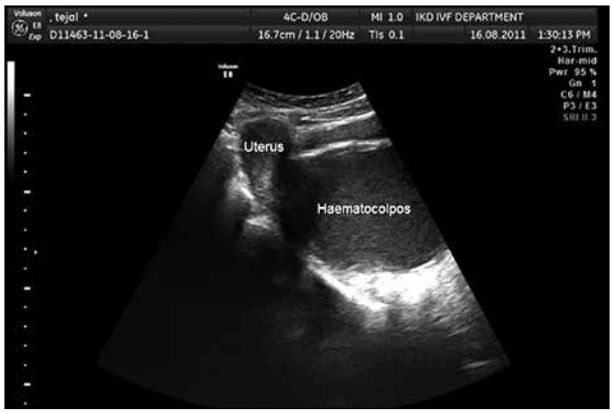

Fig 1: USG showing normal uterus with hematocolpos

Surgical Technique

On examination two dimples below urethra were identified. Dissection between the urethra and rectum was started and chocolate coloured material was drained, but no identifiable cervical opening was seen even on telescopic examination. Abdomen was opened by a pfannenstiel incision and a stab incision was given on the uterus. Through the uterus a Hagar's dilator was inserted down towards cervix after identifying cervical crypts on telescopic examination and a neocervix was created. The newly created vaginal pouch was re-checked for hemostasis

Fig 2: Mould used in complex vaginoplasty

Fig 3: Mould being inserted into space

and then the stent was inserted into the pouch for checking the stent compliance with the neovagina. A foam mould lined with a split thickness skin graft with a Malecot catheter coming out through its proximal end (Fig. 2, 3) was inserted through the cervix so that its wings are resting on the internal os so as to keep the cervix patent. The skin is usually obtained from the thigh. The edges of skin graft are sutured to each other with a reabsorbable 3.0 suture material. Skin graft is handled gently avoiding graft distortions or tear. The distal edges of skin graft can be sutured to the edges of opening incision at this time. The stent was fixed to a belt around the body (Fig. 4). The most important

Fig 4: Fixation of mould externally

point in the stent fixation is to hold a parallel fixation to normal vaginal axis, otherwise meatal or urethral necrosis may occur due to undue pressure resulting from improper stent fixation. This parallel fixation prevents the stent from prolapsing in the early postoperative period. The mould was left in situ for 7 - 10 days initially. In the early postoperative period a vaginal mould 3 cm in diameter and 10 cm in length with one hole at the distal end was used for drainage. The patient was required to wear the mould day and night for 3 - 4 months.

Case 2

A 15-year-old girl presented with suprapubic pain in the abdomen for one month. She had not attained menarche as yet and there was no associated dysuria. In the past, she was operated for bladder exostrophy on 30-5-2001 at our hospital. Later, she developed urinary incontinence and underwent investigations. Her augmentation cystoplasty with bilateral ureteric implantation with Young-Deas bladder neck reconstruction was done on 3-3-2006 at our hospital. On general physical examination her secondary sex characters were well developed. On abdominal examination an infra-umbilical vertical scar was seen. A mass was palpable in hypogastrium. On local examination the external genitalia was normal except that a vaginal opening was not seen. On per rectal examination a bulge was palpated anteriorly which was smooth and uniform. A trans-abdominal ultrasound showed an uterus, cervix, bilateral ovaries and hematocolpos of 6 x 7.5 cm with a volume of 135 ml (Fig. 1). An MRI showed hematocolpos with dilated tortuous left fallopian tube with a hemorrhagic collection. A provisional diagnosis of agenesis of the lower 1/3rd of the vagina was made. She was posted for EUA and vaginoplasty. On 3-10-2011, a trans-vaginal septum was seen on EUA which was excised. A tarry hematocolpos was drained. A hysteroscopy was done which showed a normal uterine cavity, cervical canal and upper 2/3rd of vagina. The raw area of vaginal mucosa was sutured. She was discharged after 10 days. She menstruated normally and cyclically afterwards. Her follow-up ultrasonography (USG) on day five of her periods showed no endometrial collection, an endometrial thickness of 3.5 cm and normal ovaries.

Case 3

A 25-year-old female patient presented with primary amenorrhoea and her marriage was fixed. She was evaluated clinically and gynecologic examination showed that vagina was absent with normal secondary sex characters. On USG examination her ovaries were normal and uterus was absent (Fig. 5).

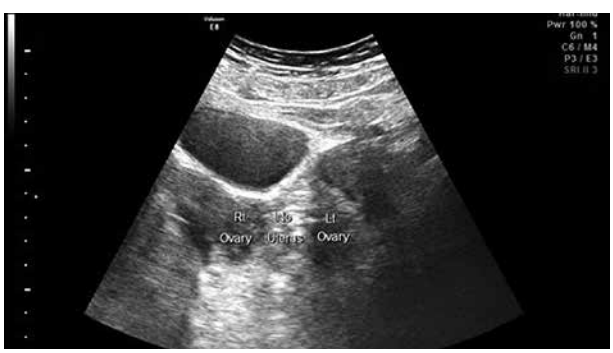

Fig 5: USG showing absent uterus and normal ovaries

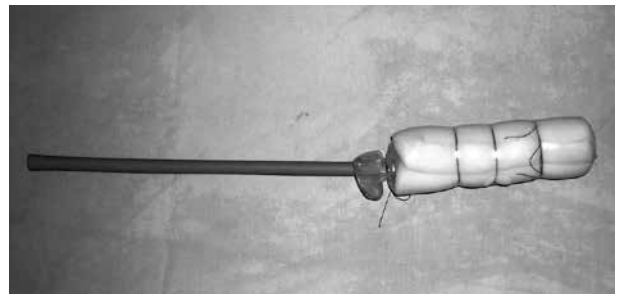

Fig 6: Mould used in simple vaginoplasty

Her gonadotrophin level and Barr body karyotyping was done. Karyotype analysis showed 46XX. She was thoroughly evaluated to exclude other causes of amenorrhoea. Other pathologies like renal, cardiological and oestrogenic abnormalities were also evaluated. Final diagnosis of MRKH syndrome was made. Her McIndoe vaginoplasty was done in November 2011. The McIndoe vaginoplasty is a technique that has the advantage of not requiring abdominal surgical entry. Split-thickness skin graft taken from inguinal region was used. Two dimples in between two labia below urethra in the location of hymen which is the lower end of the vestigial Mullerian ducts were identified. Blunt dissection was used to create space between urethera and rectum (Fig. 7, 8). The prosthesis (Fig. 6) was secured in place for

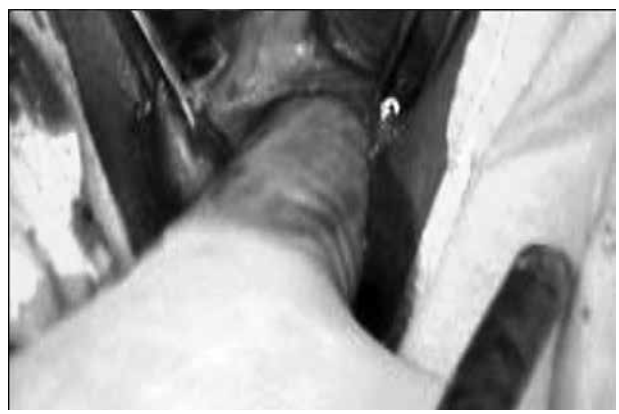

Fig 7: Dissection of space

Fig 8: Mould insertion

seven days after the operation and then it was gently removed for first look at the grafts. Then the prosthesis (Fig. 9) was kept in place with the advice of self

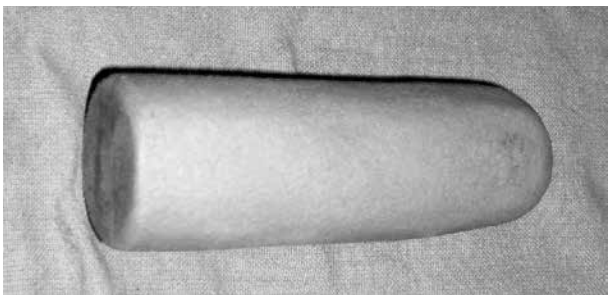

Fig 9: Mould used postoperatively

insertion and dilatation meticulously till active sexual function was established after cleansing it for once daily. Normal axis and adequate length and width of the vagina could be achieved (Fig. 10). A vaginal depth of 10 x 3 cm was obtained and maintained in the patient. The patient is still under follow-up during the early postoperative period.

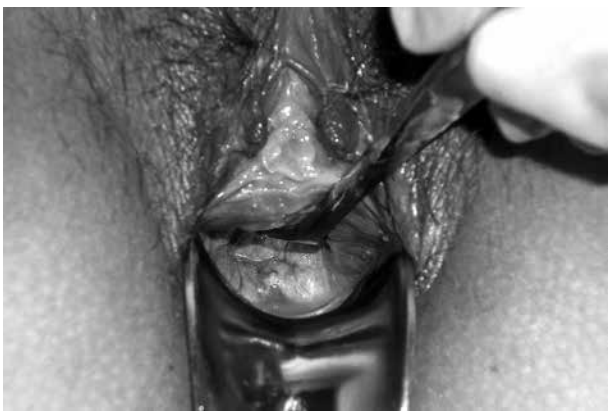

Fig 10: Postoperative appearance of vagina

DISCUSSION

Techniques of vaginal reconstruction are needed in congenital vaginal agenesis or hypoplasia or in cases of obliterated vagina secondary to radiation therapy, trauma or severe vaginal inflammation. An accurate preoperative assessment is essential. MRI is pivotal in patients of mullerian anomalies, as it can delineate the uterine and vaginal anatomy and can correlate with surgical findings in patients with complex pelvic anomalies[9]. Essential information includes the presence or absence of menstrual obstruction, duplication anomaly, and patency of cervical canal, ovarian or tubal pathology along with associated pelvic, muscular and bony anomalies. Gap between the lower end of the proximal vagina and upper end of distal vagina should be delineated so as to ascertain whether only vaginal or combined abdomino-perineal approach will suffice. On examination under anesthesia extent of scarring, vaginal tissue pliability and capacity of bony pelvis can be assessed.

Vaginal dilation should always be recommended as the first line of treatment in vaginal agenesis. It involves insertion of vaginal moulds of gradually increasing length and width into the vaginal dimple, applying local pressure and increasing the potential space between rectum and bladder. Vaginal dilation has few complications, as there are no anesthetic and surgical risks. However, it is usually a time-consuming exercise taking several months to achieve an adequate vaginal length and capacity

The Vecchietti procedure[1] was first described in 1965 and over the last 15 years has been modified to a laparoscopic approach[12]. An acrylic olive is positioned within the vaginal dimple and is connected to threads that are then passed through the rectovesical space, into the peritoneal cavity under laparoscopic control and through to the abdominal wall, where they are attached to a traction. Steady increasing traction is applied to the threads, and the neovagina is stretched by approximately 1 cm per day. The woman remains in hospital for analgesia and the traction device and olive is removed after 7 - 10 days. Vaginal dilation or intercourse is required to maintain the length of the vagina. A degree of elasticity is a prerequisite for the Vecchietti procedure to be successful and it is therefore not recommended where there is scarring from previous reconstructive surgery.

In a retrospective study 75% of McIndoe vaginoplasty patients stated that the procedure improved their quality of life[10]. The only drawback is contracture of tissue at the apex of vagina. In the Sheares procedure[11], the space between two labia is dilated with a Hegar's dilator along the vestigial mullerian ducts and central septum is excised to form a single vagina. A mould covered with graft is placed in the neovagina.

Other attempts to avoid the drawbacks of skin grafting have included the use of amnion as an alternative to line the neovagina. However, this technique is now rarely used mainly due to the possible risk of donor–participant viral infection such as hepatitis and HIV as well as the practicalities of obtaining tissue and appropriate storage.

The Davydov procedure was also initially described as an open procedure but is increasingly performed laparoscopically[12]. In this procedure, the space between rectum and bladder is developed from the perineum and abdominally under laparoscopic guidance. It is then lined by peritoneal flaps that are freed laparoscopically from the pelvis. The peritoneum is sutured circumferentially to the introitus and a soft mould is left *in situ* and removed after one week. The

vault of the neovagina is created either by a purse-string suture or by suturing large bowel serosa at the top. The Davydov procedure[14] is suitable for women who have had previous pelvic surgical procedures, and therefore, the perineum is scarred and not amenable to stretching and dilation. One of the potential complications of both the Davydov and Vecchietti procedure is the risk of prolapse, and a recent publication describes a modified McCall's culdoplasty for additional support of the vaginal vault.

Intestinal vaginoplasty[13] requires a combined perineal–abdominal approach. The procedure is major and is usually performed *via* a laparotomy although has been reported laparoscopically. It remains an operation with significant morbidity as it involves resection and anastomosis of bowel. The recto-vaginal space is dissected, while a loop of either colon or ileum is resected and fed through to the perineum with its vascular pedicle maintained. The caudal edge of the intestinal segment is then sutured to the perineum. The advantages of this technique are that it can be carried out even when there has been extensive previous surgery in the area. The neovagina is well lubricated and the capacity is usually maintained as the lumen of the bowel does not collapse. Nevertheless, the mucosal discharge can be increased, persistent and sometimes foul smelling and commonly the women have to wear pads daily or perform regular irrigations.

There are reports of adenocarcinoma occurring in the neovagina[13] and although a precise risk of the malignancy or its timescale is unclear, it is recommended that women undergoing intestinal vaginoplasty are followed up regularly.

A promising new technique that theoretically should not have the risks of a skin graft vaginoplasty is the use of autologous vaginal tissue[14]. The first successful case was reported this year in a woman with Rokitansky syndrome. A small piece of vaginal mucosa was harvested from the vestibule of the woman and cultured *in vitro*. The reconstructed tissue was then used to line the neovaginal cavity that was developed in the same way as in the McIndoe Reed vaginoplasty. This will of course avoid the problem of donor site scarring

Timing of procedure

The ideal time for intervention is at or after adolescence, when the girl has reached physical and psychological maturity .This allows the woman herself to be involved in decision making and also increases compliance with adjuvant dilation therapy to prevent postoperative stenosis.

Choice of the appropriate procedure

It is highly individual and multiple factors will influence the decision as to which procedure is best. Clearly, surgical proficiency and access to the appropriate open and laparoscopic equipment is essential. In addition, the decision will be influenced by the underlying diagnosis and previous treatment attempts if any. Prior abdominal surgery such as bladder reconstruction in women with multiple complex anomalies of the genitourinary tract will mean that laparoscopic techniques such as the Vecchietti and the Davidov procedures are hazardous and should not be attempted. Surgical treatment should only be undertaken by a multidisciplinary team that can offer all potential approaches. The majority of gynecologists would not be able to proceed to an intestinal vaginoplasty if required and so involvement in the team of the appropriate urological or colorectal surgeon is crucial. In addition, adjuvant therapy such as vaginal dilation treatment and psychological support can influence outcome and satisfaction of the participant and should be available.

Vaginoplasty can be done using split skin graft neovagina, intestinal vaginoplasty and flap / pull-through with patient's skin. Drawback of split thickness is contracture while that of intestinal is excess mucus production and need of major abdominal surgery. Neovaginal malignancy can occur and will present as bleeding. At present complex high risk surgery is all available for this group. Long-term outcomes are generally good with sexual intercourse being possible in over 90% of women, although again data on sexual satisfaction are scanty[16]. Vaginal dryness and strictures are a common problem. Also, there is a reported risk of developing squamous cell carcinoma of the neovagina, and regular follow up is required[12]. Recent work by De Filipo *et al*[17] has shown the possibility of culturing vaginal epithelium and smooth muscle in rabbits.

CONCLUSION

Vaginal agenesis is a rare condition and surgical decision-making is highly complex. The creation of vagina that has a satisfactory appearance, function and feeling is the aim of vaginoplasty and should always be considered. As with the majority of surgical procedures, the first operation is likely to be the most successful. Women undergoing vaginal reconstruction after a series of failed procedures are likely to have a worse outcome and a realistic chance of success should be discussed when taking informed consent. If surgery is required for creating a functionally useful vagina, the primary operation should be definite and performed by well-trained experts. Newer techniques using autologous vaginal tissue may in the future increase the armamentarium available to clinicians dealing with these rare conditions.

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Case Report

Idiopathic Glomerular Capillary Endotheliosis in a Male Patient with Nephrotic Syndrome: Response to Combination Therapy with Prednisone and Nitrogen Mustard

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ABSTRACT

Nephrotic syndrome (NS) in patients with glomerular capillary endotheliosis is rare; most of the few reported cases have had preeclampsia. We present an interesting case of idiopathic glomerular capillary endotheliosis in a male patient with NS. A renal biopsy showed capillary endothelial swelling, occlusion of capillary loops, fusion of epithelial cell foot processes, no electron-dense deposits, and no tubulo-interstitial and arteriolar changes. We administered a novel combination therapy with prednisone and nitrogen mustard to combat the endothelial dysfunction. The systemic symptoms and proteinuria gradually disappeared. This case is the first instance of successful treatment with prednisone plus nitrogen mustard in idiopathic glomerular capillary endotheliosis, and also provides evidence for the superiority of prednisone and nitrogen mustard-based regimens over prednisone alone.

KEY WORDS: immunosuppressant, proteinuria, thrombotic microangiopathy

INTRODUCTION

Glomerular endotheliosis is a specific variant of thrombotic microangiopathy (TMA) that leads to glomerular microvascular injury, and in most cases occurs in pregnant women. Idiopathic glomerular capillary endotheliosis refers to endothelial cell injury of unknown etiology, characterized by glomerular endothelial swelling with loss of endothelial fenestrae, occlusion of the capillary lumens, and the lack of immune complex deposition. The resultant morphological changes increase glomerular filtration membrane permeability, thus permitting the passage of protein molecules or red blood cells into urine. The massive proteinuria often leads to nephrotic syndrome (NS). Limited pregnancy-related endotheliosis has been reported in association with NS[1]. Here, we describe a case of a male patient with NS whose renal biopsy indicated idiopathic glomerular capillary endotheliosis (IGCE). Combined use of prednisone and nitrogen mustard led to clinical improvement and eventual complete recovery.

CASE PRESENTATION

An 18-year-old man with no particular past medical history presented with progressive lower-extremity swelling, oliguria and abdominal distension that had persisted for one week. He did not have any other compounding symptoms nor did he have respiratory infection prior to the onset of symptoms. He was a non-smoker / non-alcoholic consumer, and was not taking acetaminophen, aspirin, or non-steroidal anti-inflammatory drugs near the time of his illness. Upon physical examination, he had normal blood pressure and marked bilateral pitting ankle edema. There were no lesions detected in the heart, lungs, or abdomen. Similarly, the neurological examination was normal.

Routine urinalysis indicated 3+ protein, 2+ blood and two dysmorphic red blood cells per microliter. Serum chemistry test results were notable for a blood albumin level of 16 g/l, triglyceride level of 2.44 mmol/l, and a total cholesterol level of 10.76 mmol/l. Other items included a creatinine level of 1.19 mg/dl (105 μmol/l), elevated uric acid level of 433 μmol/l, and alanine

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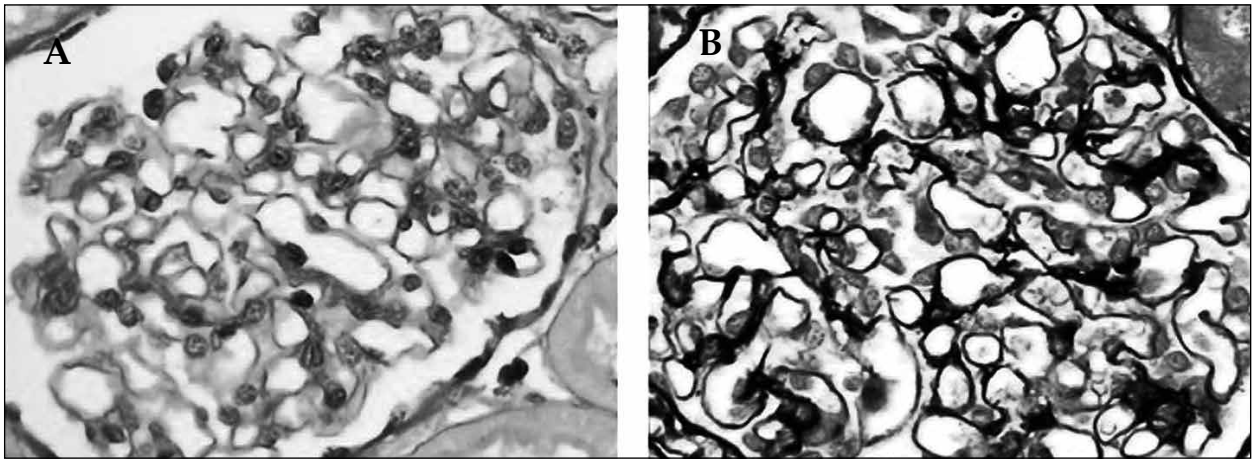

Fig. 1: (A) PAS-stained sections indicating endothelial proliferation, mild mesangial cell proliferation, and matrix expansion. (B) Masson-staining indicating basement membrane degeneration. (Original magnification x 400)

aminotransferase (ALT) level of 60 IU/l (reference range: 0 - 40 IU/l). Other test results indicated that he was negative for anti-nuclear antibody, anti-double-stranded DNA antibody, anti-extractable nuclear antigen antibody, anti-neutrophil cytoplasmic antibody, anti-nucleosome antibody, anti-cyclic citrullinated peptide antibody, and anti-glomerular basement membrane antibody. Furthermore, he had normal C3 and C4 levels. Serological test results for hepatitis B and C were negative.

A percutaneous renal biopsy was performed under ultrasound guidance, and the obtained glomerular numbers were 40. Light microscopy examination revealed endothelial cell proliferation, mild mesangial cell proliferation and matrix expansion (Fig. 1A), and basement membrane degeneration (Fig. 1B). No significant histological lesions were found in the tubulo-interstitial or vascular spaces.

Immunofluorescent staining was negative for immunoglobulin G (IgG), IgA, IgM, C3, C4, C1q, FRA and HBsAg. Electron microscopy revealed capillary endothelial swelling, occlusion of capillary loops, fusion of epithelial cell foot processes, increased lysosomal bodies (Fig. 2A), and widening of the inner less dense layer of the basement membrane (Fig. 2B). There were no electron-dense deposits and also no tubulo-interstitial and arteriolar changes, findings consistent with idiopathic capillary endothelial disease.

Based on the clinical manifestations, we made a diagnosis of NS and acute kidney injury. The patient was treated with glucocorticoids (prednisone 50 mg qd), platelet anti-aggregants (dipyridamole 50 mg tid), and combined thiazide (hydrochlorothiazide 25 mg tid for 5 days and 50 mg bid for 7 days) and potassium-sparing diuretics (spironolactone 20 mg tid for 5 days and 40 mg bid for 7 days) for 12 days. During those 12 days, the patient's edema gradually subsided, levels of creatinine, ALT, and uric acid were normalized, serum albumin level returned to 29.4 g/l, proteinuria decreased to 1+, and 0.53 g of protein was present in a 24-hour urine collection. Following

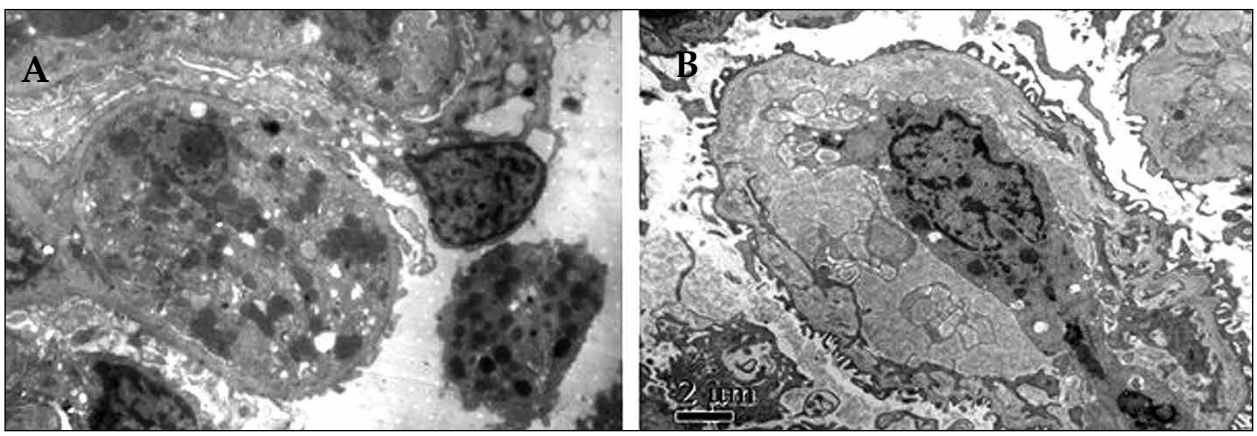

Fig. 2a: Electron microscopy showing capillary endothelial swelling with occluded capillary lumen, widespread fusion of epithelial cell foot processes and increased lysosomal bodies. **(b)** Electron microscopy showing widening of the inner layer of the basement membrane. (Original magnification x 5,000)

another 12 days of prednisone treatment, the patient was administered cefuroxime sodium (3.0 g, q12h) for six days to treat an upper respiratory tract infection. Even after these clinical treatments (occurring over 30 days), his proteinuria increased to 3+, 18 g of protein was present in a 24-hour urine collection, serum albumin level decreased to 16.2 g/lL, and ALT level increased from 19 IU/l to 66 IU/l. Thirty-four days after the initiation of pharmacological therapy we began intravenous injection of nitrogen mustard with continued prednisone treatment. The first phase of nitrogen mustard treatment consisted of an injection of 2 mg nitrogen mustard on days 34 and 36 and 3 mg on days 38. At this time, laboratory data indicated that the patient was negative for proteinuria, serum albumin levels were 27.5 g/l, and ALT levels were 49 U/l. The second phase of nitrogen mustard treatment consisted of an injection of 3 mg every other day for days 40 to 50. At this time, the total nitrogen mustard dose reached 25 mg. A routine urinalysis was negative for proteinuria and a 24-hour urine collection showed 0.16 g of protein. Serum albumin and ALT levels returned to normal. The dose of nitrogen mustard was increased to 4 mg every other day for days 52 to 64 until the patient received a total of 53 mg nitrogen mustard. In conclusion, this case of NS was sensitive to treatment and clinical abnormalities completely vanished. The clinical treatment is outlined in Fig. 3.

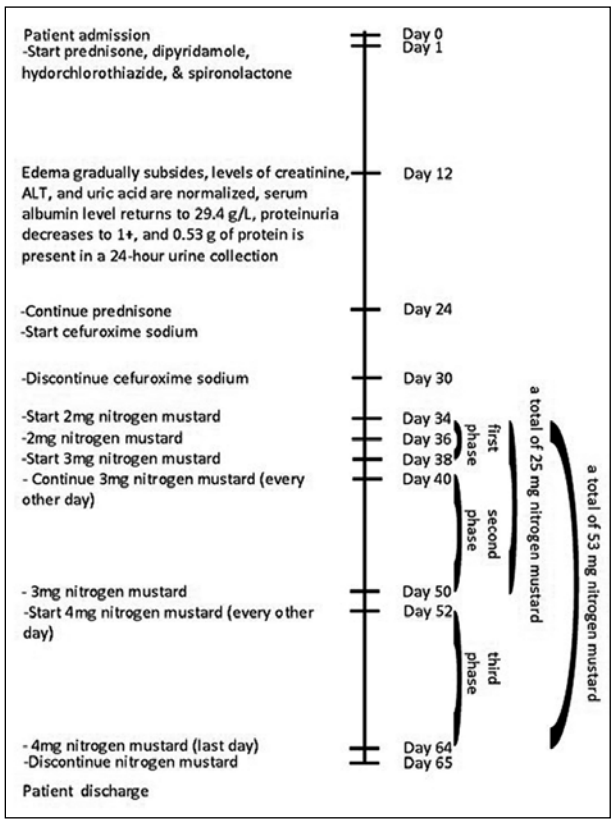

Fig. 3: Patient treatment course

DISCUSSION

In normal kidneys, the endothelial cells play a vital role in vascular tone, perfusion, permeability, and inflammation / adhesion within the glomerulus. Vascular endothelial cell injury and dysfunction, on the other hand, is believed to results in cell proliferation and apoptosis, as well as swelling and detachment of the endothelial cells from the basement membrane[2,3]. Glomerular capillary endotheliosis, a lesion associated with endothelial cell injury, is characterized by glomerular hypertrophy and a reduction in capillary lumen size caused by endothelial swelling.

Endotheliosis has rarely been described in proteinuric disorders other than acute glomerulonephritis. Recently, this lesion has been found in patients with TMA, including preeclampsia, thrombotic thrombocytopenic purpura (TTP), and hemolytic-uremic syndrome (HUS). Similar histopathologic characteristics have been reported in patients with other diseases, including active infection with parvovirus B19, polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome (POEMS), as well as IGCE[4].

Idiopathic disease is unique in that there is no special medical history or associated diseases. Preeclampsia is a hypertensive proteinuric disorder that can occur during pregnancy, and endothelial injury is often a conspicuous physiological feature. Morphological characteristics include capillary endothelial and mesangial cell swelling. Immunofluorescent microscopy usually indicates glomerular capillary wall granular deposition of fibrin, sometimes with IgM deposition. Electron-dense granules and fibrin deposits are seen under the endothelial cells and in the mesangium by electron microscopy. TTP has been classically defined by the pentad of microangiopathic hemolytic anemia, thrombocytopenia, neurological manifestation, renal dysfunction, and fever[5]. HUS is defined as the presence of thrombocytopenia, anemia, and renal dysfunction. However, it has been observed that many patients with TTP lack one or more of these criteria, while some HUS patients exhibit fever and neurologic dysfunction. Hence, differentiating between the two diseases is often difficult. The main difference is the degree of renal failure. HUS is more common in infants and young children, predominantly affecting the kidney and leading to acute renal failure. HUS is also seen in adults with poor prognosis who are progressing to chronic renal failure, often requiring long-term dialysis or renal transplantation to sustain life. The POEMS syndrome is a rare multisystem disorder associated with osteosclerotic myeloma and multicentric Castleman's disease. The age of onset of the illness is over 45 years of age, and patients with POEMS tend to have a chronic course.

IGCE refers to endothelial cell injury of unknown etiology showing diffuse swelling and proliferation of endothelial cells with glomerular ischemia, but without conspicuous mesangial proliferation and specific tubulo-interstitial changes. However, immunofluorescence is often negative. By means of electron microscopy, endothelial cell swelling and proliferation are prominent throughout the glomerular capillary, which causes the obstruction of the capillary lumen. Extensive fusion of the foot processes of the visceral epithelial cells is present, and no electron-dense deposits are observed in glomeruli.

In our patient, there were no clinical or laboratory findings of secondary NS, such as systemic lupus erythematosus, Henoch-Schönlein purpura nephritis, or hepatitis B virus-associated glomerulonephritis. Therefore, a diagnosis of primary NS was made on the basis of the severe proteinuria, hypoproteinemia, edema, and hypercholesterolemia. Electron microscopy revealed occlusive endothelial swelling of glomerular capillary loops (endotheliosis), but there were no significant endothelial changes observed in the glomeruli by light microscopy. The patient had no evidence for other causes of glomerular changes. This may presumably be due to mild lesions induced by viral infection.

Glomerular endothelial cells are a target of injury in a variety of kidney diseases. Although the underlying mechanisms leading to glomerular capillary endotheliosis are unclear, it is likely that many of them are involved in endothelial cell damage, such as dysfunction of vascular endothelial growth factor (VEGF), or abnormal vascular endothelial function[6-8]. Evidence suggests that the glomerular endotheliosis lesion of pre-eclampsia is mediated by excess placenta-derived soluble circulating molecules (soluble VEGF receptor 1 (sFlt-1), sEndoglin) that block the VEGF-A / VEGF receptor and transforming growth factor-β / endoglin signaling, leading to the loss of glomerular endothelial cell fenestrae, cell swelling and proteinuria[9-11]. Recently, haplosufficient mice for all isoforms of VEGF were shown to develop glomerular endotheliosis underscoring the importance of VEGF for the endothelial integrity[12]. In addition, in Denys-Drash syndrome (DDS) glomeruli have swollen endothelial cells, reminiscent of endotheliosis, and podocytes associated with this syndrome express high levels of the proangiogenic isoform VEGF165, but completely lack the inhibitory isoform VEGF165b[13]. This study suggests that alteration of the VEGF165 / VEGF165b ratio in DDS may provide a mechanistic insight into the pathogenesis of DDS. These results demonstrate that VEGF plays a critical role in glomerular development and function, and provides the foundation to develop novel diagnostic or therapeutic tools for patients with glomerular endotheliosis disease.

Glucocorticoids (prednisone) have powerful anti-inflammatory and immunomodulatory effects and have been used as a first-line treatment in immune-mediated / inflammatory diseases. However, glucocorticoids alone are associated with high recurrence rates, steroid-dependence, steroid-resistance or even serious side effects. The combined use of prednisone and immunosuppressants is effective in improving efficacy, reducing side effects and recurrence rate, and allows for a shortened period of glucocorticoid administration. It has been reported that in a female IGCE is responsive to steroids and cyclophosphamide (CTX)[14]. In our case, after the combined treatment of nitrogen mustard and prednisone, urine examination was negative and liver and renal function returned to normal. Nitrogen mustard administration is an ancient and effective cytotoxic regimen. Its principal lethal lesion is largely attributed to the covalent structure of a nitrogen mustard-induced DNA interstrand cross-link, which inhibits the replication of DNA and the synthesis of RNA and protein. Furthermore, nitrogen mustard can inhibit B-cell proliferation, differentiation and antibody production, inhibit T-cell activation and release of inflammatory mediators, and also inhibit fibril formation. Compared with CTX, nitrogen mustard has no bladder toxicity, no obvious gonadal suppression (this is very important for young men), and directly depresses the immune response without hydrolysis in vivo, suggesting that the immunosuppressive effect of nitrogen mustard is stronger than that of CTX. Thus, the novel combination therapy with prednisone and nitrogen mustard can significantly reduce proteinuria by improving glomerular filtration membrane permeability and improve renal function. The main side effects of nitrogen mustard are nausea, vomiting, phlebitis, leukopenia, etc., which limit its use. In our study, the patient showed slight gastrointestinal symptoms that were observed in the early treatment phase due to the use of antiemetic drugs (metoclopramide). However, this did not warrant discontinuation of treatment. The patient also had a reduced white cell count during the post-treatment period that was effectively treated using vitamin B4. Thus there were no serious complications such as infection or injury of vessels. Therefore, we think that nitrogen mustard is an effective and relatively safe immunosuppressant.

Despite the limited evidence provided by the present case, combination therapy with prednisone and nitrogen mustard may be a potential therapy for IGCE .

ACKNOWLEDGMENT

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Case Report

Severe Bilateral Pulmonary Edema after Video-Assisted Thoracic Surgery for Pneumothorax

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ABSTRACT

A 36-year-old woman was admitted to our hospital complaining of chest tightness and progressive shortness of breath. Chest radiography showed a collapsed lung in the left hemithorax. After tube thoracostomy, persistent air-leakage was noted even after five days. Video-assisted thoracoscopic surgery was carried out under general anesthesia, and one-lung ventilation was performed for one hour and 15 minutes. Eighteen hours after extubation, the patient experienced severe dyspnea with hypoxia. A chest radiograph revealed bilateral pulmonary edema. She was re-intubated, and her symptoms improved with mechanical ventilation and PEEP in the intensive care unit (ICU). Although video-assisted thoracic surgery (VATS) is a safe and effective procedure, clinicians should keep in mind that bilateral re-expansion pulmonary edema (RPE) may occur after anesthesia for the treatment of pneumothorax.

KEY WORDS: mechanical ventilation, pneumothorax, re-expansion pulmonary edema

INTRODUCTION

Re-expansion pulmonary edema (RPE) occurring after re-expansion of a lung with pneumothorax by means of thoracentesis was first reported by Carlson in 1959[1]. Although the incidence of RPE is low, it may lead to severe hypoxemia with a mortality rate of up to 21%[2]. RPE is associated with the treatment of lung diseases such as pneumothorax, hemothorax, and pleural effusion. The pathogenesis of RPE is not clear but a treatment regimen with supportive therapy has been established. We present a rare case of bilateral pulmonary edema after video-assisted thoracic surgery (VATS) to treat left-side recurrent pneumothorax. The patient was successfully treated with mechanical ventilation and positive end-expiratory pressure (PEEP).

CASE REPORT

A 36-year-old woman with no history of smoking presented at our emergency department with progressive shortness of breath and a 7-day history of left-sided chest tightness. She had left spontaneous pneumothorax while undergoing tube thoracostomy two years ago. There was no trauma or any medical history, and physical examination revealed absent breath sounds over the left thorax. A chest radiograph revealed left-sided pneumothorax with a totally collapsed left lung (Fig. 1A). The patient underwent thoracostomy with a 28-Fr chest tube, and her symptoms improved. After tube insertion, a persistent air-leak was noted for five days. The patient agreed to undergo video-assisted thoracoscopic surgery for treatment of persistent pneumothorax. The operation was performed under general anesthesia with one-lung ventilation using a double-lumen endobronchial tube, and the site was confirmed using a flexible bronchoscope. The patient was placed in the right lateral recumbent position. The entire operative time was 75 minutes, after which the endobronchial tube was removed. After 18 hours, the patient showed dyspnea with marked inspiratory efforts. Her SpO_2 decreased from

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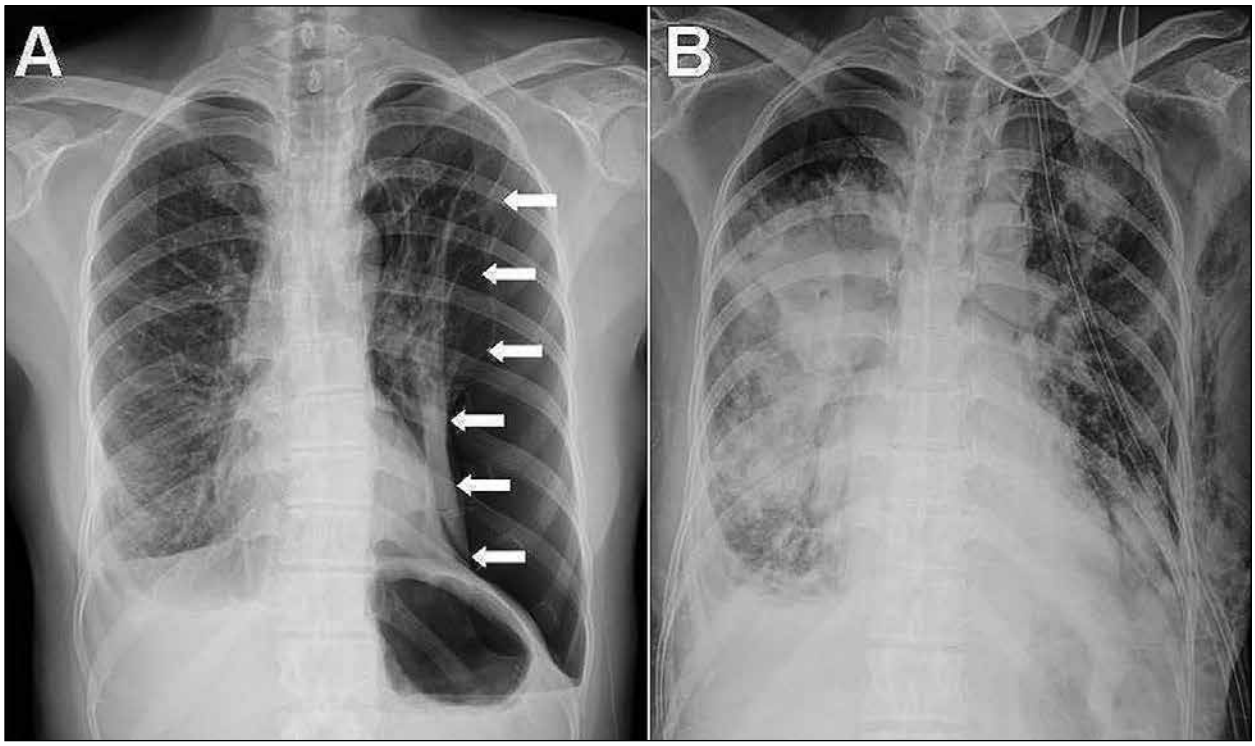

Fig. 1: (A) Chest X-ray showing left pneumothroax with a totally collapsed left lung (white arrow) and (B) pulmonary edema of right lung after video-assisted thoracic surgery for left pneumothroax

100% to 80%, and blood oxygen levels could not be maintained. Arterial blood gas analysis showed a pH of 7.424, a PaO_2 of 71.9 mmHg, and a $PaCO_2$ of 40.0 mmHg. A chest radiograph revealed pulmonary edema in both lungs with left-sided subcutaneous emphysema (Fig. 1B). The patient was re-intubated and ventilated with 100% oxygen. Subsequently, the patient received mechanical ventilation with positive end-expiratory pressure (PEEP) in the intensive care unit. The pulmonary edema improved after four days of ventilation treatment, and the patient was extubated without oxygen administration. The chest tube was removed without complications, and the patient was discharged from the hospital on the eighth postoperative day.

DISCUSSION

RPE is a rare complication, and its incidence ranges from 1% to 14%[3]. The pathophysiology of RPE is not clearly understood, and increased vascular permeability has been recognized as a possible cause because of hypoxic injury to the capillary and alveolar membranes and diminished surfactant production[4]. According to a previous report, RPE is more common among men, and the average age of occurrence is 42 years[5]. All patients are symptomatic in 24 hours[2]. Although RPE is usually ipsilateral and develops immediately after resolution of the pneumothorax[6], it can also occur on the contralateral side or both sides[4]. Risk factors of RPE include young age, degree of lung collapse, rapid re-expansion, duration of pneumothorax, and re-expansion of a pneumothorax of short duration[7, 8]. The total reported number of cases of contralateral pulmonary edema caused by lung re-expansion after pneumothorax is only five, and this condition has a mortality rate of up to 40%[4,7,9]. The possible reasons for contralateral pulmonary edema include unrecognized aspiration, increased cardiac output, inflammatory response caused by the activity of interleukin-8, and mediastinal shift with contralateral lung compression[7]. The treatment for RPE is supportive with administration of oxygen, diuretics, inotropes, and mechanical ventilation[10]. The usage of extracorporeal membrane oxygenation has been reported to successfully treat bilateral RPE with worsened oxygenation[4,7]. The cornerstones of treatment for RPE are adequate oxygenation and support of circulation.

CONCLUSION

Clinicians should always be aware of the possibility of RPE after VATS with one-lung ventilation. Careful perioperative management is necessary, and early diagnosis and immediate supportive treatment will decrease the mortality and result in a good clinical outcome.

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Letter to the Editor

Adopting E-learning and Social Networking in Medical Education

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Kuwait Medical Journal 2014; 46 (2): 163 - 164

E-learning is one of the popular technologies in education system[1-3]. It is also known as the World Wide Web (WWW) learning, web-based learning, online learning, distributed learning, computer-assisted instruction, or internet-based learning. This gives tremendous amount of benefits and flexibilities in learning and education. Recent concepts like virtual patients, virtual medical homes (VMH), virtual intensive care units (VICUs) and advanced simulation techniques have given new directions to E-learning in medical education[4,5]. In parallel, social networking is also getting popular day-by-day and billions of users are participating in social networking activities. That social networking has also given a number of benefits to education systems and medical education is not an exception. Thousands of groups related to medical education are available today on popular networking sites such as Facebook, Twitter, LinkedIn and SecondLife. Thousands of groups are available on Facebook having the term "medical" in their name. Guidelines for using Twitter in medical education are also given in earlier works[6].

The latest technologies on the web like blogs, podcasts, social networking sites, wikis, and video streaming have given a new thought in directions for using e-learning in medical education[7, 8]. A numbers of top universities in the world have adopted the e-learning models for educating medical students[2].

Harvard Medical School (Department of Continuing Education - http://cmeonline.med.harvard.edu) and Johns Hopkins University (School of Medicine - http://webapps.jhu.edu) are two such topmost universities where e-learning is implemented and used effectively in medical education. A working group namely, MedBiquitous consortium (http://www.medbiq.org/) was also established in this direction in 2005, with the objective to create a free and open data standard for expressing and exchanging virtual patients between different authoring and delivery systems[9]. MedBiquitous is accredited by the American National Standards Institute (ANSI) to develop information technology standards supporting the health professions in education. In this article, challenges, opportunities, advantages and future direction for adopting E-learning and social networking in medical education are discussed.

Social networking sites provide a number of online tools and techniques using which users can share their knowledge and can participate individually or in a network group across the world for information sharing. A number of social networking sites are available over the web; some of the most popular sites are Facebook, Twitter, LinkedIn and Google Circles. All these online social networking sites have their own way of managing and sharing information and all these sites provides an excellent way of knowledge sharing which can be utilized efficiently in medical education. Social sites allow medical students, practitioners, educators and experts to communicate openly and equally in a more efficient manner than traditional way of teaching and learning. There are an enormous number of ways available on social sites which can be used in medical learning and education system including discussing questions, case based learning and participating in online activities and conferences.

Several organizations are also working on creating standards on E-learning for medical education. One such organization is FOAM (Free Open Access Medical Education)[10]. FOAM manages the continuously growing collection of online resources related to medical education which can be accessed by anyone, anytime, anywhere. FOAM also works in direction

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of improving the existing practices and adoption of E-learning in medical education.

E-learning is not a new concept in medical education; it has been adopted by various countries in educating medical students. Apart from this, a number of useful softwares, tools and online resources are managed and provided by various organizations[11-13]. Recent focus of E-learning in medical education is in four major areas. These areas are problem-based learning (PBL) or case based learning (CBL), evidence-based medicine (EBM), medical teacher training, and simulators and virtual patients. PBL or CBL is a learner-centered educational method. It uses appropriate problems to increase knowledge and understanding[14]. PBL or CBL is designed to help students to understand the basics of medicine and at the same time, they develop the reasoning process used by physicians and other health professional in their clinical practice. In EBM best evidence is referred to in making clinical decisions while caring for individual patients. The practitioners, consultants, and students perform a literature search and identify the best literature evidence available on the clinical condition, critically evaluate it, and determine the "Best Evidence" to diagnose or treat the patient.

Virtual patients are the advanced simulators which are adopted by a number of top medical institutions across the world. Virtual patients are specialized computer programs where the medical students and practicing professionals apply their knowledge to learn and practice on these computer-generated patients. E-learning also provides early clinical exposure to fresh medical students. Medical students can get an overview of diagnosis and treatment methodologies using computer programs using E-learning approaches which prepare the medical students mentally for the traditional learning of medical science. E-learning also provides for implementation of logbook management which can be used effectively for trainee medical students and junior doctors to monitor their activities and learning processs. VMH and VICUs are other variants of popular simulator programs in medical education.

Apart from the above, Mobile learning (M-learning) can be introduced and used effectively. M-learning provides a virtual learning environment (VLE) in education by handheld portable devices[15]. It provides feasibility for online content access by portable devices through internet.

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Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Kuwait Medical Journal 2014, 46 (2): 165 - 167

Use of Fingolimod in Patients with Relapsing Remitting Multiple Sclerosis in Kuwait

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[3]Neurology Clinic, Department of Medicine, Dasman Diabetes Institute, Dasman, Kuwait
[4]Department of Neurology, Ibn Sina Hospital, Kuwait City, Kuwait; Department of Medicine, Kuwait University, Jabriya, Kuwait

Clin Neurol Neurosurg 2014; 119:17-20

Background: Post-marketing studies are important to confirm what was established in clinical trials, and to assess the intermediate and long-term efficacy and safety.
Objective: To assess efficacy and safety of fingolimod in multiple sclerosis (MS) in Kuwait.
Methods: We retrospectively evaluated MS patients using the MS registries in 3 MS clinics. Relapsing remitting MS patients according to revised 2010 McDonald criteria who had been treated with fingolimod for at least 12 months were included. Primary endpoint was proportion of relapse-free patients at last follow-up. Secondary endpoints were mean change in EDSS and proportion of patients with MRI activity (gadolinium-enhancing or new/enlarging T2 lesions).
Results: 76 patients met the inclusion criteria. Mean age and mean disease duration were 34.43 and 7.82 years respectively. Mean duration of exposure to fingolimod was 18.50 months. Proportion of relapse-free patients was 77.6% at last follow-up. Mean EDSS score significantly improved (2.93 versus 1.95; $p<0.0001$) while 17.1% of patients continued to have MRI activity versus 77.6% at baseline ($p<0.0001$). Four patients stopped fingolimod due to disease breakthrough (n = 3) and lymphadenitis (n = 1).
Conclusion: Fingolimod is safe and effective in reducing clinical and radiological disease activity in relapsing remitting MS patients. Our results are comparable to reported results of phase III studies.

Patient Satisfaction with Primary Health-Care Services in Kuwait

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Int J Nurs Pract 2014 Mar 12. doi: 10.1111/ijn.12257. [Epub ahead of print]

The study aims to evaluate patient satisfaction with respect to primary health-care services in Kuwait.A total of 245 patients completed the General Practice Assessment Questionnaire postconsultation version 2.0. Two statistically significant differences of patients' satisfaction with sex and level of education were found. Overall satisfaction was higher among men than women ($P = 0.002$), and it was also higher among those with university degree of education than the other levels of education ($P = 0.049$). We also found statistically significant differences of patients' responses over sex for three themes, namely: satisfaction with receptionists, satisfaction with access and satisfaction with communication; and over the age for one theme: satisfaction with access. There was no statistically significant differences of patients' responses over nationality for all themes. Satisfaction is a multifactorial and no one factor alone could provide satisfaction with primary health services in Kuwait.

Analysis of Neoadjuvant Therapies in Breast Cancer with Respect to Pathological Complete Response, Disease-Free Survival and Overall Survival: 15 Years Follow-Up Data from Kuwait

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E-mail: yams23in@yahoo.com

Asia Pac J Clin Oncol 2013 Aug 12. doi: 10.1111/ajco.12118. [Epub ahead of print]

Aims: Optimizing neoadjuvant chemotherapy regimens is essential for achieving maximal pathological complete response (pCR) in patients with breast cancer. pCR is usually considered as a surrogate marker for survival. The aim of this study was to analyze pCR with respect to various neoadjuvant regimens and its effect on survival.
Methods: This retrospective analysis included 377 patients with stages II and III breast cancer treated between 1998 and 2009 with neoadjuvant chemotherapy. Neoadjuvant regimens were analyzed with respect to pCR, disease-free survival (DFS) and overall survival (OS).
Results: The median age of our population was 50 years with the majority being premenopausal and locally advanced. The overall pCR rate was 13.7% with higher rates seen in patients receiving combination of anthracyclines and taxanes (14.2%). The practice of sandwiching surgery and chemotherapy was inferior to true neoadjuvant chemotherapy of eight cycles. Addition of trastuzumab to Her2 positive patients resulted in higher pCR rates (P = 0.006). Achievement of pCR with neoadjuvant chemotherapy resulted in significantly higher DFS and OS.
Conclusion: pCR is associated with better survival in breast cancer patients receiving neoadjuvant chemotherapy. Initial anthracycline-based chemotherapy followed by non-cross-resistant taxane-based chemotherapy along with the addition of trastuzumab in Her2 positive patients might be the optimal neoadjuvant regimen in breast cancer patients.

ND4L Gene Concurrent 10609T>C and 10663T>C Mutations are Associated with Leber's Hereditary Optic Neuropathy in a Large Pedigree from Kuwait

Behbehani R[1], Melhem M, Alghanim G, Behbehani K, Alsmadi O
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Br J Ophthalmol 2014 Feb 25. doi: 10.1136/bjophthalmol-2013-304140. [Epub ahead of print]

Background: Leber's hereditary optic neuropathy (LHON) is a condition characterised by a rapid bilateral central vision loss due to death of the retinal ganglion cells, leading to visual impairment commonly occurring during young adulthood. The disease manifests itself more in male patients than female patients. The mtDNA mutations m.11778G>A, m.3460G>A and m.14484T>C are by far more frequent in LHON than any other mutation. In this report, a multi-generational Arab family from Kuwait with 14 male members with LHON was investigated.
Methods: Complete mtDNA mutational analysis by direct Sanger's sequencing was carried out to detect pathogenic mutations, polymorphisms and haplogrouping.
Results: All maternally related subjects from this study who were examined expressed the L3 haplotype background, with two concurrent mtDNA mutations, 10609T>C and 10663T>C, that led to non-conservative amino acid changes of Ile47Thr and Val65Ala, respectively. The two variations were absent in 144 normal and ethnicity-matched controls.
Conclusions: The two identified mutations associated with LHON in this family may exert their pathogenicity through a cumulative or haplogroup effect. This is the first report of the presence of two concurrent mutations in the ND4L gene in individuals with LHON who carry the L3 haplogroup.

Adverse Pregnancy Outcome among Teenagers: A Reality?

Chibber R[1], Fouda M, Al-Hijji J, Al-Dossary M, Sadeq EH, Amen A, Shishtawy W, Tasneem A
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J Obstet Gynaecol 2014 Jan 31. [Epub ahead of print]

The objective of this retrospective analysis was to evaluate maternal, fetal and neonatal outcomes in primi-adolescent pregnancies in Kuwait. Case records of primigravidae under 29 years of age, attending the antenatal clinic at our tertiary hospital, between January 2002 and December 2010, were analysed. The study group (up to 19 years of age at first pregnancy) consisted of 3,863 women and the control group (20-29 years of age at first pregnancy) comprised of 4,416 women. Maternal obstetric, fetal and neonatal complications were compared between the groups. Rates of ectopic pregnancy, pre-eclampsia, eclampsia, preterm labour, premature rupture of membrane and caesarean section were significantly higher among adolescents < 15 years of age; the risk then decreased steadily with age and became comparable with the control group after 16 years of age.

Screening for PCDD/Fs and dl-PCBs in Local and Imported Food and Feed Products Available Across the State of Kuwait and Assessment of Dietary Intake

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[4]BioDetection Systems BV (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands

Ecotoxicol Environ Saf. 2014; 100:27-31. doi: 10.1016/j.ecoenv.2013.12.002. Epub 2013 Dec 20

A total of 318 local and imported meat, milk, eggs, fish, and animal feed samples collected in Kuwait were analyzed by cell-based reporter gene assay (Dioxin-Responsive Chemical Activated LUciferase gene eXpression DR-CALUX) for PCDD/Fs and dl-PCBs. The bioanalytical equivalents (BEQs) obtained by DR-CALUX bioassay were compared with the official maximum limits according to the European Commission (EC) regulations. Suspected and randomly chosen negative samples were analyzed by gas chromatography-high resolution mass spectrometry (GC-HRMS). The results showed that among suspected samples, one sample was confirmed to be non-compliant. The positive sample was of imported origin. The correlation coefficient of 0.98 between DR-CALUX and GC-HRMS was found. Moreover, the average daily intakes of PCDD/Fs and dl-PCBs for the Kuwaiti population were estimated. Results obtained in this study were discussed and compared with other published data.

Forthcoming Conferences and Meetings

Compiled and edited by
Babichan K Chandy

Kuwait Medical Journal 2014; 46 (2): 168 - 182

Symposia at Sea™ **Head and Neck Imaging**: What You Need to Know
Jul 1 - 12, 2014
Greece / Athens
Contact: Educational Symposia
Phone: 800-338-5901

2014 International **Cartilage Repair Society** (ICRS) Focus Meeting
Jul 3 - 5, 2014
Switzerland / Zurich
Contact: Sandra Kessler, Secretariat, ICRS
Phone: 011-41-44-503-7371; Fax: 011-41-44-503-7372
Email: sandra.kessler@cartilage.org

2nd International Congress on **Naturopathic Medicine**
Jul 4 - 6, 2014
France / Paris
Contact: Congress Secretariat, Paragon Group
Phone: 011-41-22-533-0948
Email: gtito@paragong.com

Infectious Disease Medicine for Primary Care
Jul 4 - 6, 2014
Canada / Ontario
Contact: Medical Education Resources
Phone: 800-421-3756 or 303-798-9682; Fax: 303-798-5731
Email: info@mer.org

13th International Congress on **Neuromuscular Diseases**
Jul 5 - 10, 2014
France / Nice
Contact: Congress Office, MCO Congrès SAS
Phone: 011-33-4-9509-3800; Fax: 011-33-4-9509-3801
Email: contact@icnmd2014.org

International Conference on **Geriatrics & Gerontology**
Jul 8 - 10, 2014
United States / Illinois
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: geriatrics2014-@omicsonline.net

Adolescent Health
Jul 9 - 10, 2014
United Kingdom / London
Contact: Centre for Continuing Professional Development, Imperial College London
Phone: 011-44-20-7594-6882
Email: cpd@imperial.ac.uk

Anticoagulation in Emergency Care
Jul 9, 2014
United Kingdom / London
Contact: Kerry Tarrant, Programme Director, Healthcare Conferences UK
Phone: 011-44-19-3242-9933; Fax: 011-44-20-8181-6491
Email: kerry@hc-uk.org.uk

International Society for Magnetic Resonance in Medicine (ISMRM) Workshop on **Motion Correction in MRI**
Jul 11 - 14, 2014
Norway / Tromso
Contact: Melisa Martinez, Meetings Coordinator, ISMRM
Phone: 510-841-1899; Fax: 510-841-2340
Email: info@ismrm.org

2nd International Conference and Exhibition on **Physical Medicine & Rehabilitation**
Jul 14 - 16, 2014
United States / Maryland
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: physicalmedicine2014@omicsonline.net

4th International Conference on Clinical & Experimental **Ophthalmology**
Jul 14 - 16, 2014
United States / Maryland / Baltimore
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: ophthalmology2014@omicsonline.net

Primary Care: **ECG & Arrhythmia Interpretation** with Focus on a Clinical Approach
Jul 14 - 24, 2014
United Kingdom / Harwich
Contact: Continuing Education, Inc, Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711; Fax: 727-522-8304

2014 International Congress for **Joint Reconstruction** (ICJR) Pan Pacific Congress
Jul 16 - 18, 2014
United States / Hawaii
Contact: Sylke Anderson, Meeting Administrator, ICJR
Phone: 760-942-7859; Fax: 760-942-1140
Email: sanderson@icjr.net

Renal Biopsy in Medical Diseases of the Kidneys
Jul 16 - 19, 2014
United States / New York
Contact: Columbia CME, Columbia University College of Physicians & Surgeons
Phone: 212-305-3334
Email: cme@columbia.edu

10th World Congress of International Academy of **Cosmetic Dermatology**
Jul 18 - 20, 2014
Brazil / Rio de Janeiro
Contact: Executive Secretariat, JZ Brasil
Phone: 011-55-21-2286-2846; Fax: 011-55-21-2286-2839
Email: contato@iacdRio2014.com.br

2014 Mental Health **Aegean & Ionian Sea Cruise**
Jul 19 - 26, 2014
Turkey / Istanbul
Contact: Dr. Martin Gerretsen, Sea Courses Inc.
Phone: 888-647-7327 or 604-684-7327; Fax: 888-547-7337 or 604-684-7337
Email: cruises@seacourses.com

Otolaryngology: Comprehensive Review for Primary Care & Emergency Care Providers Mediterranean Cruise
Jul 19 - 26, 2014
Turkey / Istanbul
Contact: Continuing Education, Continuing Education, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

1st Annual International Conference on Advanced Research: **Pediatrics**
Jul 21 - 22, 2014
Singapore / Singapore
Contact: GSTF Global Science, Conference Secretariat, Global Science and Technology Forum
Phone: 011-65-6327-0166; Fax: 011-65-6327-0162
Email: info@pediatrics-conf.org

2014 Annual International Conference on Advanced Research: **Obstetrics & Gynecology**
Jul 21 - 22, 2014
Singapore / Singapore
Contact: GSTF Global Science, Conference Secretariat, Global Science and Technology Forum
Phone: 011-65-6327-0166; Fax: 011-65-6327-0162
Email: info@obgynae-conf.org

2014 Annual International Conference on **Microscopic & Macroscopic Anatomy**
Jul 21 - 22, 2014
Singapore / Singapore
Contact: GSTF Global Science, Conference Secretariat, Global Science and Technology Forum
Phone: 011-65-6327-0166; Fax: 011-65-6327-0162
Email: info@anatomy-conf.org

23rd Annual Congress for **Endosurgery in Children**
Jul 22 - 26, 2014
United Kingdom / Edinburgh
Contact: International Pediatric Endosurgery Group
Phone: 310-437-0553; Fax: 310-437-0585

19th World Congress on **Heart Disease**
Jul 25 - 28, 2014
United States / Massachusetts
Contact: Cardiology Online
Phone: 310-657-8777; Fax: 310-659-4781
Email: klimedco@ucla.edu

3rd International Conference and Exhibition on **Orthopedics & Rheumatology**
Jul 28 - 30, 2014
United States / California / San Francisco
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: orthopedics.rheumatology2014@omicsonline.net

3rd International Conference on **Gastroenterology & Urology**
Jul 28 - 30, 2014
United States / California / San Francisco
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: gastroenterology2014@omicsonline.net

4th International Conference on **Proteomics & Bioinformatics**
Aug 4 - 6, 2014
United States / Illinois
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: proteomics2014@omicsonline.net

Primary Care Update: **Type 2 Diabetes, Metabolic Syndrome and Obesity**
Aug 4 - 15, 2014
United Kingdom / Harwich
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711; Fax: 727-522-8304
Email: contactus@continuingeducation.net

Oral Dermatology and Oral Pathology
Aug 8 - 15, 2014
United States / Washington
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711; Fax: 727-522-8304
Email: contactus@continuingeducation.net

12th International Symposium of **Spermatology**
Aug 10 - 14, 2014
Australia / Newcastle, AUS
Contact: ASN Events Pty Ltd
Phone: 011-61-3-5983-2400
Email: participation@asnevents.net.au

2nd International Conference on **Epidemiology & Evolutionary Genetics**
Aug 18 - 19, 2014
China / Beijing
Contact: Sydney James, Conference Coordinator, Omics Publishing Group
Phone: 650-268-9744
Email: epidemiology2014@omicsonline.net

2014 **Ultrasound Guided Regional Anesthesia** & Vascular Access Workshop
Aug 20, 2014
United States / Pennsylvania
Contact: Northwest Anesthesia Seminars
Phone: 800-222-6927; Fax: 509-547-1265

10th Asia Pacific Congress in **Maternal Fetal** Medicine
Aug 22 - 24, 2014
Singapore / Singapore
Contact: Clara Lau, APCMFM Secretariat, Department of Obstetrics & Gynaecology, Chinese University of Hong Kong
Phone: 852-2632-1535; Fax: 852-2636-0008
Email: apcmfm@med.cuhk.edu.hk

Medical CBT: Ten-Minute Cognitive Behaviour Therapy Techniques for Real Doctors
Aug 22 - 23, 2014
Canada / Ontario
Contact: Greg Dubord, MD, CME Director, CBT Canada
Phone: 877-466-8228
Email: registrar@cbt.ca

2014 International Society for **Clinical Biostatistics** Conference
Aug 24 - 28, 2014
Austria / Vienna
Contact: Conference Organization Bureau, AIM Group International
Email: iscb2014@aimgroup.eu

7th World Congress for **Psychotherapy**
Aug 25 - 29, 2014
South Africa / Durban
Contact: Janine Koeries, Conference Secretariat, Paragon Conventions
Phone: 011-27-21-552-8679; Fax: 011-27-21-552-1218
Email: jkoeries@paragon-conventions.com

Pulmonary Medicine for the Primary Care Provider Adriatic Cruise
Aug 25 - September 5, 2014
Italy / Rome
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711 or 727-526-1571; Fax: 727-522-8304
Email: contactus@continuingeducation.net

19th World Congress of the International Federation for the Surgery of **Obesity & Metabolic Disorders**
Aug 26 - 30, 2014
Canada / Quebec
Contact: Manuela Mazzarella, IFSO Executive Secretariat, International Federation for the Surgery of Obesity
Phone: 011-39-081-761-1085; Fax: 011-39-081-664-372
Email: secretariat@ifso.com

21st Budapest **Nephrology** School
Aug 26 - 31, 2014
Hungary / Budapest
Contact: Prof. Dr. Laszlo Rosivall, Local Coordinator, Department of Pathophysiology, Semmelweis University
Phone: 011-36-1-210-0100
Email: rosivall.laszlo@med.semmelweis-univ.hu

6th Kuala Lumpur International Conference on **Biomedical Engineering**
Aug 27 - 29, 2014
Malaysia / Kuala Lumpur
Contact: Dr. Nahrizul Adib Kadri
Phone: 011-60-3-7967-7661
Email: biomed2014@um.edu.my

Fundamentals of **Spine Surgery & Interventional Pain** Management
Aug 29 - 30, 2014
United States / Illinois
Contact: North American Spine Society Headquarters
Phone: 630-230-3600; Fax: 630-230-3700

74th World Congress of **Pharmacy & Pharmaceutical** Sciences of International Pharmaceutical Federation (FIP)
Aug 30 - Sep 4, 2014
Thailand / Bangkok
Contact: FIP Congresses & Conferences
Phone: 011-31-70-302-1982; Fax: 011-31-70-302-1998
Email: congress@fip.org

5th International Course in **Nutritional Epidemiology**
Sep 1 - 12, 2014
United Kingdom / London
Contact: Nikki Whitelock, Faculty of Medicine, Imperial College London
Phone: 011-44-20-7594-2116
Email: nutrition-epi-course@imperial.ac.uk

40th Anniversary Conference of International Society for **Pediatric & Adolescent Diabetes** (ISPAD)
Sep 3 - 6, 2014
Canada / Ontario
Contact: ISPAD Executive Office, Secretariat, K.I.T. Group GmbH
Phone: 011-49-30-2460-3210; Fax: 011-49-30-2460-3200
Email: secretariat@ispad.org

World Congress on **Cancer of the Skin**
Sep 3 - 6, 2014
United Kingdom / Edinburgh
Contact: Danielle, Conference and Event Services, British Association of Dermatologists
Phone: 011-44-20-7391-6343
Email: danielle@bad.org.uk

16th International Conference on **Chronic Myeloid Leukemia:** Biology & Therapy
Sep 4 - 7, 2014
United States / Pennsylvania
Contact: Nicolas Jaillard, Conference Coordinator, European School of Haematology
Phone: 011-33-1-5727-6833; Fax: 011-33-1-5727-6838
Email: nicolas.jaillard@univ-paris-diderot.fr

22nd International **Pigment Cell** Conference (IPCC)
Sep 4 - 7, 2014
Singapore / Singapore
Contact: IPCC 2014 Conference Organiser
Phone: 011-65-6411-6686
Email: info@ipcc2014.org

9th International Symposium on **Knee & Shoulder Arthroscopy & Arthroplasty** (ISKSAA)
Sep 4 - 7, 2014
India / New Delhi
Contact: Dr Pushpinder Bajaj, President, ISKSAA
Phone: 011-91-98-11-056525; Fax: 011-91-98-11-056525
Email: psbajaj@hotmail.com

Advanced MR Imaging in **Paediatric Radiology**
Sep 4 - 6, 2014
Greece / Ioannina
Contact: Ms. Elena Skocek, Coordinator of Educational Activities, European Society for Magnetic Resonance in Medicine and Biology
Phone: 011-43-1-535-1306; Fax: 011-43-1-535-7041
Email: eskocek@esmrmb.org

World Congress on **Neurotherapeutics**: Dilemmas, Debates & Discussions (Dddn)
Sep 4 - 7, 2014
Switzerland / Basel
Contact: Ruthi Yahav, Secretariat, Congressmed
Phone: 011-972-73-706-6950; Fax: 011-972-73-706-6959
Email: Dddn@Congressmed.Com

8th Annual Conference of International **Liver Cancer Association** (Ilca)
Sep 5 - 7 , 2014
Japan / Kyoto
Contact: Ilca
Phone: 011-32-2-789-2345; Fax: 011-32-2-743-1550
Email: Info@Ilca-online.org

15th Congress of the International Society for **Peritoneal Dialysis**
Sep 7 - 10, 2014
Spain / Madrid
Contact: Congress Organizer, Tilesa Kenes Spain, Sl
Phone: 011-34-91-361-2600
Email: ispd2014@kenes.Com

2nd International Conference on **Radiology & Imaging**
Sep 8 - 9, 2014
United States / North Carolina / Raleigh
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: radiology2014@omicsonline.net

Glaucoma
Sep 8 - 12, 2014
Switzerland / Lugano
Contact: European School for Advanced Studies in Ophthalmology
Phone: 011-41-91-921-1154

20th International Congress on **Palliative Care**
Sep 9 - 12, 2014
Canada / Quebec / Montreal
Contact: Congress Secretariat, O'donoughue & Associates Event Management
Phone: 450-292-3456 Ext. 227; Fax: 450-292-3453
Email: secretariat@pal2014.com

14th **Euretina** Congress
Sep 11 - 14, 2014
United Kingdom / London
Contact: Euretina
Phone: 011-353-1-210-0092; Fax: 011-353-1-209-1112
Email: euretina@euretina.org

3rd World Congress on **Controversies in Hematology**
Sep 11 - 13, 2014
Turkey / Istanbul
Contact: Congress Secretariat, Comtecmed
Phone: 011-972-3-566-6166; Fax: 011-972-3-566-6177
Email: cohem@comtecmed.com

2014 International **Breast Ultrasound** Seminar (Ibus) Poland
Sep 12 - 13, 2014
Poland / Warsaw
Contact: Ibus Secretariat
Email: info@ibus.org

8th Reena: Clinical Cases & **Renal Histopathology**
Sep 12, 2014
Ukraine / Kiev
Contact: Prof Dmytro Ivanov, Local Coordinator, National Medical Academy
Email: ivanovdd@i.kiev.ua

Medical CBT: Ten-Minute **Cognitive Behaviour Therapy** Techniques for Real Doctors
Sep 12 - 13, 2014
Canada / British Columbia
Contact: Greg Dubord, Md, Cme Director, Cbt Canada
Phone: 877-466-8228
Email: registrar@cbt.ca

Paediatrics & Child Health in Vietnam
Sep 13 - 24, 2014
Vietnam / Hanoi
Contact: Jon Baines Tours
Phone: 011-44-20-7223-5618; Fax: 011-44-20-7228-7290
Email: info@jonbainestours.co.uk

16th World Congress of **Psychiatry:** Focusing on Quality, Access & Humane Care
Sep 14 - 18, 2014
Spain / Madrid
Contact: Ms. Carolina G. Sicilia, Secretariat, Tilesa Opc
Phone: 011-34-91-361-2600
Email: secretariat@wpamadrid2014.com

24th World Congress on **Ultrasound** in Obstetrics and Gynecology
Sep 14 - 17, 2014
Spain / Barcelona
Contact: International Society of Ultrasound In Obstetrics And Gynecology
Phone: 011-44-20-7471-9955; Fax: 011-44-20-7471-9959
Email: info@isuog.org

29th International Association for **Breast Cancer** Research, National Breast Cancer Foundation Conference
Sep 14 - 17, 2014
Australia / Sydney
Contact: Asn Events Pty Ltd
Phone: 011-61-3-5983-2400
Email: participation@asnevents.net.au

Medical Ethics
Sep 15 - 19, 2014
United Kingdom / London
Contact: Centre for Continuing Professional Development, Imperial College London
Phone: 011-44-20-7594-6882
Email: cpd@Imperial.ac.uk

6th World Congress on **Mental Health & Deafness**
Sep 16 - 19, 2014
United Kingdom / Belfast
Contact: Renata Sarmento, Manager, Esmhd
Phone: 011-34-6-4757-3679
Email: info@wcmhd2014.org

Abdomen and Urogenital MRI
Sep 17 - 19, 2014
Turkey / Ankara
Contact: Walter Rijsselaere, Erasmus Course On Magnetic Resonance Imaging
Phone: 011-32-2-477-5322; Fax: 011-32-2-477-5362
Email: Walter.rijsselaere@uzbrussel.be

Cancer Antibodies Vaccines/Adjuvants & Delivery
Sep 17 - 19, 2014
Switzerland / Lausanne Immunology/Allergy, Oncology
Contact: Meetings Management
Phone: 011-44-14-8342-7770; Fax: 011-44-14-8342-8516

19th International Symposium on **Endoscopic Ultrasonography**
Sep 18 - 20, 2014
India / Chennai
Contact: Shelja Sethi, Apm, Kenes India
Phone: 011-91-11-4519-9100; Fax: 011-91-11-2551-3052
Email: info@eus2014.org

1st Euro-Asian **Melanoma** Congress
Sep 18 - 21, 2014
Bosnia-Herzegovina / Sarajevo
Contact: Hana Helppikangas, Reuf Karabeg, Society For Prevention And Fight Against Skin Cancer, Melanoma In B&H
Email: dr.hana_helppikangas@yahoo.com

Rheumatology & Critical Care Mediterranean Cruise
Sep 18 – 2, 2014
Spain / Barcelona
Contact: Continuing Education, Continuing Education, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

12th Turkish Society of **Sports Traumatology, Arthroscopy & Knee Surgery** Congress
Sep 23 - 27, 2014
Turkey / Izmir
Contact: Secretariat, Ege Congress And Tourism Services
Phone: 011-90-850-333-5343; Fax: 011-90-232-464-4105
Email: tusyad2014@egekongre.com

22nd International Conference on **Alzheimer's Disease & Dementia**
Sep 23 - 25, 2014
Spain / Valencia
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: dementia2014@omicsonline.net

3rd International Conference & Exhibition on **Nutrition & Food Sciences**
Sep 23 - 25, 2014
Spain / Valencia
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: contact@nutritionalconference.com

2014 **Tissue Engineering** International Regenerative Medicine Society (TERMIS) Asia Pacific
Sep 24 - 27, 2014
South Korea / Daegu
Contact: Termis-Ap 2014 Secretariat
Phone: 011-82-53-746-9969; Fax: 011-82-53-742-9007
Email: info@termis-ap2014.org

3rd International Conference on **Clinical Microbiology & Microbial Genomics**
Sep 24 - 26, 2014
Spain / Valencia
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: clinicalmicrobiology2014@omicsonline.net

3rd International Conference on **Tissue Science & Regenerative Medicine**
Sep 24 - 26, 2014
Spain / Valencia
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: tissuescience2014@omicsonline.net

4th International Conference on **Vaccines & Vaccination**
Sep 24 - 26, 2014
Spain / Valencia
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: vaccines2014@omicsonline.net

International Conference of **Repair, Regeneration and Reconstruction**
Sep 25 - 27, 2014
United Kingdom / London
Contact: Sammy Al-Benna, Institute Of Surgery and Innovation
Phone: 011-44-87-1288-5135; Fax: 011-44-87-1288-5135
Email: info@Instituteofsurgery.org

2nd International Conference on **Hematology & Blood Disorders**
Sep 29 - Oct 1, 2014
United States / Maryland
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: hematology2014@omicsonline.net

Musculoskeletal MRI Ii
Sep 29 - Oct 3, 2014
Portugal / Porto
Contact: Walter Rijsselaere, Erasmus Course on Magnetic Resonance Imaging
Phone: 011-32-2-477-5322; Fax: 011-32-2-477-5362
Email: walter.rijsselaere@uzbrussel.be

2014 European Association for **Vision and Eye Research** (EVER) Congress
Oct 1 - 4, 2014
France / Nice
Contact: EVER
Phone: 011-32-16-233-849; Fax: 011-32-16-234-097
Email: ever@ever.be

Core Skills in **Laparoscopic Surgery**
Oct 1 - 3, 2014
United Kingdom / Colchester
Contact: Colchester General Hospital
Phone: 011-44-12-4568-6791
Email: daisy.martlew@anglia.ac.uk

Emergency Skills in **Oral & Maxillofacial Surgery**
Oct 1 – 2, 2014
United Kingdom / London
Contact: Education, Royal College of Surgeons of England
Phone: 011-44-20-7869-6300
Email: education@rcseng.ac.uk

10th Congress of the Asia Pacific Federation of Societies for **Surgery of the Hand**
Oct 2 - 4, 2014
Malaysia / Kuala Lumpur
Contact: Marcus, Console Communications
Email: apfssh2014@console.com.my

2014 World Congress of International Society of **Addiction Medicine** (ISAM)
Oct 2 - 6, 2014
Japan / Yokohama
Contact: Marilyn Dorozio, Office Administration, ISAM
Phone: 403-813-7217
Email: ISAM.mdorozio@gmail.com

Anesthesia Spectrum
Musculoskeletal Navigator Canary Islands Cme Cruise
Oct 2 - 13, 2014
United Kingdom / Southampton
Contact: Dr. Martin Gerretsen, Director of CME, Sea Courses Cruises
Phone: 888-647-7327; Fax: 888-547-7337
Email: cruises@seacourses.com

Purulent Bone Surgery
Oct 2 - 3, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

Society for **Cardiovascular Angiography & Interventions** (SCAI)-Fortis Fellows Course
Oct 2 - 4, 2014
India / New Delhi
Contact: SCAI
Phone: 202-741-9854; Fax: 800-863-5202
Email: info@scai.org

7th Annual **Breast Cancer** Meeting: Hot Topics in Breast Cancer
Oct 3, 2014
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden
Phone: 011-44-20-7808-2921
Email: conferencecentre@rmh.nhs.uk

Neurology
Oct 3, 2014
United Kingdom / Edinburgh
Contact: Christine Berwick, Education Co-ordinator, Royal College of Physicians of Edinburgh
Phone: 011-44-13-1247-3634; Fax: 011-44-13-1220-4393
Email: c.berwick@rcpe.ac.uk

Eso-Esso Masterclass in **Breast Cancer Surgery**
Oct 4 - 7, 2014
Switzerland / Ermatingen (Lake Constance)
Contact: European School of Oncology
Phone: 011-39-2-854-6451
Fax: 011-39-2-8546-4545
Email: eso@eso.net

Oral Dermatology and Oral Pathology Canada and New England Cruise
Oct 4 - 11, 2014
Canada /Quebec / Montreal
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711 or 727-526-1571; Fax: 727-522-8304
Email: contactus@continuingeducation.net

13th World Congress of the **Human Proteome**
Oct 5 - 8, 2014
Spain / Madrid
Contact: Scientific Secretariat, Tilesa Kenes Spain
Phone: 011-34-91-361-2600
Email: hupo2014@kenes.com

Short Course on **Systems Genetics**
Oct 5 - 11, 2014
Germany /Maine / Bar Harbor
Contact: The Jackson Laboratory
Phone: 207-288-6000
Email: coursesandconferences@jax.org

15th International Association for the Study of **Pain (LASP)** World Congress On Pain
Oct 6 - 11, 2014
Argentina / Buenos Aires
Contact: IASP Secretariat
Phone: 202-524-5300; Fax: 202-524-5301
Email: IASPdesk@iasp-pain.org

4th World Congress on **Virology**
Oct 6 - 8, 2014
United States / Texas / San Antonio
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: virology2014@omicsonline.net

9th International Conference of **Anticancer Research**
Oct 6 - 10, 2014
Greece / Sithonia
Contact: John G. Delinasios, Dr., International Institute of Anticancer Research
Phone: 011-30-22950-53389; Fax: 011-30-22950-52945
Email: conference@iiar-anticancer.org

Central Nervous System **MRI LI**
Oct 6 - 10, 2014
Latvia / Riga
Contact: Walter Rijsselaere, Erasmus Course on Magnetic Resonance Imaging
Phone: 011-32-2-477-5322; Fax: 011-32-2-477-5362
Email: walter.rijsselaere@uzbrussel.be

2nd Annual Single Cell **Genomics & Transcriptomics** Asia Congress
Oct 7 - 8, 2014
Singapore / Singapore
Contact: Freda Shi, Marketing Exec, 2014 Oxford Global Asia Conferences
Phone: 011-65-6-570-2208
Email: f.shi@oxfordglobalasia.com

4th Annual Next **Generation Sequencing** Asia Congress
Oct 7 - 8, 2014
Singapore / Singapore
Contact: Freda Shi, Marketing Exec, Oxford Global Asia Conferences
Phone: 011-65-6570-2208
Email: f.shi@oxfordglobalasia.com

2014 **Health & Wellbeing in Children**, Youth & Adults with Developmental Disabilities
Oct 8 - 10, 2014
Canada / British Columbia / Vancouver
Contact: Interprofessional Continuing Professional Education, University of British Columbia
Phone: 604-827-3112; Fax: 604-822-4835
Email: katia.ipce@ubc.ca

Family Medicine Mediterranean Cruise
Oct 8 - 19, 2014
Italy / Rome
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711 or 727-526-1571; Fax: 727-522-8304
Email: contactus@continuingeducation.net

Infectious Diseases
Oct 8 - 9, 2014
Ukraine
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

2014 **Diabetic Limb Salvage** (DLS) Conference
Oct 9 - 11, 2014
United States / District of Columbia / Washington
Contact: DLS
Phone: 337-235-6606; Fax: 337-235-7300
Email: info@dlsconference.com

42nd Society for Neuroscience in **Anesthesiology & Critical Care** (SNACC) Annual Meeting
Oct 9 - 10, 2014
United States / Louisiana / New Orleans
Contact: SNACC
Phone: 804-565-6360; Fax: 804-282-0090
Email: snacc@snacc.org

Current Practice of **Vascular Ultrasound**
Oct 10 - 12, 2014
United States / District of Columbia / Washington
Contact: Institute for Advanced Medical Education
Phone: 802-824-4433

Laparoscopic TME Cadaveric Course
Oct 10, 2014
United Kingdom / Newcastle Upon Tyne
Contact: Ethicon Professional Education Department
Email: profed@its.jnj.com

Royal Marsden **Bladder & Testicular Cancer** Conference
Oct 10, 2014
United Kingdom / London
Contact: Education and Conference Centre, the Royal Marsden
Phone: 011-44-20-7808-2921
Email: conferencecentre@rmh.nhs.uk

Novel Strategies in the Treatment of CKD Complications – CKD-MBD, PEW, **Renal Anaemia, Arterial Hypertension**
Oct 11, 2014
Bulgaria / Hissarya
Contact: Emil Paskalev, M.D. D.sc., Local Coordinator, University hospital "Alexandrovska"
Phone: 011-35-92-923-0539
Email: emilpaskalev@abv.bg

2014 Hands-On **Cardiac Ultrasound Imaging & Doppler**
Oct 13 - 17, 2014
United States / Texas / Dallas
Contact: Amy Donaldson, Registrar, Keith Mauney & Associates Ultrasound Training Institutes
Phone: 972-353-3200 (USA, CDT, UTC-6), 800-845-3484 (North America, Caribbean);
Fax: 817-577-4250
Email: info@kmaultrasound.com

Medical and Surgical **Retina**
Oct 13 - 17, 2014
Turkey / Ankara
Contact: European School for Advanced Studies in Ophthalmology
Phone: 011-41-91-921-1154

Management of the **Term Breech**
Oct 14, 2014
United Kingdom / London
Contact: Barbara Mettle-Olympio, Royal College of Obstetricians and Gynaecologists
Phone: 011-44-20-7772-6279
Email: bmettle-olympio@rcog.org.uk

Operative Skills in **Orthopaedic Surgery**
Oct 14 - 16, 2014
United Kingdom / Liverpool
Contact: Royal Liverpool University Hospital
Phone: 011-44-15-1706-3580
Email: rlb-tr.MASTERUnit@nhs.net

7th International Congress of the **Growth Hormone** Research Society & International Society for Insulin-like Growth Factors Research
Oct 15 - 18, 2014
Singapore / Singapore
Contact: GRS-IGF 2014 Secretariat, The Meeting Lab Pte Ltd
Phone: 011-65-6346-4402; Fax: 011-65-6346-4403
Email: secretariat@grs-igf2014.org

8th International Symposium on Objective Measures in **Auditory Implants**
Oct 15 - 18, 2014
Canada / Ontario / Toronto
Contact: Continuing Professional Development, University of Toronto
Phone: 888-512-8173 or 416-978-2719
Email: info-ENT1409@cepdtoronto.ca

Anaesthesia for Major Surgery
Oct 16 - 17, 2014
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden
Phone: 011-44-20-7808-2921
Email: conferencecentre@rmh.nhs.uk

Pan GHQ Medical Conference
Oct 16 - 18, 2014
United Arab Emirates / Abu Dhabi
Contact: DiaEdu Management Consultancy, DiaEdu Management Consultancy
Phone: 011-971-50-929-9239
Email: bkadara@diaedu.com

2014 Practical **Dermatology**
Oct 17 - 18, 2014
United States / Minnesota / Duluth
Contact: Jolene Bell Makowesky, Education Coordinator, Office of Continuing Medical Education, University of Minnesota
Phone: 612-626-1712; Fax: 612-626-7766
Email: jolenem@umn.edu

International School of **Thyroid Ultrasonography**
Oct 17 - 18, 2014
Italy / Pisa
Contact: Denise Rizzitelli, Congress Coordinator, Meridiano Congress International
Phone: 011-39-6-8859-5210; Fax: 011-39-6-8859-5234
Email: d.rizzitelli@meridiano.it

New Trend in Management of **Genitourinary Malignancy**
Oct 17 - 18, 2014
Romania / Brasov
Contact: American Society of Clinical Oncology
Phone: 571-483-1300
Email: meetings@asco.org

Family Medicine: Palliative Care Tahiti and Society Islands Cruise
Oct 18 - 25, 2014
Tahiti / Papeete
Contact: Continuing Education, Inc., Meeting Planner, Continuing Education, Inc.
Phone: 800-422-0711 or 727-526-1571; Fax: 727-522-8304
Email: contactus@continuingeducation.net

2014 **Endocrinology**
Oct 20 – 22, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NB Science
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

3rd International Summit on **Toxicology & Applied Pharmacology**
Oct 20 – 22, 2014
United States / Illinois / Chicago
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: toxicology2014@omicsonline.net

4th World Congress on **Cancer Science & Therapy**
Oct 20 – 22,
United States / Illinois
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: cancerscience2014@omicsonline.net

Basic Practical Skills in **Obstetrics & Gynaecology**
Oct 20 – 21, 2014
United Kingdom / London
Contact: Royal College of Obstetricians and Gynaecologists
Phone: 011-44-20-7772-6245
Email: events@rcog.org.uk

Up-To-Date Management of **Venous Thromboembolism**
Oct. 21, 2014
United Kingdom / London
Contact: Conferences Team, Royal College of Physicians of London
Phone: 011-44-20-3075-2389
Email: conferences@rcplondon.ac.uk

30th Annual Fall Conference on **Pediatric Emergencies**
Oct 22 - 25, 2014
United States / Hawaii / Big Island
Contact: Symposia Medicus
Phone: 800-327-3161 or 925-969-1789; Fax: 925-969-1795

46th Congress of the International Society of **Paediatric Oncology**
Oct 22 - 25, 2014
Canada / Ontario / Toronto
Contact: Linda Friedman, APM, Kenes International
Phone: 011-41-22-908-0488; Fax: 011-41-22-906-9140
Email: siop@kenes.com

9th World **Stroke** Congress
Oct 22 - 25, 2014
Turkey / Istanbul
Contact: Vanessa Fisher, APM, Kenes International
Phone: 011-41-22-908-0488; Fax: 011-41-22-906-9140
Email: stroke@kenes.com

2014 International Meeting of **International Psychogeriatric Association** (IPA)
Oct 23 - 26, 2014
China / Beijing
Contact: IPA
Phone: 847-501-3310; Fax: 847-501-3317
Email: membership@ipa-online.org

Advanced **MR Imaging** of the Musculoskeletal System
Oct 23 - 25, 2014
Uzbekistan / Tashkent
Contact: Ms. Elena Skocek, Coordinator of Educational Activities, European Society for Magnetic Resonance in Medicine and Biology
Phone: 011-43-1-535-1306; Fax: 011-43-1-535-7041
Email: eskocek@esmrmb.org

Head & Neck IM/IGRT Education Course
Oct 23 - 25, 2014
Canada / Ontario / Toronto
Contact: Accelerated Education Program, Radiation Medicine Program, Princess Margaret Hospital
Email: aep@rmp.uhn.on.ca

Pulmonary Rehabilitation
Oct 23 - 25, 2014
Netherlands / Horn
Contact: European Respiratory Society
Fax: 011-41-21-213-0100
Email: school@ersnet.org

2014 International **Kidney Cancer** Symposium
Oct 24 - 25, 2014
United States / Illinois / Chicago
Contact: NIU Outreach
Fax: 815-753-6900
Email: outreachregistration@niu.edu

2014 World Congress of **Internal Medicine** (WCIM 2014)
Oct 24 - 28, 2014
South Korea / Seoul
Contact: Secretariat, WCIM 2014
Phone: 011-82-2-566-2229; Fax: 011-82-2-6254-8049
Email: wcim2014@intercom.co.kr

30th International Conference on **Pharmacoepidemiology & Therapeutic** Risk Management
Oct 24 – 27, 2014
Taiwan / Taipei
Contact: International Society for Pharmacoepidemiology
Phone: 301-718-6500; Fax: 301-656-0989
Email: ISPE@paimgmt.com

2014 Masters Experience **Foot/Ankle**
Oct 25 - 26, 2014
United States / Illinois / Rosemont
Contact: Arthroscopy Association of North America
Phone: 847-292-2262; Fax: 847-292-2268
Email: info@aana.org

International **Resident Leadership** Summit
Oct 25 - 26, 2014
Canada / Ontario / Toronto
Contact: Royal College of Physicians & Surgeons of Canada Services Centre
Phone: 800-461-9598 and/or 613-730-6243
Fax: 613-730-2410
Email: cpd@royalcollege.ca

2nd International Conference on **HIV/AIDS,** Stds, & Stis
Oct 27 - 29, 2014
United States / Nevada / Las Vegas
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: std-aids2014@omicsonline.net

3rd International Conference & Exhibition on **Cell & Gene Therapy**
Oct 27 - 29, 2014
United States / Nevada / Las Vegas
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: celltherapy2014@omicsonline.net

Advanced Cadaveric Trauma Emergency Surgery Course
Oct 27 - 28, 2014
United Kingdom / Newcastle
Contact: Lorraine Waugh, Newcastle Surgical Training Centre
Phone: 011-44-19-1223-1264; Fax: 011-44-19-1223-7248
Email: Lorraine.waugh@nuth.nhs.uk

7th Asia-Pacific **Heart Rhythm Scientific** Session (Aphrs)
Oct 29 - Nov. 1, 2014
India / New Delhi
Contact: Secretariat, APHRS
Email: secretariat@aphrsindia.com

Diabetes and Endocrinology
Oct 29, 2014
United Kingdom / Edinburgh
Contact: Felicity Garvie, Education Co-ordinator, Royal College of Physicians of Edinburgh
Phone: 011-44-13-1247-3607; Fax: 011-44-13-1220-4393
Email: f.garvie@rcpe.ac.uk

Preceptorship on MRI in **Multiple Sclerosis**
Oct 30 – 3, 2014
Italy / Milan
Contact: David H. Slangen, Congress Coordinator, Meridiano Congress International
Phone: 011-39-6-8859-5211; Fax: 011-39-6-8859-5234
Email: d.slangen@meridiano.it

World Congress on **Controversies in Thrombosis and Hemostasis**
Oct 30 - Nov. 2, 2014
Germany / Berlin
Contact: Secretariat, CongressMed
Phone: 011-972-73-706-6950; Fax: 011-972-73-706-6959
Email: cith@congressmed.com

6th International Conference on the **Epididymis**
Oct 31 - Nov. 3, 2014
China / Shanghai
Contact: Shanghai Institute of Biochemistry & Cell Biology
Phone: 011-86-21-5492-0000; Fax: 011-86-21-5492-1011
Email: sibcb@sibs.ac.cn

Chronic Kidney Disease
Oct 31 - Nov. 1, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

Introductory Course on **Epidemiology**
Oct 31 - Nov. 1, 2014
Ukraine / Kiev
Contact: Natalia Stepanova, Local Coordinator
Phone: 011-38-44-455-9377; Fax: 011-38-44-455-9387
Email: palalo@yandex.ru

2014 **HIV** Glasgow
Nov 2 - 6, 2014
United Kingdom / Glasgow
Contact: Georgina Palmer, Congress Assistant, KP360 Group
Phone: 011-44-16-2566-4127
Email: hivglasgow@kp360group.com

3 Day Course on **Obstetric Anaesthesia & Analgesia**
Nov 3 - 5, 2014
United Kingdom / London
Contact: Obstetric Anaesthetists' Association
Phone: 011-44-20-7631-8883
Fax: 011-44-20-7631-4352

3rd International Conference on **Translational Medicine**
Nov 3 - 5, 2014
United States / Nevada / Las Vegas
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: translationalmedicine2014@conferenceseries.net

5th World Congress on **Diabetes & Metabolism**
Nov 3 - 5, 2014
United States / Nevada / Las Vegas
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: diabetes2014@omicsonline.net

Medicine and Society in Ethiopia
Nov 3 - 16, 2014
Ethiopia / Addis Ababa
Contact: Jon Baines Tours
Phone: 011-44-20-7223-5618; Fax: 011-44-20-7228-7290
Email: info@jonbainestours.co.uk

Writing a **Journal Article**
Nov 3, 2014
United Kingdom / Edinburgh
Contact: Royal College of Physicians of Edinburgh
Phone: 011-44-13-1225-7324

Obstetrics & Gynaecology in South Africa
Nov 4 - 15, 2014
South Africa / Johannesburg
Contact: Jon Baines Tours
Phone: 011-44-20-7223-5618; Fax: 011-44-20-7228-7290
Email: info@jonbainestours.co.uk

Gynaecological Cancers
Nov 5, 2014
United Kingdom / London
Contact: Education and Conference Centre, The Royal Marsden
Phone: 011-44-20-7808-2921
Email: conferencecentre@rmh.nhs.uk

Cardiology
Nov 6, 2014
United Kingdom / Edinburgh
Contact: Eileen Strawn, Symposium Co-ordinator, Royal College of Physicians of Edinburgh
Phone: 011-44-13-1247-3619; Fax: 011-44-13-1220-4393
Email: e.strawn@rcpe.ac.uk

2014 **Infectious Diseases** Update
Nov 7 - 8, 2014
Canada / British Columbia / Victoria
Contact: Nova Clinical Services
Phone: 250-658-6056; Fax: 250-658-6109
Email: info@novaclinical.com

2014 **Transplantology**
Nov 7, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

7th **Medication Safety** Conference
Nov 7 - 9, 2014
United Arab Emirates / Abu Dhabi
Contact: Synovetics
Phone: 011-971-2-443-4331; Fax: 011-971-2-491-8626
Email: info@synovetics.com

European School of Haematology (ESH) International Conference on Multiple Myeloma
Nov 7 - 9, 2014
Portugal / Madeira
Contact: Nicolas Jaillard, Conference Coordinator, ESH
Phone: 011-33-1-5727-6833; Fax: 011-33-1-5727-6838
Email: nicolas.jaillard@univ-paris-diderot.fr

12th International Congress of **Neuroimmunology**
Nov 9 - 13, 2014
Germany / Mainz
Contact: ISNI Secretariat, International Society of Neuroimmunology
Phone: 011-39-6-519-3499; Fax: 011-39-6-519-4009
Email: secretariat@isniweb.org

22nd Annual Scientific Meeting of **International Society of Hair Restoration Surgery** (ISHRS)
Nov 12 - 16, 2014
Thailand / Bangkok
Contact: ISHRS
Phone: 630-262-5399; Fax: 630-262-1520
Email: info@ishrs.org

2014 Meeting of International Federation for **Adipose Therapeutics & Science** (IFATS)
Nov 13 - 16, 2014
Netherlands / Amsterdam
Contact: IFATS
Phone: 603-643-2325; Fax: 603-643-1444

5th Bit World **Gene Convention**
Nov 13 - 16, 2014
China / Hai Kou
Contact: Teresa Xiao
Phone: 011-86-411-8457-5669 ext. 872
Email: teresa@gene-congress.com

Advanced **Head & Neck MR** Imaging
Nov 13 - 15, 2014
Croatia / Zagreb
Contact: Ms. Elena Skocek, Coordinator of Educational Activities, European Society for Magnetic Resonance in Medicine and Biology
Phone: 011-43-1-535-1306; Fax: 011-43-1-535-7041
Email: eskocek@esmrmb.org

Gastroenterology
Nov 13, 2014
United Kingdom / Edinburgh
Contact: Felicity Garvie, Education Co-ordinator, Royal College of Physicians of Edinburgh
Phone: 011-44-13-1247-3607; Fax: 011-44-13-1220-4393
Email: f.garvie@rcpe.ac.uk

Emirates Oncology Conference
Nov 14 - 16, 2014
*United Arab Emirate*s / Abu Dhabi
Contact: American Society of Clinical Oncology
Phone: 571-483-1300
Email: meetings@asco.org

2014 Hands-On **Carotid & Vertebral Duplex Imaging & Doppler**
Nov 15 - 16, 2014
United States / Texas / Dallas
Contact: Amy Donaldson, Registrar, Keith Mauney & Associates Ultrasound Training Institutes Est. 1981
Phone: 972-353-3200 (USA, CDT, UTC-6), 800-845-3484 (North America, Caribbean)
Fax: 817-577-4250
Email: info@kmaultrasound.com

Ophthalmic Block Hands-On Workshop
Nov 15 - 16, 2014
United States / Florida / Orlando
Contact: Northwest Anesthesia Seminars
Phone: 800-222-6927; Fax: 509-547-1265

Twins 2014: Joint 3rd World Congress on **Twin Pregnancy** & 15th International Congress of International Society of Twin Studies
Nov 16 - 19, 2014
Hungary / Budapest
Contact: MCA Scientific Events
Phone: 011-39-2-3493-4404; Fax: 011-39-2-3493-4397
Email: info@twin2014.eu

2nd International Congress on **Bacteriology & Infectious** Diseases
Nov 17 - 19, 2014
United States / Illinois / Chicago
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: bacteriology2014@omicsgroup.us

3rd International Conference on **Surgery & Anesthesia**
Nov 17 - 19, 2014
United States / Illinois / Chicago
Contact: Conference Secretariat, Omics Publishing Group
Phone: 650-268-9744; Fax: 650-618-1414
Email: surgery-anesthesia2014@omicsonline.net

Virtual Colonography Course
Nov 17 - 19, 2014
United States / Ontario / Toronto
Contact: Theresa A. Findlay, Hons B.A., M.Ed., Office of Continuing Education & Professional Development, University of Toronto
Phone: 416-340-4800 ext. 5108
Email: Theresa.Findlay@uhn.ca

Molecular Pathology & Targeted Treatments for Non-Small Cell Lung Cancer
Nov 18, 2014
United Kingdom / Manchester
Contact: Education Events, The School of Oncology, The Christie NHS Foundation Trust
Phone: 011-44-16-1446-3403
Email: education.events@christie.nhs.uk

2014 Acquired **Brain Injury** Conference
Nov 20 – 21, 2014
Canada / Ontario / Toronto
Contact: Toronto ABI Network
Phone: 416-597-3057; Fax: 416-597-7021
Email: info@abinetwork.ca

2014 **Arrhythmology**
Nov 20 – 21, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

2014 Association for **Behavioral and Cognitive Therapies** (ABCT) Convention
Nov 20 - 23, 2014
United States / Pennsylvania / Philadelphia
Contact: ABCT
Phone: 212-647-1890; Fax: 212-647-1865

Baha **Hearing Implant** Course
Nov 20 – 21, 2014
United Kingdom
Contact: Carol Anne Cockayne, Cochlear
Email: CCockayne@cochlear.com

6th International **Hip Arthroscopy** Meeting
Nov 21 – 22, 2014
Germany / Munich
Contact: Juliane Fricke, Assistant Conventions, Intercongress GmbH
Phone: 011-49-761-6969-9240
Email: juliane.fricke@intercongress.de

4th World Congress of **Regional Anaesthesia and Pain Therapy**
Nov 24 - 28, 2014
South Africa / Cape Town
Contact: Robert Nesbitt, APM, Kenes International
Phone: 011-41-22-908-0488; Fax: 011-41-22-908-9140
Email: wcrapt2014@kenes.com

Basic Practical Skills in **Obstetrics & Gynaecology** - London
Nov 24 - 25, 2014
United Kingdom / London
Contact: Royal College of Obstetricians and Gynaecologists
Phone: 011-44-20-7772-6245
Email: events@rcog.org.uk

15th Asia-Pacific Congress of Clinical **Microbiology & Infection**
Nov 26 - 29, 2014
Malaysia / Kuala Lumpur
Contact: Ms. Shikha, Reliance Conventions and Events
Phone: 011-60-3-2170-2000; Fax: 011-60-3-2730-9972 / 73
Email: apccmi@relianceconventions.com

Issues in **Pediatric Gastroenterology & Nutritiology**
Nov 26 - 27, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

Tropical Medicine Excursion to Ghana
Nov 26 - December 6, 2014
Ghana / Accra
Contact: Kay Schaefer, MD, Tropical Medicine Excursions
Phone: 011-49-221-340-4905; Fax: 011-49-321-2147-5305
Email: contact@tropmedex.com

Traumatology and Orthopedics
Nov 27 - 28, 2014
Ukraine / Kiev
Contact: Kristina Zadorina, NBScience
Phone: 011-380-4-4233-2770
Email: uk@nbscience.com

Laparoscopic Incisional & Groin Hernia Training (LIGHT) Cadaver Course
Nov 28, 2014
United Kingdom / Newcastle
Contact: Ethicon Professional Education Department
Email: profed@its.jnj.com

Update on **Living Kidney Donation**
Nov 28 - 29, 2014
Germany / Munich
Contact: Prof. Dr. Dr. h.c. Uwe Heemann, Local Coordinator, Klinikum rechts der Isar
Phone: 011-49-89-4140-2231; Fax: 011-49-89-4140-7734
Email: uwe.heemann@lrz.tum.de

Diagnostic and Operative **Hysteroscopy**
Dec 2 - 4, 2014
United Kingdom / London
Contact: Sarah Monro, Royal College of Obstetricians and Gynaecologists
Phone: 011-44-20-7772-6437
Email: smonro@rcog.org.uk

2014 World **Cancer Conference**
Dec 3 - 6, 2014
Australia / Melbourne
Contact: American Society of Clinical Oncology
Phone: 571-483-1300
Email: meetings@asco.org

10th International Congress on Non-Motor Dysfunctions In **Parkinson's Disease & Related Disorders**
Dec 4 - 7, 2014
France / Nice
Contact: Ronit Eisenbach, APM, Kenes International
Phone: 011-41-22-908-0488; Fax: 011-41-22-906-9140

20th World Congress on Controversies in **Obstetrics, Gynecology & Infertility**
Dec 4 - 7, 2014
France / Paris
Contact: Secretariat, CongressMed
Phone: 011-972-73-706-6950
Email: cogi@congressmed.com

2nd Workshop of **Chronic Kidney Disease** - Mineral Bone Disorders Era-Edta Working Group
Dec 5, 2014
Italy / Milan
Contact: Mario Cozzolino, Local Coordinator
Phone: 011-39-2-8184-4381
Email: Mario.cozzolino@unimi.it

2nd World Congress on **Clinical Lipidology**
Dec 5 - 7, 2014
Austria / Vienna
Contact: Cheryl Marsh, Project Manager, Paragon Group
Phone: 011-27-21-409-7878; Fax: 011-27-21-409-7050
Email: secretariat@clinical-lipidology.com

10th Annual **Liver Transplant Symposium**
Dec 6, 2014
United States / Pennsylvania / Hershey
Contact: Continuing Education, Penn State Hershey College of Medicine
Phone: 717-531-6483; Fax: 717-531-5604
Email: ContinuingEd@hmc.psu.edu

Update in Current Management of **Sexually Transmitted Infections & HIV Infection**
Dec 8, 2014
United Kingdom / London
Contact: Conferences Team, Royal College of Physicians of London
Phone: 011-44-20-3075-2389
Email: conferences@rcplondon.ac.uk

International School of **Musculoskeletal Ultrasound**: Elbow to Fingers
Dec 9 - 11, 2014
Italy / Genoa
Contact: Denise Rizzitelli, Meridiano Congress International
Phone: 011-39-6-8859-5210; Fax: 011-39-6-8859-5234
Email: d.rizzitelli@meridiano.it

2014 Experts in **Stone Disease**
Dec 11 - 13, 2014
South Africa / Cape Town
Contact: Erasmus S.A.
Phone: 011-30-210-741-4700; Fax: 011-30-210-725-7532
Email: info@esdconference.com

Psoriasis: From Gene to Clinic
Dec 11 - 13, 2014
United Kingdom / London
Contact: Conference and Event Services, British Association of Dermatologists
Email: conference@bad.org.uk

2014 **Brain & Behavior**
Dec 12 - 13, 2014
United States / Louisiana / New Orleans
Contact: Center for Continuing Education, Tulane University Health Sciences Center
Phone: 504-988-5466; Fax: 504-988-1779
Email: cme@tulane.edu

2014 Amsterdam Live **Endoscopy**
Dec 15 - 16, 2014
Netherlands / Amsterdam
Contact: Mrs. Jacqueline van der Woude, European Postgraduate Gastro-surgical School
Phone: 011-31-20-566-3926 or 566-6468; Fax: 011-31-20-697-5594
Email: info@amsterdamendoscopy.com

Targeted Treatments for **Cancers of the Digestive System**
Dec 16, 2014
United Kingdom / Manchester
Contact: Education Events, the School of Oncology, the Christie NHS Foundation Trust
Phone: 011-44-16-1446-3403
Email: education.events@christie.nhs.uk

7th International **Hemodialysis** Course
Dec 22 - 26, 2014
Egypt / Mansoura
Contact: Hussein Sheashaa, Local Coordinator, Mansoura University, Egypt
Email: sheashaa@mans.edu.eg

6th International Course on **Ophthalmic & Oculoplastic Reconstruction & Trauma** Surgery
Jan 14 - 16, 2015
Austria / Vienna
Contact: Helmut Weissmann, MD, Advanced Ophthalmic Trainings
Phone: 011-43-22-432-0898
Fax: 011-43-22-432-0898 ext. 15
Email: office@ophthalmictrainings.com

9th Asia Pacific Conference on **Clinical Nutrition** (APCCN 2015)
Jan 26 - 29, 2015
Malaysia / Kuala Lumpur
Contact: Shu Shan, Conference Secretariat, Console Communications Sdn Bhd
Phone: 011-60-3-2162-0566; Fax: 011-60-3-2161-6560
Email: apccn2015@console.com.my

2014 **Osteoporosis** Conference
Nov 30 – Dec. 2, 2014
United Kingdom / Birmingham
Contact: The Events Team, National Osteoporosis Society
Phone: 011-44-17-6147-3281; Fax: 011-44-17-6147-9271
Email: conferences@nos.org.uk

Geriatric Medicine & Palliative Care Eastern Caribbean Cruise
Dec 6 - 13, 2014
United States / Florida / Fort Lauderdale
Contact: Continuing Education, Continuing Education, Continuing Education, Inc
Phone: 800-422-0711
Email: registrar@continuingeducation.net

Advanced Surgery **Cadaver Skills** in GI Surgery (ASICS) Course
Dec 17 - 18, 2014
United Kingdom / Glasgow
Contact: Joanne Woollard, CASC Supervisor, Royal College of Physicians and Surgeons of Glasgow
Phone: 011-44-14-1330-2063
Email: joanne.woollardED@rcpsg.ac.uk

2015 Advances in **Fetal & Neonatal** Imaging Course
Jan 23 - 25, 2015
United States / Florida / Orlando
Contact: Barbara Quattrone, Membership Services, Society for Pediatric Radiology
Phone: 703-648-0680 ext. 4907
Email: bquattrone@acr.org

WHO-Facts Sheet

1. Trachoma
2. Chikungunya
3. Leishmaniasis
4. Yaws (*Framboesia*)
5. Taeniasis/Cysticercosis
6. Human African Trypanosomiasis

Compiled and edited by
Babichan K Chandy

Kuwait Medical Journal 2014, 46 (2): 183 - 193

1. TRACHOMA

Overview

Trachoma is the leading cause of infectious blindness in the world. It is caused by an obligate intracellular micro-organism called Chlamydia trachomatis. The disease is transmitted through contact with eye and nose discharge of infected people, particularly young children who form the reservoir of infection. It is also spread by flies which have been in contact with the eyes and nose of infected people.

KEY FACTS

- Trachoma is estimated to be endemic in 53 countries and is responsible for the visual impairment of about 2.2 million people, of whom 1.2 million are irreversibly blind.
- Approximately 229 million people live in trachoma endemic areas and are at risk of infection.
- An estimated 47 million were treated with antibiotics in 2012 and 169,000 received surgical treatment.
- Infection spreads through personal contact (hands, clothing) and by flies that have been in contact with discharge from the eyes and nose of infected persons.
- With repeated episodes of infection over many years, chronic sequelae may occur, with pain and discomfort and permanently damage to the cornea of the eye, leading to irreversible blindness.

Clinical characteristics and morbidity

In areas where trachoma is endemic active trachoma is common among preschool-aged children, with prevalence rates which might be as high as 60-90%. The infection becomes less frequent and shorter in duration with increasing age. Infection is usually acquired through living in close proximity to a person with the active disease, and the family is the principal unit for transmission.

After years of repeated infection, the inside of the eyelid can become so severely scarred (conjuctival scarring) that it turns inwards and the eyelid border causes the eye-lashes to rub against the eyeball (trichiasis) resulting in severe discomfort and pain; this and other alterations of the eye can lead to the scarring of the cornea. Left untreated, this condition leads to the formation of irreversible opacities with resulting visual impairment and blindness typically between the ages 30 - 40.

Visual impairment and blindness results in a worsening of the life experience of affected individuals and their families, which are normally already among the poorest of the poor. Women are blinded 2 - 3 times more often than men, probably due to their close contact with affected children.

Environmental risk factors influencing the transmission of the disease include:

- poor hygiene;
- crowded households;
- water shortage; and
- inadequate latrines and sanitation facilities.

Distribution

Trachoma is hyperendemic in many of the poorest and most rural areas of 53 countries of Africa, Asia, Central and South America, Australia and the Middle East. It is responsible for approximately 1% of the world's blindness and for the visual impairment of about 2.2 million people, of whom 1.2 million are irreversibly blind.

Address correspondence to:
Office of the Spokesperson, WHO, Geneva. Tel.: (+41 22) 791 2599; Fax (+41 22) 791 4858; Email: inf@who.int; Web site: http://www.who.int/

Overall, Africa remains the most affected continent and the one with the most intensive control efforts. In 2012, 47 million people were treated with antibiotics and 169,000 cases of trichiasis were operated in 29 endemic countries of WHO's Africa Region.

A number of countries have reported achieving intervention goals, which signify a major milestone in the campaign to trachoma elimination and the move to post-endemic surveillance. These countries are: The Gambia, Ghana, Iran, Morocco, Myanmar, Oman and Viet Nam

Prevention and control

Control programmes in endemic countries are being implemented through the WHO recommended SAFE strategy. This consists of:

- surgery to treat the blinding stage of the disease (trachomatous trichiasis or TT);
- antibiotics to treat infection from chlamydia trachomatis;
- facial cleanliness, to educate the at risk population on the preventive measures; and
- environmental improvements, such as providing access to safe water and improved sanitation.

Most endemic countries have agreed to accelerate the implementation of this strategy to achieve their respective elimination targets, all within the year 2020. Data reported to WHO by Member States in 2012 shows that about 47 million people in endemic communities were treated with antibiotics to eliminate trachoma.

Elimination efforts need to continue to satisfy the target set by the World Health Assembly resolution (WHA 51.11), which is elimination of trachoma as a public health problem by 2020. Particularly important will be, the full engagement of other sectors involved in sanitation and socioeconomic development.

2. CHIKUNGUNYA

Overview

Chikungunya is a mosquito-borne viral disease first described during an outbreak in southern Tanzania in 1952. It is an RNA virus that belongs to the alphavirus genus of the family Togaviridae. The name 'chikungunya' derives from a word in the Kimakonde language, meaning "to become contorted" and describes the stooped appearance of sufferers with joint pain (arthralgia).

KEY FACTS

- Chikungunya is a viral disease transmitted to humans by infected mosquitoes. It causes fever and severe joint pain. Other symptoms include muscle pain, headache, nausea, fatigue and rash.
- The disease shares some clinical signs with dengue, and can be misdiagnosed in areas where dengue is common.
- There is no cure for the disease. Treatment is focused on relieving the symptoms.
- The proximity of mosquito breeding sites to human habitation is a significant risk factor for chikungunya.
- Since 2004, chikungunya fever has reached epidemic proportions, with considerable morbidity and suffering.
- The disease occurs in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of chikungunya have spread to Europe and the Americas. In 2007, disease transmission was reported for the first time in a localized outbreak in north-eastern Italy.

Signs and symptoms

Chikungunya is characterized by an abrupt onset of fever frequently accompanied by joint pain. Other common signs and symptoms include muscle pain, headache, nausea, fatigue and rash. The joint pain is often very debilitating, but usually lasts for a few days or may be prolonged to weeks.

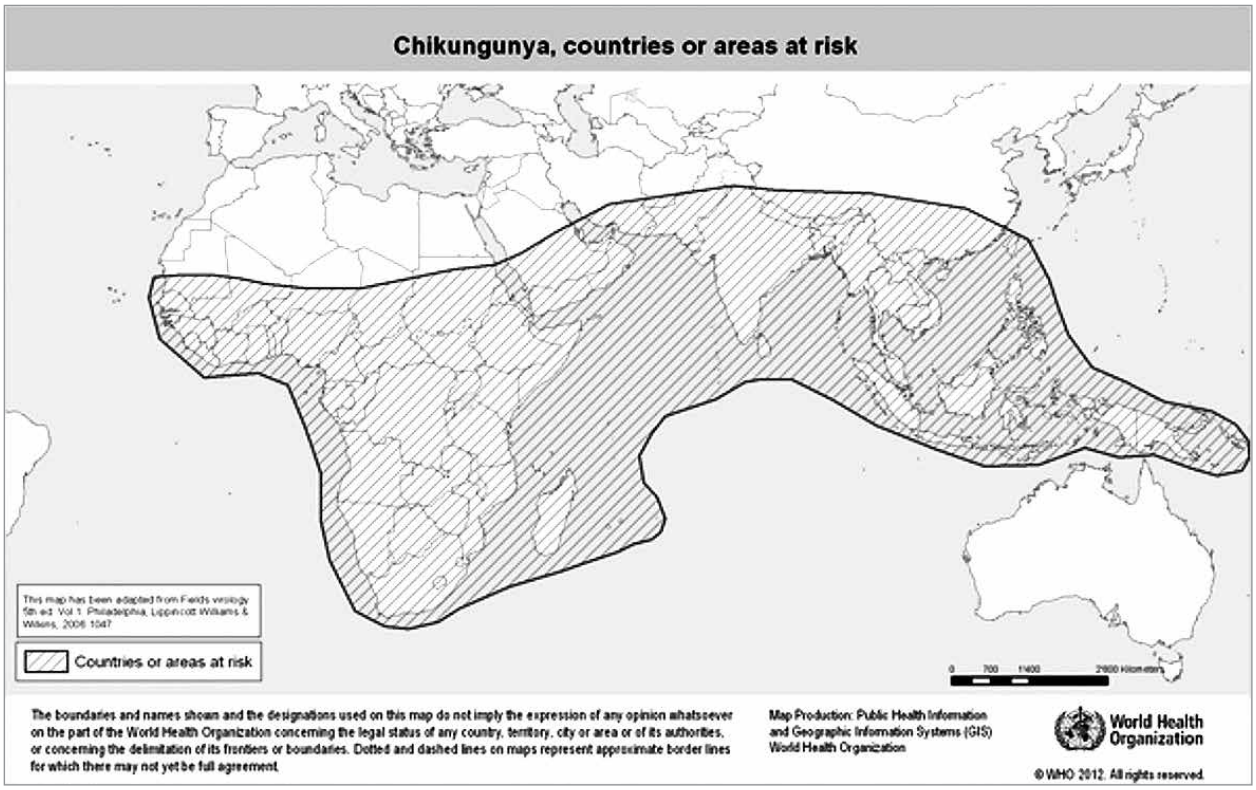
Most patients recover fully, but in some cases joint pain may persist for several months, or even years. Occasional cases of eye, neurological and heart complications have been reported, as well as gastrointestinal complaints. Serious complications are not common, but in older people, the disease can contribute to the cause of death. Often symptoms in infected individuals are mild and the infection may go unrecognized, or be misdiagnosed in areas where dengue occurs.

Transmission

Chikungunya has been identified in nearly 40 countries in Asia, Africa, Europe and also in the Americas (picture follows).

WHO

The virus is transmitted from human to human by the bites of infected female mosquitoes. Most commonly, the mosquitoes involved are *Aedes aegypti* and *Aedes albopictus*, two species which can also transmit other mosquito-borne viruses, including dengue. These mosquitoes can be found biting throughout daylight hours, though there may be peaks of activity in the early morning and late afternoon. Both species are found biting outdoors, but *Ae. aegypti* will also readily feed indoors.



After the bite of an infected mosquito, onset of illness occurs usually between four and eight days but can range from two to 12 days.

Diagnosis

Several methods can be used for diagnosis. Serological tests, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of IgM and IgG anti-chikungunya antibodies. IgM antibody levels are highest three to five weeks after the onset of illness and persist for about two months. Samples collected during the first week after the onset of symptoms should be tested by both serological and virological methods (RT-PCR).

The virus may be isolated from the blood during the first few days of infection. Various reverse transcriptase–polymerase chain reaction (RT–PCR) methods are available but are of variable sensitivity. Some are suited to clinical diagnosis. RT–PCR products from clinical samples may also be used for genotyping of the virus, allowing comparisons with virus samples from various geographical sources.

Treatment

There is no specific antiviral drug treatment for Chikungunya. Treatment is directed primarily at relieving the symptoms, including the joint pain using anti-pyretics, optimal analgesics and fluids. There is no commercial chikungunya vaccine.

Prevention and control

The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for chikungunya as well as for other diseases that these species transmit. Prevention and control relies heavily on reducing the number of natural and artificial water-filled container habitats that support breeding of the mosquitoes. This requires mobilization of affected communities. During outbreaks, insecticides may be sprayed to kill flying mosquitoes, applied to surfaces in and around containers where the mosquitoes land, and used to treat water in containers to kill the immature larvae.

For protection during outbreaks of chikungunya, clothing which minimizes skin exposure to the day-biting vectors is advised. Repellents can be applied to exposed skin or to clothing in strict accordance with product label instructions. Repellents should contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester) or icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). For those who sleep during the daytime, particularly young children, or sick or older people, insecticide treated mosquito nets afford good protection. Mosquito coils or other insecticide vaporizers may also reduce indoor biting.

Basic precautions should be taken by people traveling to risk areas and these include use of

repellents, wearing long sleeves and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering.

Disease outbreaks

Chikungunya occurs in Africa, Asia and the Indian subcontinent. Human infections in Africa have been at relatively low levels for a number of years, but in 1999 - 2000 there was a large outbreak in the Democratic Republic of the Congo, and in 2007 there was an outbreak in Gabon.

Starting in February 2005, a major outbreak of chikungunya occurred in islands of the Indian Ocean. A large number of imported cases in Europe were associated with this outbreak, mostly in 2006 when the Indian Ocean epidemic was at its peak. A large outbreak of chikungunya in India occurred in 2006 and 2007. Several other countries in South-East Asia were also affected. Since 2005, India, Indonesia, Thailand, Maldives and Myanmar have reported over 1.9 million cases. In 2007 transmission was reported for the first time in Europe, in a localized outbreak in north-eastern Italy. There were 197 cases recorded during this outbreak and it confirmed that mosquito-borne outbreaks by *Ae. Albopictus* are plausible in Europe.

In December 2013, France reported 2 laboratory-confirmed autochthonous (native) cases of chikungunya in the French part of the Caribbean island of St Martin. Since then, local transmission has been confirmed in the Dutch part of Saint Martin [St Maarten], Anguilla, British Virgin Islands, Dominica, French Guiana, Guadeloupe, Martinique and St Barthelemy. Aruba only reported imported cases.

This is the first documented outbreak of chikungunya with autochthonous transmission in the Americas. As of 6 March 2014, there have been over 8000 suspected cases in the region.

More about disease vectors

Both *Ae. aegypti* and *Ae. albopictus* have been implicated in large outbreaks of chikungunya. Whereas *Ae. aegypti* is confined within the tropics and sub-tropics, *Ae. albopictus* also occurs in temperate and even cold temperate regions. In recent decades *Ae. albopictus* has spread from Asia to become established in areas of Africa, Europe and the Americas.

The species *Ae. albopictus* thrives in a wider range of water-filled breeding sites than *Ae. aegypti*, including coconut husks, cocoa pods, bamboo stumps, tree holes and rock pools, in addition to artificial containers such as vehicle tyres and saucers beneath plant pots. This diversity of habitats explains the abundance of *Ae. albopictus* in rural as well as peri-urban areas and shady city parks.

Ae. aegypti is more closely associated with human habitation and uses indoor breeding sites, including flower vases, water storage vessels and concrete water tanks in bathrooms, as well as the same artificial outdoor habitats as *Ae. albopictus*.

In Africa several other mosquito vectors have been implicated in disease transmission, including species of the *A. furcifer-taylori* group and *A. luteocephalus*. There is evidence that some animals, including non-primates, rodents, birds and small mammals may act as reservoirs.

For more information contact:WHO Media centre,Telephone: +41 22 791 2222
E-mail: mediainquiries@who.int

3. LEISHMANIASIS

Overview

Leishmaniasis is caused by a protozoa parasite from over 20 Leishmania species and is transmitted to humans by the bite of infected female phlebotomine sandflies. There are three main forms of the disease:

1. Visceral leishmaniasis (VL also known as kala-azar) is fatal if left untreated. It is characterized by irregular bouts of fever, weight loss, enlargement of the spleen and liver, and anaemia. It is highly endemic in the Indian subcontinent and in East Africa. An estimated 200,000 to 400,000 new cases of VL occur worldwide each year. Over 90% of new cases occur in six countries: Bangladesh, Brazil, Ethiopia, India, South Sudan, and Sudan.
2. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and causes ulcers on exposed parts of the body, leaving life-long scars and serious disability. About 95% of CL cases occur in the Americas, the Mediterranean basin, and the Middle East and Central Asia. Over two-third of CL new cases occur in six countries: Afghanistan, Algeria, Brazil, Colombia, Iran (Islamic Republic of) and the Syrian Arab Republic. An estimated 0.7 million to 1.3 million new cases occur worldwide annually.
3. Mucocutaneous leishmaniasis leads to partial or total destruction of mucous membranes of the nose, mouth and throat. Almost 90% of mucocutaneous leishmaniasis cases occurs in the Plurinational State of Bolivia, Brazil and Peru.

KEY FACTS

- There are three main forms of leishmaniases – visceral (often known as kala-azar and the most serious form of the disease), cutaneous (the most common), and mucocutaneous.
- Leishmaniasis is caused by the protozoan Leishmania parasites which are transmitted by the bite of infected sandflies.

- The disease affects the poorest people on the planet, and is associated with malnutrition, population displacement, poor housing, a weak immune system and lack of resources.
- Leishmaniasis is linked to environmental changes such as deforestation, building of dams, irrigation schemes and urbanization.
- An estimated 1.3 million new cases and 20,000 to 30,000 deaths occur annually.

Transmission

Leishmaniasis is transmitted through the bites of infected female phlebotomine sandflies. The epidemiology of leishmaniasis depends on the characteristics of the parasite species, the local ecological characteristics of the transmission sites, current and past exposure of the human population to the parasite and human behaviour.

Mediterranean Basin: In the Mediterranean Basin, visceral leishmaniasis is the main form of the disease. It occurs in rural areas, in villages in mountainous regions and also in some periurban areas, where Leishmania parasites live on dogs and other animals.

South-East Asia: In South-East Asia, visceral leishmaniasis is the main form of the disease. Transmission generally occurs in rural areas below 600 m above sea level, with a heavy annual rainfall, with a mean humidity above 70%, a temperature range of 15 – 38 °C, abundant vegetation, subsoil water and alluvial soil. The disease is most common in agricultural villages where houses are frequently constructed with mud walls and earthen floors, and cattle and other livestock live close to humans.

East Africa: In East Africa, there are frequent outbreaks of visceral leishmaniasis in in the northern Acacia–Balanite savanna and the southern savanna and forest areas where sandflies live around termite mounds.

Cutaneous leishmaniasis occurs in the highlands of Ethiopia and other places in East Africa, where increased human–fly contact occurs in villages built on rock hills or river banks, which are the natural habitat of hyraxes.

Afro-Eurasia: In Afro-Eurasia, cutaneous leishmaniasis is the main form of the disease. Agricultural projects and irrigation schemes can increase the prevalence of cutaneous leishmaniasis as people who have no immunity to the disease move in to work on the projects.

Large outbreaks in densely populated cities also occur, especially during war and large-scale population migration.

The parasites causing cutaneous leishmaniasis live mainly on humans or rodents.

Americas: Kala-azar in the Americas is very similar to that found in the Mediterranean Basin. The habit of keeping dogs and other domestic animals inside the house is thought to promote human infection. The epidemiology of CL in the Americas is complex, with variations in transmission cycles, reservoir hosts, sandfly vectors, clinical manifestations and response to therapy, and multiple circulating Leishmania species in the same geographical area.

Post kala-azar dermal leishmaniasis (PKDL)

PKDL is a sequel of visceral leishmaniasis that appears as macular, papular or nodular rash usually on face, upper arms, trunks and other parts of the body. It occurs mainly in East Africa and on the Indian subcontinent, where up to 50% and 5–10% of patients with kala-azar, respectively, develop the condition. It usually appears six months to one or more years after kala-azar has apparently been cured. But it can occur earlier. People with PKDL are considered to be a potential source of kala-azar infection.

Leishmania-HIV co-infection

Leishmania-HIV coinfected people have high chance of developing the full blown clinical disease, high relapses and mortality rates. Antiretroviral treatment reduces the development of the disease, delays relapses and increases the survival of the coinfected patients.

Major risk factors

Socioeconomic conditions: Poverty increases the risk for leishmaniasis. Poor housing and domestic sanitary conditions (*e.g.*, lack of waste management, open sewerage) may increase sandfly breeding and resting sites, as well as their access to humans. Sandflies are attracted to crowded housing as these provide a good source of blood-meals. Human behaviour, such as sleeping outside or on the ground, may increase risk. The use of insecticide-treated bednets reduces risk.

Malnutrition: Diets lacking protein-energy, iron, vitamin A and zinc increase the risk that an infection will progress to kala-azar.

Population mobility: Epidemics of both main forms of leishmaniasis are often associated with migration and the movement of non-immune people into areas with existing transmission cycles. Occupational exposure as well as widespread deforestation remain important factors. For example, people settling in areas that used to be forests may be moving near sandflies' habitat. This can lead to a rapid increase in cases.

Environmental changes: Environmental changes that can affect the incidence of leishmaniasis include urbanization, domestication of the transmission cycle and the incursion of agricultural farms and settlements into forested areas.

Climate change: Leishmaniasis is climate-sensitive, and strongly affected by changes in rainfall, temperature and humidity. Global warming and land degradation together affect the epidemiology of leishmaniasis in a number of ways:

- changes in temperature, rainfall and humidity can have strong effects on vectors and reservoir hosts by altering their distribution and influencing their survival and population sizes;
- small fluctuations in temperature can have a profound effect on the developmental cycle of Leishmania promastigotes in sandflies, allowing transmission of the parasite in areas not previously endemic for the disease;
- drought, famine and flood resulting from climate change can lead to massive displacement and migration of people to areas with transmission of leishmaniasis, and poor nutrition could compromise their immunity.

Diagnosis and treatment

In visceral leishmaniasis, diagnosis is made by combining clinical signs with parasitological, or serological tests (rapid diagnostic tests and others). In cutaneous and mucocutaneous leishmaniasis serological tests have limited value. In cutaneous leishmaniasis, clinical manifestation with parasitological tests confirms the diagnosis.

The treatment of leishmaniasis depends on several factors including type of disease, parasite species and geographic location. Leishmaniasis is a treatable and curable disease. All patients diagnosed as visceral leishmaniasis require prompt and complete treatment. Detailed information on treatment of the various forms of the disease by geographic location is available in the WHO technical report series 949 on the control of leishmaniasis.

Prevention and control

Prevention and control of leishmaniasis require a combination of intervention strategies because transmission occurs in a complex biological system involving the human host, parasite, sandfly vector and in some causes an animal reservoir.

Key strategies include

- Early diagnosis and effective case management reduces the prevalence of the disease and prevents disabilities and death. Currently there are highly effective and safe anti-leishmanial medicines particularly for VL and access to these medicines is improving.
- Vector control helps to reduce or interrupt transmission of disease by controlling sandflies, especially in domestic conditions. Control methods include insecticide spray, use of insecticide–treated nets, environmental management and personal protection.
- Effective disease surveillance is important. Early detection and treatment of cases helps reduce transmission and helps monitor the spread and burden of disease.
- Control of reservoir hosts is complex and should be tailored to the local situation.
- Social mobilization and strengthening partnerships – mobilization and education of the community with effective behavioral change interventions with locally tailored communication strategies. Partnership and collaboration with various stakeholders and other vector-borne disease control programmes is critical at levels.

4. YAWS (*FRAMBOESIA*)

Overview

Yaws forms part of a group of chronic bacterial infections caused by treponemes which include endemic syphilis (bejel) and pinta and are commonly known as endemic treponematoses. Yaws is the most common of these infections. The disease is found primarily in poor communities in warm, humid and tropical forest areas of Africa, Asia, Latin America and the Pacific.

Yaws is also known as framboesia (German or Dutch) and pian (French) and affects the skin, bone and cartilage. It is caused by T. pallidum subspecies pertenue. This organism belongs to the same group of bacteria that cause venereal syphilis.

Yaws is transmitted through direct (person-to-person) non-sexual contact with the fluid from the lesion of an infected person. Most lesions occur on the limbs. The initial lesion of yaws is teemed with the bacteria. Contact with this fluid, especially among children who play together and sustain minor injuries, leads to transmission of infection. The incubation period is 9 - 90 days (average 21 days).

About 75% of people affected are children under 15 years old (peak incidence occurs in children aged 6–10 years). Males and females are equally affected. Overcrowding and poor socio-economic conditions facilitate the spread of the yaws. Without treatment, infection can lead to chronic disfigurement and disability.

KEY FACTS

- Yaws is a neglected tropical disease that affects the skin, bone and cartilage.
- The disease is caused by a bacterium from the same group of organisms that cause venereal syphilis; however, the transmission of yaws is not sexually-related.
- Yaws can be eradicated as humans are the only reservoir.
- A recent finding has shown that that a single, oral dose of the antibiotic azithromycin can completely cure yaws, opening up prospects for large-scale treatment of affected populations.
- 2 countries - Ecuador and India - which were once endemic reported interruption of transmission in 2003.
- 12 currently endemic countries need support to implement WHO's new eradication strategy.

Scope of the problem

The eradication campaigns of 1952 - 1964 targeted 46 countries. Since 1990, formal reporting of yaws to WHO stopped due to the discontinuation of yaws eradication programes in many countries. Only a few countries kept yaws as part of their public health agenda.

A review of historic documents from the 1950s shows that at least 85 countries within the tropical belt 20 degrees north and south of the equator, were endemic for yaws. However, only 12 are known to be currently endemic for yaws, while 2 countries, Ecuador and India, which claim to have interrupted transmission in 2003, need to be verified. Furthermore, WHO also plans to assess the status of yaws in 71 previously endemic countries.

Reporting of yaws is not mandatory so the available data, published in a recent edition of the Weekly Epidemiological Record are only indications of the global distribution of the disease.

Surveys are currently in progress to assess the full extent of the disease.

Diagnosis

Clinical: There are two basic stages of yaws: early (infectious) and late (non-infectious).

- In early yaws, an initial papilloma (a circular, solid, swelling on the skin, with no visible fluid) develops at the site of entry of the bacterium. This papilloma is full of the organisms and may persist for 3–6 months followed by natural healing. Bone pain and bone lesions may also occur in the early stage.
- Late yaws appears after five years of the initial infection and is characterized by disfigurement of the nose and bones, and thickening and cracking of the palms of the hand and the soles of the feet. These complications on the soles of the feet make it difficult for patients to walk.

In the field, diagnosis is primarily based on clinical and epidemiological findings.

Serology : Serological tests are widely used to diagnose treponemal infections (e.g. syphilis and yaws). Rapid tests however cannot distinguish between active yaws and treated infections. New rapid dual non-treponemal and treponemal point-of-care syphilis tests hold promise for rapid confirmation of active yaws in the field. Studies are in progress in Ghana, Papua New Guinea, Solomon Islands and Vanuatu to evaluate this new test.

PCR: Genomic analysis using polymerase chain reaction (PCR) can be used to definitely confirm yaws. The PCR technique can also be used to determine azithromycin resistance from swabs taken from yaws lesions.

Treatment

Two antibiotics may be used to treat yaws.

- Azithromycin (single oral dose) at 30 mg/kg (maximum 2 gm).
- Benzathine penicillin (single intramuscular dose) at 1.2 million units (adults) and 600 000 units (children).

Complications

Without treatment, about 10% of affected people develop disfiguring and disabling complications – deformities of the legs and nose - after five years. The disease and its complications cause school absenteeism and prevent adults from farming activities.

Prevention

There is no vaccine for yaws. Prevention is based on the interruption of transmission through early diagnosis and treatment of individual cases and mass or targeted treatment of affected populations or communities. Health education and improvement in personal hygiene are essential components of prevention.

Past eradication efforts

Between 1952 and 1964, WHO and UNICEF provided assistance to 46 countries with the aim of eradicating endemic treponematoses. Mass campaigns in these countries examined over 300 million people and treated 50 million.

By 1964, the prevalence of these diseases had decreased by 95% (2.5 million). This achievement is

considered to be one of the success stories in public health but this was not sustained until the end goal – eradication. However, premature integration of yaws control activities into the weak primary health-care systems and lack of continued surveillance were partly responsible for the world's inability to eradicate yaws. Resurgence in the 1970s prompted a World Health Assembly Resolution WHA 31.58.

Perspective

Yaws is eradicable as humans are the only reservoir. Covering all at-risk populations through large scale treatment programmes with oral azithromycin will interrupt transmission and eliminate the disease in a given area.

The momentum to achieve this is gathering pace and WHO, together with partners, is spearheading renewed efforts to eradicate yaws.

The provision of azithromycin in sufficient quantities, the availability of a rapid diagnostic test and adequate funding are critical to ensure the smooth implementation of activities to reach 2020 target.

5. TAENIASIS/CYSTICERCOSIS

Overview

Taeniasis is an intestinal infection caused by two species of tapeworms. The most important human Taenia tapeworm infections are caused by *Taenia solium* (pork tapeworm) and *T. saginata* (beef tapeworm).

Humans become infected with *T. saginata* when they consume beef which has not been adequately cooked. Taeniasis due to *T. saginata* usually has a minor impact on human health.

Infection also occurs in humans when they eat raw or undercooked pork (*T.solium*). *Taenia solium* tapeworm infection is of significant importance as it can cause cysticercosis – a serious disease.

Cysticercosis is the infection with the tapeworm at the larval stage (cysticerci). Inside the body, cysticerci can develop in a number of tissues such as the muscles, subcutaneous tissues, eyes and brain; those that are located in the central nervous system cause neurocysticercosis, the most severe form of the disease.

Neurocysticercosis is considered to be a common infection of the human nervous system and is the most frequent preventable cause of epilepsy in the developing world. More than 80% of the world's 50 million people who are affected by epilepsy live in low-income and lower-middle income countries, many of which are endemic for *T. solium* infections in people and pigs.

Cysticercosis mainly affects the health and livelihoods of subsistence farming communities in developing countries of Africa, Asia and Latin America since it can lead to epilepsy and death in humans it reduces the market value of pigs and cattle and makes pork and beef unsafe to eat.

Although theoretically amenable to control and declared eradicable by the International Task Force for Disease Eradication in 1993, *T. solium* cysticercosis remains a neglected disease and was added by WHO to the list of major neglected tropical diseases in 2010.

KEY FACTS

- Taeniasis is an intestinal infection caused by adult tapeworms.
- Taeniasis is acquired by humans through the inadvertent ingestion of tapeworm larval cysts (*cysticerci*) in undercooked pork or beef.
- Human tapeworm carriers contaminate the environment with tapeworm eggs which pass out with feces.
- Cysticercosis is the infection of tissues caused by cystercerci as a result of ingesting Taenia eggs. Cysticerci of *T. solium*, but not *T. saginata*, can infect humans.
- Cysts that develop in the central nervous system cause neurocysticercosis – the most severe form of the disease and one of the main preventable causes of epilepsy (seizures) in many developing countries.
- More than 80% of the world's 50 million people who are affected by epilepsy live in low-income and lower-middle-income countries, many of which are endemic for *T. solium* infections in people and pigs.

Transmission

Taeniasis is acquired by humans through the inadvertent ingestion of their cysticerci in undercooked pork or beef. Once in the human body, cysticerci develop into adult tapeworms that live in the intestine and release egg-bearing gravid proglottids (segments) which are passed out with faeces.

Cysticercosis is acquired when proglottids or eggs are ingested. It is a natural infection of pigs and cattle, but, in the case of *T. solium*, it can also affect humans, usually when they swallow *T. solium* egg-contaminated soil, water or food (mainly vegetables). Taeniasis and cysticercosis are common in areas where animal husbandry practices are such that pigs and cattle come into contact with human faeces.

Symptoms

Taeniasis due to *T. solium* or *T. saginata* is usually characterized by mild and non-specific symptoms. Abdominal pain, nausea, diarrhoea or constipation might arise, 6 - 8 weeks after ingestion of the cysticerci when the tapeworms become fully developed.

These symptoms may continue until the tapeworm dies following treatment (otherwise it may live many years).

In the case of cysticercosis due to T. solium, the incubation period is variable, and infected people may remain asymptomatic for years.

In some endemic regions (in particular Asia), infected people may develop visible or palpable nodules (a small bump or node which is solid that can be detected by touch) beneath the skin (subcutaneous).

When cysts are recognized by the host following spontaneous degeneration or after treatment, an inflammatory reaction may occur.

This usually results in clinical symptoms which, depending on the location of the cysts and may include chronic headaches, blindness, seizures (epilepsy if they are recurrent), hydrocephalus, meningitis, dementia and symptoms caused by lesions occupying spaces of the central nervous system.

Treatment

Taenaisis is easily treated with praziquantel (5 -10 mg/kg, single-administration) or niclosamide (adults and children over 6 years: 2 g, single-administration after a light breakfast, followed after 2 hours by a laxative; children aged 2 - 6 years: 1 g; children under 2 years: 500 mg).

Treating human cysticercosis is difficult with varying success and may include long courses with praziquantel and/or albendazole, as well as supporting therapy with corticosteroids and/or anti-epileptic drugs, and possibly surgery.

Prevention and control

Infections with *T. saginata* can be managed through an individual clinical approach due to its low pathogenicity (low ability to spread from host to host).

By contrast, infections due to *T. solium* require proper public health interventions aimed at their prevention, control and possibly elimination.

Prevention measures involve strict meat inspection regimens, health education, thorough cooking of pork, sound hygiene, adequate water and sanitation (elimination of open defecation), and improved pig farming practices.

Easy access to treatment should be provided to infected individuals and people who are in close contact with pigs.

However, difficulties linked to the implementation of prevention measures are increased by the fact that reliable epidemiological data on geographical distribution of *T. solium* taeniasis/cysticercosis in people and pigs is often missing.

Appropriate surveillance mechanisms should enable new cases of human or porcine cysticercosis to be reported to national authorities in order to facilitate the identification of communities at high risk and focus prevention and control measures in such areas.

6. HUMAN AFRICAN TRYPANOSOMIASIS

Overview

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. It is caused by infection with protozoan parasites belonging to the genus *Trypanosoma*. They are transmitted to humans by tsetse fly (*Glossina genus*) bites which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites.

Tsetse flies are found just in sub-Saharan Africa though only certain species transmit the disease. For reasons that are so far unexplained, there are many regions where tsetse flies are found, but sleeping sickness is not. Rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse fly and therefore to the disease. The disease develops in areas ranging from a single village to an entire region. Within an infected area, the intensity of the disease can vary from one village to the next.

KEY FACTS

- Sleeping sickness occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that transmit the disease.
- The people most exposed to the tsetse fly and therefore the disease live in rural areas and depend on agriculture, fishing, animal husbandry or hunting.
- *Trypanosoma brucei gambiense* accounts for more than 98% of reported cases of sleeping sickness.
- Sustained control efforts have lowered the number of new cases. In 2009, the number of cases reported dropped below 10 000 (9878) for first time in 50 years and in 2012 there were 7216 cases recorded.
- Diagnosis and treatment of the disease is complex and requires specifically skilled staff.

Forms of human African trypanosomiasis

Human African trypanosomiasis takes two forms, depending on the parasite involved:

- *Trypanosoma brucei gambiense* is found in 24 countries in west and central Africa. This form currently accounts for over 98% of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months or even years without major signs or symptoms of the disease.

When more evident symptoms emerge, the patient is often already in an advanced disease stage where the central nervous system is affected.

- *Trypanosoma brucei* rhodesiense is found in 13 countries eastern and southern Africa. Nowadays, this form represents under 2% of reported cases and causes an acute infection. First signs and symptoms are observed a few months or weeks after infection. The disease develops rapidly and invades the central nervous system. Only Uganda presents both forms of the disease.

Another form of trypanosomiasis occurs mainly in Latin America. It is known as American trypanosomiasis or Chagas disease. The causal organism is a different subgenus from those causing the African form of the disease.

Animal trypanosomiasis

Other parasite species and sub-species of the *Trypanosoma genus* are pathogenic to animals and cause animal trypanosomiasis in wild and domestic animals. In cattle the disease is called Nagana.

Animals can host the human pathogen parasites, especially *T.b. rhodesiense*; thus domestic and wild animals are an important parasite reservoir. Animals can also be infected with *T.b. gambiense* and act as a reservoir. However the precise epidemiological role of the animal reservoir in the gambiense form of the disease is not yet well known. The disease in domestic animals, particularly cattle, is a major obstacle to the economic development of affected rural areas.

Major human epidemics

There have been several epidemics in Africa over the last century:

- one between 1896 and 1906, mostly in Uganda and the Congo Basin;
- one in 1920 in a number of African countries; and
- the most recent epidemic occurred in 1970 and lasted up to late 1990s.

The 1920 epidemic was controlled thanks to mobile teams which organized the screening of millions of people at risk. By the mid-1960s, the disease was under control with less than 5000 cases reported in the whole Continent. After this success, surveillance was relaxed, and the disease reappeared in several regions. The efforts of WHO, national control programmes, bilateral cooperation and nongovernmental organizations (NGOs) during the 1990s and the beginning of the 21st century stopped and reversed the upward trend of new cases. Since the number of new human African trypanosomiasis cases reported between 2000 and 2012 has dropped by 73%, the WHO NTD Roadmap has targeted its elimination as a public health problem by 2020.

Distribution of the disease

Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. Many of the affected populations live in remote areas with limited access to adequate health services, which complicates the surveillance and therefore the diagnosis and treatment of cases. In addition, displacement of populations, war and poverty are important factors that facilitate transmission. In 1998, almost 40 000 cases were reported, but estimates were that 300 000 cases were undiagnosed and therefore untreated.

During epidemic periods prevalence reached 50% in several villages in the Angola, Democratic Republic of Congo, and South Sudan. Sleeping sickness was the first or second greatest cause of mortality in those communities, ahead of even HIV/AIDS.

In 2009, after continued control efforts, the number of cases reported dropped below 10,000 (9878) for first time in 50 years. This decline in number of cases has continued with 7216 new cases reported in 2012. However, the estimated number of actual cases is 20,000 and the estimated population at risk is 70 million people.

Current situation in endemic countries

The prevalence of the disease differs from one country to another as well as in different parts of a single country.

- In the last 10 years, over 70% of reported cases occurred in the Democratic Republic of Congo (DRC).
- The DRC is the only country that has reported more than 1000 new cases annually and accounts for 83% of the cases reported in 2012.
- Central African Republic, Chad and South Sudan declared between 100 and 500 new cases in 2012.
- Countries such as, Angola, Cameroon, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Ghana, Guinea, Kenya, Malawi, Nigeria, Uganda, United Republic of Tanzania, Zambia and Zimbabwe are reporting fewer than 100 new cases per year.
- Countries like Benin, Botswana, Burkina Faso, Burundi, Ethiopia, Gambia, Guinea Bissau, Liberia, Mali, Mozambique, Namibia, Niger, Rwanda, Senegal, Sierra Leone, Swaziland and Togo have not reported any new cases for over a decade. Transmission of the disease seems to have stopped but there are still some areas where it is difficult to assess the exact situation because the unstable social circumstances and/or remote accessibility hinders surveillance and diagnostic activities.

Infection and symptoms

The disease is mostly transmitted through the bite of an infected tsetse fly but there are other ways in which people are infected with sleeping sickness.

- Mother-to-child infection: the trypanosome can cross the placenta and infect the fetus.
- Mechanical transmission through other blood sucking insects is possible. However, it is difficult to assess the epidemiological impact of transmission.
- Accidental infections have occurred in laboratories due to pricks from contaminated needles.

In the first stage, the trypanosomes multiply in subcutaneous tissues, blood and lymph. This is known as a first stage or haemolymphatic phase, which entails bouts of fever, headaches, joint pains and itching.

In the second stage the parasites cross the blood-brain barrier to infect the central nervous system. This is known as the neurological or meningoencephalic phase. In general this is when more obvious signs and symptoms of the disease appear: changes of behaviour, confusion, sensory disturbances and poor coordination. Disturbance of the sleep cycle, which gives the disease its name, is an important feature of the second stage of the disease. Without treatment, sleeping sickness is considered fatal although cases of healthy carriers have been reported.

Disease management: diagnosis

Disease management is made in three steps.

- Screening for potential infection. This involves using serological tests (only available for *T.b.gambiense*) and checking for clinical signs - generally swollen cervical glands.
- Diagnosing whether the parasite is present.
- Staging to determine the state of disease progression. This entails examining cerebro-spinal fluid obtained by lumbar puncture and is also used to determine the outcome of treatment.

Diagnosis must be made as early as possible to avoid progressing to the neurological stage in order to elude complicated, difficult and risky treatment procedures.

The long, relatively asymptomatic first stage of *T. b. gambiense* sleeping sickness is one of the reasons why an exhaustive, active screening of the population at risk is recommended, in order to identify patients at an early stage and reduce transmission. Exhaustive screenings require a major investment in human and material resources. In Africa such resources are often scarce, particularly in remote areas where the disease is mostly found. As a result, some infected individuals may die before they can ever be diagnosed and treated.

Treatment

The type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease are of lower toxicity and easier to administer. The earlier the disease is identified, the better the prospect of a cure.

Treatment success in the second stage depends on a drug that can cross the blood-brain barrier to reach the parasite. Such drugs are toxic and complicated to administer. Four drugs are registered for the treatment of sleeping sickness. These drugs are donated to WHO by manufacturers and distributed free of charge to countries endemic for the disease.

First stage treatment

- Pentamidine: discovered in 1941, used for the treatment of the first stage of *T.b. gambiense* sleeping sickness. Despite non-negligible undesirable effects, it is in general well tolerated by patients.
- Suramin: discovered in 1921, used for the treatment of the first stage of *T.b. rhodesiense*. It provokes certain undesirable effects, in the urinary tract and allergic reactions.

Second stage treatment:

- **Melarsoprol:** discovered in 1949, it is used in both forms of infection. It is derived from arsenic and has many undesirable side effects. The most dramatic is reactive encephalopathy (encephalopathic syndrome) which can be fatal (3% to 10%). An increase in resistance to the drug has been observed in several foci particularly in central Africa.
- **Eflornithine**: this molecule, less toxic than melarsoprol, was registered in 1990. It is only effective against *T.b. gambiense*. The regimen is strict and difficult to apply.
- A combination treatment of nifurtimox and eflornithine was introduced in 2009. It simplifies the use of eflornithine in monotherapy, but unfortunately it is not effective for *T.b. rhodesiense*. Nifurtimox is registered for the treatment of American trypanosomiasis but not for human African trypanosomiasis. Nevertheless, after safety and efficacy data provided by clinical trials, its use in combination with eflornithine has been accepted and included in the WHO List of Essential Medicine, and it is provided free of charge for this purpose by WHO to endemic countries.

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