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Congratulations and best wishes to all our contributors and collaborators.

Professor Adel Kahader Ayed
Editor
Editorial

Secret of Healthy Living in a Hostile World

Belle M Hegde

*The Journal of the Science of Healing Outcomes, Maryland, USA and Mangalore, India
**Manipal University, Manipal India
#London University, UK
##Northern Colorado University, USA

“*The greatest truths are the simplest, and so are the greatest men.”
JC and AW Hare

When one ponders over the multitude of risk factors in and around us in this hostile world, one wonders as to how we are alive at all. Medical claptrap informs us day in and day out about the multitude of potions-chemical drugs, surgeries, special foods, tonics etc, to keep us alive. The truth is that most, if not all, of those heavily advertised items damage the system further than helping us. The truth is that there is a very complicated, yet simple system inside each of the one hundred thousand billion cells inside the human body which function better than a super computer to compute all the internal and external inputs through multiple sources to transduce them into coherent methods to eventually put in place two fascinating energy systems, a low energy and high energy system, to keep us going[1].

Let us first examine this nature’s computer inside every cell which existed for nearly a billion and half years before the first nerves ever appeared in Jellyfish. It took another half a billion years for cranial nerves and brain to appear in organisms including man. The myth that the brain and the nervous systems alone keep us going has to give place to a more holistic view that there are many other important systems that help keep man alive. Then we will progress further in our understanding of human physiology and pathology. Life is a complicated system of individual cell function in the body in an interdependent manner to keep us alive and healthy. If the ten thousand odd proteins that are present inside each one of the trillions of cells in our bodies do not work well we will have disease states. For recovery from any illness, body cells will have to function normally again[2].

Our approach in the “so-called” evidence based modern medicine is to try and correct those changes (not knowing what they are) in disease states using chemicals or surgery. As a quick-fix, apparently, they help some people some times but all of them damage some part of the human system almost always, some times as late as five years after the event. One good example is a pain killer trasylol which is now known to kill the recipient as late as five years after he/she had it! What happened to another pain killer wyoxo is now common knowledge. These measures have now resulted in considerable misery for mankind. To quote the most authentic scientific body of the USA : “The National Academy’s data attributes 100,000 deaths per year to physicians’ errors, added to well over 100,000 deaths due to severe drug interactions and another 100,000 fatalities from hospital-based-infections. (For a detailed analysis, see Death By Medicine, by Gary S. Null, et al)”. This is from a country with less than one third the population of India. Thank God, we do not have statistics like this for India[3].

What is the remedy? We must get to know the true physiology of cell function and try and see how we could restore that in the unlikely event of disease in a more natural way rather than inflicting chemical and surgical damage to the cells where possible. The fruitless research of modern medicine is based on statistical science and not true hard science. The “failure of millions of dollars spent on AIDS vaccine, failure of interferon as a wonder drug for cancer management with the latter still

Address correspondence to:
Professor B. M. Hegde, Manjunath, Pais Hills, Bijai, Mangalore 575004, India. Tel: +91 824221 7575, E-mail: hegdebm@gmail.com;
web: www.bmhegde.com
*Editor in Chief, The Journal of the Science of Healing Outcomes, Maryland, USA and Mangalore, India
**Vice Chancellor (Retd), Manipal University, Manipal India
#Former Visiting Professor of Cardiology, London University, UK
##Affiliate Professor of Human Health, Northern Colorado University, USA

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eluding a cure despite billions being wasted on cancer research and cancer screening (the latter has been shown to be useless and dangerous), and the ravages caused by drugs like thalidomide and thorazine,“ are there for all of us to see.

We need to arrange an urgent marriage between the beneficial remedies in modern medicine “like the excellent emergency care methods, brilliant surgical successes, time tested and harmless pharmaceuticals as also the newer life style changes” with the best and scientifically authenticated multitude of methods in many other systems of medicine into a judicious integrated system of medical care that is inexpensive, safe, and effective under all circumstances. Unfortunately, the vested interests in modern medicine are scuttling every effort in this field by hitting those efforts with an old but, effective whip “there is no evidence base” in other systems of medical care. This is the biggest lie.

Let us examine how we can use natural methods to get the damaged cells back to normalcy. The ten thousand odd proteins in each cell are functionally better than our supercomputers. They have two energy systems- the Low energy system and the High energy system. Initially, the proteins process all the information they collect from the body as also the outside world into a low energy information system which primes the other proteins to a high energy functional system that could power the body as a whole. In this milieu there are certain specific proteins that do the directing or chaperoning job very effectively. One such chaperone protein is the Stress Responsive Protein (SRP 70) as it responds to every kind of stress in the cell[4].

The HSP 70 protein is supervised by the HSP 70 gene. HSP 70 protein could be re-primed by heating the cell to 47°C but, that can never be done in the human body. The other method is to use some kind of natural energy to do the job. In health the cell uses the energy coming from the main source, sunlight, as also the magnetic energy generated by lightening throwing a halo of Schuman energy field around the earth (Schumann effect). All proteins are but carbon, hydrogen, nitrogen and oxygen as in the DNA along with amino-acids that come from food. The electromagnetic energy used by the cell proteins are then transduced to fire the mitochondria inside every cell to produce energy needed for life[5].

Glen Gordon was one of NIH’s brilliant young scientists, 4th in hierarchy at one stage. He was a pioneer in this field of trying to regenerate the damaged (ischemic) cells back to normal. This made the American Medical Association to file a law suit against him, which did not materialize at the end. He lost his entire grant support, though. He would not

re lent. He has come up with a small Pulsed Electro-Magnetic Field (PEMF) generator powered by a battery to stimulate and up-regulate the depressed HSP 70 protein and thereby regenerate the cells again. My own initial enthusiasm with this toy of his is exciting. Time is not ripe to disclose the final data as the study is ongoing but the preliminary reports are very encouraging indeed. None the less, the results are an opening for us to look more deeply into many such natural methods of making the sick cells (individuals) to regain their strength and health without any long term detriment to the owner in the bargain[6-8].

The marriage between the best in both the worlds, as suggested above, is our only solution in this dangerous state in which modern medical claptrap and statistical science have landed us. We need a holistic approach to human, nay, all problems of this world.

“... and all the loveliest things there be
Come simply, so it seems to me.”

Edna St. Vincent Millay

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Thanks to Glen Gordon for his inputs, help with gadgets and love. To Professor Rustum Roy of Penn. State, for many things that cannot be enumerated.

REFERENCES

The Immune System in Pregnancy: Friend or Foe?

Raj Raghupathy
Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

ABSTRACT

Pregnancy is an intriguing immunological paradox; how does an allogeneic fetus survive despite a potentially antagonistic maternal immune system, while tissue allografts succumb to rapid immunological rejection? While several hypothetical models have been proposed in the last five decades to explain the immunological success of pregnancy, the model that has survived the test of experimentation is the one that proposes a state of immunomodulation during pregnancy. Several factors appear to prevent the rejection of the fetus. Yet, pregnancy can be compromised by a variety of complications such as recurrent spontaneous miscarriage, preeclampsia and preterm delivery. Research in the field of immunology of pregnancy has opened up the possibility of cellular immune effectors that might underlie these pregnancy complications. Particularly interesting are the effects that pro-inflammatory and anti-inflammatory cytokines have on the conceptus and thus on the success and failure of pregnancy. This review focuses on the association between some cytokines and successful pregnancy on the one hand, and between other cytokines and complications of pregnancy as also the possible pathways of effector function of cytokines in pregnancy loss. This review proceeds to discuss the therapeutic redirection of cytokine profiles towards one that is favorable to the success of pregnancy.

KEY WORDS: allogeneic fetus, cellular immune effectors, immunomodulation in pregnancy

INTRODUCTION

One of the basic tenets of immunology is that the host immune system recognizes anything that is foreign or “non-self”, mounts an immune response against it and then eliminates it. Thus, pathogens, toxins and foreign antigens in general are recognized and eliminated. This holds true for foreign cells and tissues as well, which is why tissues from other individuals are recognized as foreign and then rejected. How then is the fetus not rejected? The fetus is derived from both paternal and maternal genes, thus the fetus expresses both paternal and maternal antigens. Paternal antigens, such as the strongly antigenic human leukocyte antigens (HLA) could well be foreign to the maternal host and could stimulate maternal immune responses which could potentially lead to the immunological rejection of the fetus. The fact that pregnancy does succeed has long been considered an immunological paradox, a puzzle of sorts, which prompted the British immunologist Peter Medawar more than 55 years ago to ask “How does the mother contrive to nourish within herself an antigenically foreign fetus?”

Medawar went on to propose several mechanisms for the protection of the fetus from maternal immune rejection; one of these was that pregnancy induces a state of immunosuppression in the mother because of which rejection reactions are either not induced or are not effective[1]. Subsequent experimentation has demonstrated the lack of a generalized, systemic state of immunosuppression, though a sort of immunomodulation, or manipulation of maternal immunity towards a less harmful and more conducive status seems to be in effect. In fact, some immune system factors actually act as positive growth factors for the placenta, as we shall see later; thus, the maternal immune system may actually contribute to nurturing the conceptus and to the success of pregnancy.

The Maternal Immune System during Normal Pregnancy

Evidence from animal experiments and clinical evidence from humans indicate that humoral responses are enhanced during pregnancy[2]. On the other hand, cell-mediated immune reactions such as delayed-type hypersensitivity, natural killer (NK) activity, cellular responses to intracellular infections and the course of cell-mediated autoimmune disorders are downregulated. These observations

Address Correspondence to:
Dr Raj Raghupathy, PhD, FRCPath, Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait. Tel: 25319602, Fax: 25332719, E-mail: raj@hsc.edu.kw
suggest that there is a downregulation of Th1-type immunity and upregulation of Th2-type immunity during pregnancy\textsuperscript{[2]}. Th1-type immunity is primarily mediated by Th1 cells and Th2-type immunity is induced by Th2 cells. Th1 and Th2 cells are the two major subsets of CD4\textsuperscript{+} T helper cells; these two subsets have different patterns of cytokine production and different roles in immune responses. Each subset induces immune responses that are effective at handling certain types of pathogens, but can be ineffective, or even pathological if made in response to other types of pathogens\textsuperscript{[3,4]}. Th1 cells secrete the pro-inflammatory cytokines interferon-\(\gamma\) (IFN-\(\gamma\)), tumor necrosis factor-\(\beta\) (TNF\(\beta\)), interleukin (IL)-2 and TNF\(\alpha\); these Th1 cytokines activate macrophages and cell-mediated reactions important in resistance to infection with intracellular pathogens, and in cytotoxic and delayed-type hypersensitivity (DTH) reactions. Th2 cells secrete IL-4, IL-5, IL-6, IL-10 and IL-13; these Th2-type cytokines induce strong antibody production and are therefore commonly found in association with strong antibody responses that are important in combating infections with extracellular organisms. Which type of reactivity, Th1 or Th2, is activated first may influence the subsequent outcome; if a particular T cell subset is activated first or preferentially in a response, it can suppress the development of the other subset. The overall effect is that certain responses are dominated by either humoral (Th2) or cell-mediated (Th1) immunity\textsuperscript{[3,4]}. Furthermore, Th1 cytokines such as IL-2, TNF-\(\alpha\) and IFN-\(\gamma\) generally tend to provoke inflammatory cellular immune reactions and are thus also referred to as pro-inflammatory cytokines, while Th2 cytokines such as IL-10, IL-13 and IL-4 are considered anti-inflammatory cytokines as they tend to downregulate inflammatory reactions.

What is the Th1/Th2 status during normal pregnancy? Pregnancy seems to bring about a shift towards Th2 bias. Th2 cytokines have been detected in post-partum human placental tissue\textsuperscript{[5]}, amniotic fluid\textsuperscript{[6]}, endometrium and decidua of early pregnancy\textsuperscript{[7]}, and in supernatants from cultures of endometrial cells, decidual stroma\textsuperscript{[7]}, cytotrophoblast\textsuperscript{[5]} and amnion epithelial cells. Piccinni et al\textsuperscript{[8]} demonstrated significantly higher levels of IL-4- and IL-10-producing T cell clones from the decidua of women with normal pregnancy than from women with unexplained recurrent spontaneous miscarriage (RSM). Some Th2 cytokines have been shown to be expressed in the human placenta; IL-10 is synthesized by the decidua, chorion and amnion cells of the placenta\textsuperscript{[9]}, IL-4 is expressed by the decidua, amniochorionic membranes, cytotrophoblast and both maternal and fetal endothelial cells\textsuperscript{[10]}

IL-13, an anti-inflammatory Th2 cytokine has the capacity to limit inflammatory reactions that may arise locally at the site of implantation and within the placenta during pregnancy. Dealtry et al\textsuperscript{[11]} demonstrated the expression of IL-13 by human trophoblast cells and suggested that IL-13 may play important roles in this dialogue that aids in the establishment and maintenance of the placenta. There are reports of significantly higher IL-10 production by mitogen-activated PBMC in pregnant women as compared with non-pregnant women\textsuperscript{[12]}. Using a sensitive on-line quantitative RT-PCR Kruse et al\textsuperscript{[13]} reported significantly reduced expression of Th1 cytokine mRNA during normal human pregnancy. Th1/Th2 ratios revealed a shift to a pronounced Th2 status. Significantly increased IL-4-producing CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells were demonstrated in normal pregnant women as compared to non-pregnant women. In contrast, Th1 cytokine-producing T cells were significantly reduced in pregnancy, which is suggestive of a Th2 shift in pregnancy\textsuperscript{[14]}. Th2-type cytokine production reportedly predominates in the second and third trimesters of pregnancy\textsuperscript{[15,16]} leading to a hypothesis that successful pregnancy is correlated with and perhaps depends on the preferential stimulation of Th2 cytokine-producing T cells.

Besides an anti-inflammatory effects mediated by Th2 cytokines, several other non-Th2 cytokines have positive influences on the conceptus; cytokines such as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF) and colony-stimulating factor-1 (CSF-1) have been shown to act as growth factors for the placenta\textsuperscript{[2]}

Thus, we can view normal, successful pregnancy as a situation in which the maternal immune system acts in a “friendly” manner, either not generating strong rejection reactions or keeping such reactions under check. We can view this as a sort of a “friendly”, protective role of the immune system in nurturing the conceptus and ensuring the success of pregnancy.

**Maintenance of a Protective Th2 Status during Pregnancy**

If pregnancy brings about a protective Th2-bias, how is this bias maintained? Cytokines, hormones and other molecules appear to play critical roles in directing the immune reactivity towards a Th2 bias and then maintaining it that way. The Th2-inducing effect of IL-4 predominates over other cytokines, so that if IL-4 levels reach a given threshold, differentiation of Th cells into the Th2 phenotype occurs.
One of the most vital cytokines that helps maintain a Th2 bias, once initiated, is IL-10; this anti-inflammatory, immunosuppressive cytokine interferes with antigen presentation, downregulates cytokine production by Th1 cells and directly or indirectly inhibits NK responses. Trophoblast cells produce IL-10 in a gestational age-dependent manner. Chaouat and colleagues have reported high expression of IL-10 on murine and human trophoblast and describe the placenta as a “strong Th2 cytokine shield”. Evidence for possibly crucial roles for IL-10 came from the studies of Chaouat et al. in which the administration of IL-10 to abortion-prone mice was shown to reverse the high rate of fetal resorption in these mice. Likewise, Rivera’s group showed that the increased uterine TNFα, release of nitric oxide and apoptosis of uterine epithelia that followed administration of LPS were all normalized upon injection of IL-10.

Hormones have been shown to have important roles in promoting the differentiation of Th cells or in favoring the shifting of already differentiated Th cells from one cytokine profile to another. Progesterone at concentrations comparable to those present at the materno-fetal interface, favors the development of human T cells producing Th2-type cytokines. Thus, Piccinni proposes that the high levels of progesterone present at the materno-fetal interface during gestation may contribute, at least in part, to the development of a prevalent Th2-type profile which allows successful pregnancy. Progesterone may therefore be responsible at least in part for a Th1→Th2 switch at the maternal-fetal interface. Hill’s laboratory and our laboratory have demonstrated that the production of Th1 cytokines by trophoblast antigen-activated peripheral blood mononuclear cells (PBMC) cultures was significantly inhibited by co-culture with progesterone.

Besides bringing about a skew towards Th2 reactivity, progesterone has an indirect and very interesting mediatory role in influencing the Th1/Th2 balance; Szekeres-Bartho et al have lymphocytes from pregnant females, when exposed to progesterone, produce an immunomodulatory factor, the progesterone-induced blocking factor (PIBF). This protein suppresses various cell-mediated immune reactions. In mice and in humans PIBF upregulates Th2 cytokine production and thus brings about a shift in the Th1 to Th2 cytokine bias. PIBF is proposed to play an important role in pregnancy by helping the conceptus escape immune surveillance. Thus, priming and maturation of T cells in pregnancy would seem to occur in an environment that is gradually progesterone-enriched. This would result in T cell priming, activation and differentiation in the presence of lower levels of the Th1 cytokines IL-2 and IFNγ and higher levels of the Th2 cytokines IL-4 and IL-10, an environment that has been shown to favor the development of Th2 cells. The high levels of progesterone secreted at the beginning of pregnancy may well be necessary for the orientation of the maternal immune response against the conceptus towards a Th2-like pattern; these Th2 cytokines downregulate the potentially antagonistic Th1 response, thus preventing fetal rejection.

It is likely that prostaglandin E (PGE) plays useful roles in this immunomodulation given its ability to inhibit IL-2 production, to inactivate Th1 cells and to inhibit cytotoxic activity of NK cells. PGE may serve as one of the factors that skew the balance in favor of Th2 responses. Glucocorticoids enhance Th2 activity, and synergize with IL-4; thus the steroid alterations that occur in pregnancy may influence decidual lymphocytes in favor of a Th2 response.

CYTOKINES AND PREGNANCY COMPLICATIONS

We have seen how some cytokine patterns (i.e., anti-inflammatory Th2 cytokines) are conducive to successful pregnancy; evidence accruing from experiments on animal models and humans supports the contention that some cytokines are detrimental to the success of the conceptus. The activation of some forms of maternal cellular immunity is potentially hazardous for fetal development. Cellular immunity mediated by effector cells and/or cytokines released by them have been shown to have significant deleterious effects on the conceptus. The pro-inflammatory, Th1 cytokines TNFα, IFNγ and IL-2 contribute adversely to the survival of the conceptus. The injection of TNFα, IFNγ and IL-2 into pregnant mice causes abortions while the injection of antibodies to TNFα results in a reduction in abortion rates in a murine model of natural, immunologically-mediated abortion. TNFα and IFNγ inhibit the outgrowth of human trophoblast cells in vitro and synergistically stimulate the programmed death of human primary villous trophoblast cells.

Gradually, substantial evidence was obtained to demonstrate an interesting connection between Th1 cytokines and pregnancy loss just as researchers observed interesting connections between successful pregnancy and Th2 immunity. How are cytokines associated with clinical complications of pregnancy? Most laypersons may think of pregnancy as a very successful phenomenon, but it is not nearly as successful as one might think; the actual rate of early pregnancy loss after implantation may be as high as 31%.
About 15-20% of fertilized embryos are lost within the first 2 weeks of fertilization and another 10-15% are lost during the next 12 weeks of gestation. A variety of conditions threaten the success of the fetus; these include spontaneous miscarriage, pre-term delivery, pre-eclampsia and premature rupture of membranes. For these conditions, not all cases can be attributed to the so called “known” or “explained” causes such as chromosomal anomalies, endocrinologic abnormalities, infections, anatomic problems and humoral factors. For example, as many as 60% of the cases of RSM are consigned to “unknown” or “unexplained” etiology. The existence of such a large proportion of cases with unidentified etiologies has inspired interest in the investigation of possible immunologic etiologies of pregnancy failure.

Cytokine Patterns in Recurrent Spontaneous Miscarriage

RSM is one of the most common and challenging complications of pregnancy faced by obstetricians. Spontaneous miscarriage is defined as a clinically detectable pregnancy loss prior to 20 weeks of gestation; one of every four pregnant women suffers from one or more pregnancy losses. Only about 40-50% of RSM, defined as the occurrence of three or more pregnancy losses before the 20th week of gestation, is attributable to the so called “known” causes such as chromosomal anomalies, endocrinologic abnormalities, infections, anatomic problems and humoral factors, with as much as 60% relegated to “unknown” or “unexplained” etiology. Thus, the causes of RSM remain “unexplained” in the majority of women.

Extensive research into immunologic etiologies and pathogenesis of RSM has shown that the conceptus appears to be fairly impervious to attack by humoral immunity except for anti-phospholipid antibodies. This then raised the possibility of cell-mediated immune effectors as possible etiologic agents for RSM. This includes cellular immunity mediated by effector cells and / or cytokines released by them, and these have been shown to have significant deleterious effects on the conceptus.

Most studies to date suggest that women with RSM have a greater Th1-type or pro-inflammatory cytokine bias as compared to normal pregnant women. Hill and colleagues have shown that PBMC of women with a history of RSM when stimulated with a human trophoblast antigen extract produce higher levels of the Th1 cytokines and embryotoxic activity as compared to normal pregnancy. They concluded that Th1 immunity to trophoblast antigens is associated with recurrent miscarriage and may play a role in reproductive failure while Th2 immunity may be the natural response to the trophoblast, in contributing to normal, successful pregnancy.

We have published convincing evidence for a clear Th1 cytokine bias in RSM. Peripheral blood lymphocytes from normal pregnant women at the end of the first trimester and at delivery, and from recurrent aborters at the time of abortion, were stimulated with a mitogen and the supernatants tested for Th1 and Th2 cytokines. Levels of several Th2 cytokines were higher at the end of the first trimester and at delivery in normal pregnancy than in RSM, while the levels of pro-inflammatory Th1 cytokines were uniformly higher in RSM than in normal pregnancy. This was substantiated by studies on specific maternal immunity to placental antigens assessed by co-culturing maternal lymphocytes with autologous placental cells and exposing maternal lymphocytes to a trophoblast antigen preparation. Since the absolute concentrations of secreted cytokines may not reveal a Th1- or Th2-bias per se, we analysed the ratios of Th1 to Th2 cytokines in the various permutations. In every combination of Th1 to Th2 cytokines, the ratios were higher in the RSM group as compared to the normal pregnancy group, indicating a greater Th1-bias in RSM and a greater Th2-bias in normal pregnancy.

Decreased production of Th2 cytokines and increased production of Th1 cytokines by antigen-stimulated lymphocytes were demonstrated in women with RSM as opposed to those from normal pregnancy. Piccinni et al demonstrated significantly higher levels of Th2 cytokine-producing T cell clones from the decidua of women with normal pregnancy than from women with unexplained RSM. Most reports thus support the contention that women with recurrent miscarriage exhibit a predominantly Th1 cytokine pattern, whereas healthy women exhibited decreased Th1 cytokines and increased Th2 cytokines; there is thus an increased pro-inflammatory cytokine bias in recurrent miscarriage.

How might Th1 cytokines bring about spontaneous miscarriage? Several mechanisms involving natural killer (NK) cells, activated macrophages and direct apoptosis have been suggested as being responsible for Th1 cytokine mediated effects. Increased NK activity in the blood and uterus has been linked to miscarriage; in fact, elevated NK levels in the blood has been shown to be predictive of RSM. While direct cell-mediated lysis of trophoblast cells by NK cells has not been demonstrated, it has been proposed that NK cells, like activated Th1 cells, could release inflammatory
cytokines that are harmful to the trophoblast. Hill and Choi speculate that cells in the decidua respond to trophoblast invasion by generating a Th1-dominated response which could be detrimental to early placental growth and may be toxic to the development of the embryo\cite{59}. Alternatively Th1 cytokines may convert NK cells to lymphokine-activated killer (LAK) cells that have been shown to lyse trophoblast cells; levels of LAK cells in the blood correlate with high miscarriage rates\cite{61}. Decidual NK cells are not cytolytic, but produce IFNγ which activates decidual macrophages which then secrete toxic levels of nitric oxide\cite{61}. For their part, activated macrophages may bring about damage to the conceptus not by direct lysis of trophoblast cells, but via the production of nitric oxide and TNFα\cite{62}. The role of Th1 cytokines in this scenario would be to activate such cellular effectors and to also cause apoptotic damage to the placenta.

Th1 cytokines, such as TNFα and IFNγ, may directly damage the conceptus by apoptosis of trophoblast cells\cite{37} and by inhibiting the secretion of the growth-stimulating GM-CSF from the uterine epithelium\cite{36}. Clark et al propose that maternal “rejection” of the implanted conceptus may be due to the process of what they term “cytokine-triggered vascular autoamputation” which involves the activation of coagulation mechanisms, leading to vasculitis affecting maternal blood supply to the implanted embryo\cite{53}.

Cytokine Patterns in Preterm Delivery

Preterm delivery (PTD) is an important cause of perinatal morbidity and mortality and a condition for which there is a dearth of treatment modalities\cite{64}. It occurs at a rate of 12.5%. Preterm labor is believed to be initiated by inappropriately early activation of elements that initiate normal parturition at term. Factors that predispose to preterm labor and preterm delivery include premature rupture of fetal membranes, pregnancy-induced hypertension, amniotic fluid infection, faulty placenta, serious maternal disease and genetic factors. Several factors such as changes in the levels of progesterone, oxytocin, relaxin, prostaglandins, cortisol, and corticosteroids have been studied in relation to the onset of PTD; however, the etiology of many cases of PTD is unexplained\cite{58}.

There is strong evidence available to suggest that pro-inflammatory cytokines are involved in the sequence of events leading to preterm labor and delivery associated with intrauterine infection. Increased levels of the pro-inflammatory cytokines IL-1, TNFα, IL-6 and IL-8 have been found in the amniotic fluid of women with infection-associated preterm labor\cite{58}. Higher levels of IFNγ in cervicovaginal fluid, IL-1, TNFα and IL-6 in placental cells and IL-1β, IL-6 and IL-8 in amniotic and chorionic-decidual tissues and in cervical secretions\cite{57} are found in PTD as compared to normal term delivery. Indeed Romero et al propose that preterm labor in the setting of infection results from the actions of pro-inflammatory cytokines secreted as part of the fetal and/or maternal host response to microbial invasions and suggest that a fetal pro-inflammatory cytokine response is followed by the onset of spontaneous preterm parturition\cite{58}. Dudley suggests that pre-term labor associated with subclinical infection may trigger a dysregulation of a local inflammatory response leading to a so called “intra-uterine inflammatory response syndrome” leading to pre-term labor and delivery\cite{59}. Even in the absence of intrauterine infection, pre-term labor has been shown to be associated with enhanced placental cytokine production; elevated levels of IL-1, IL-6 and IL-8 have been demonstrated in premature parturition with no signs of infection\cite{60}.

We have demonstrated that higher levels of the Th1 cytokines IL-2 and IFNγ are produced by women with unexplained PTD, while greater concentrations of the Th2 cytokines IL-4, IL-5 and IL-10 are produced by mitogen-stimulated peripheral blood lymphocytes from women with normal pregnancy\cite{61}. Furthermore, the ratios of Th1 to Th2 cytokines in PTD versus normal term delivery are indicative of a bias toward stronger pro-inflammatory cytokine reactivity in PTD. These observations support the existence of a so-called intrauterine cytokine-based inflammatory response syndrome which may account for preterm labor with both infectious and non-infectious etiologies, suggesting that the production of inflammatory cytokines may form the pathophysiologic basis for this association.

Thus, we can view the positive and negative influences of the immune system on pregnancy as a sort of balancing act that needs to be performed to ensure the success of pregnancy (Fig. 1).

Based on these observations, therapies that down-regulate Th1 cytokine reactivity may well be valuable in the clinical management of recurrent miscarriage and prematurer delivery and delivery with a predominant Th1 cytokine bias. Various strategies are worth pursuing; these include the down-regulation of Th1 cytokines, the neutralization of Th1 cytokines and the upregulation of Th2 cytokines.

MODULATION OF CYTOKINE RESPONSES TOWARDS A PREGNANCY-FRIENDLY PATTERN

The demonstration of an association between conditions such as RSM and PTD and maternal Th1 cytokine bias on the other, has led to research on
Fig. 1: The possible influences of maternal Th1 and Th2 immune reactivity on pregnancy. Th1-type cytokines acting directly and via effector cells such as activated macrophages, activated natural (NK) cells and lymphokine-activated killer (LAK) cells may lead to pregnancy complications and pregnancy loss. On the other hand, Th2 cytokines, progesterone, progesterone-induced blocking factor (PBBF), cytokines such as IL-3, GM-CSF and CSF-1 that have placental growth-promoting properties help prevent Th1-mediated deleterious effects on the conceptus.

manipulating the cytokine balance so as to down-regulate pro-inflammatory cytokines such as IFN-\(\gamma\) and TNF-\(\alpha\); this is expected to create an environment that is more conducive to the success of pregnancy. One approach would be to use a hormone such as progesterone, which has been shown to have anti-inflammatory and immunosuppressive properties\(^{[23]}\). Piccinni and colleagues demonstrated that progesterone induces the development of human T cells producing Th2 cytokines\(^{[23]}\). They propose that as the Th1 cytokines IFN-\(\gamma\) and TNF-\(\alpha\) may compromise pregnancy, the production of Th1-inhibitory Th2-type cytokines may allow allograft tolerance and fetus survival and that progesterone-mediated immunosuppression is needed for the “natural” maintenance of normal gestation.

These observations inspired us to investigate dydrogesterone (6-dehydro-9\(\beta\), 10\(\alpha\)-progesterone) for potential immunomodulatory properties. Dydrogesterone is a potent orally-administered progestogen, similar to endogenous progesterone in its molecular structure and pharmacological effects, with a high affinity for the progesterone receptor. We exposed lymphocytes from women with RSM or with PTD to the progestogens, dydrogesterone and progesterone, and observed that dydrogesterone brings about a significantly reduced secretion of the Th1 cytokines IFN-\(\gamma\) and TNF-\(\alpha\), as does progesterone. On the contrary, levels of IL-4 and IL-6, both Th2 cytokines, are significantly elevated in the presence of dydrogesterone or progesterone. Thus, the levels of Th1 cytokines decreased significantly while levels of Th2 cytokines increased significantly when cells were exposed to dydrogesterone or progesterone\(^{[23]}\). We found a marked reduction in the ratios of Th1 to Th2 cytokines indicating a decrease in Th1 cytokine bias in lymphocytes from both women with RSM\(^{[23]}\) and with PTD\(^{[24]}\).

In order to ascertain whether dydrogesterone and progesterone mediate their cytokine-modulating effects via the progesterone receptor, we tested the influence of the progesterone-receptor antagonist, RU486 on cytokine modulation by dydrogesterone and progesterone. Since RU486 competes with progesterone (and dydrogesterone) for the progesterone-receptor, if the addition of RU486 reverses the effects of these substances, it would indicate that dydrogesterone and progesterone have to interact with the progesterone-receptor in order to affect cytokine production. When PBMC from subjects with unexplained RSM were cultured with dydrogesterone or progesterone in the presence or absence of RU486, we observed increased levels of IFN-\(\gamma\) and TNF-\(\alpha\) which are otherwise suppressed by dydrogesterone and progesterone, and decreased levels of IL-4 and IL-6 which are otherwise upregulated by dydrogesterone and progesterone. Thus RU486 reverses the effects of dydrogesterone indicating that the effect of dydrogesterone and progesterone is mediated via the progesterone receptor\(^{[23]}\).

Our data based on laboratory studies indicate that significantly lower levels of the Th1-type cytokines IFN-\(\gamma\) and TNF-\(\alpha\) are produced by lymphocytes exposed to dydrogesterone. These two cytokines have been shown to deter embryo development, implantation events and proliferation of the trophoblast\(^{[34]}\) and to have apoptotic effects on human trophoblast cells\(^{[36]}\). In animals, these cytokines bring about fetal demise when injected during gestation\(^{[34]}\). Furthermore, IFN-\(\gamma\) and TNF-\(\alpha\) may mediate placental / fetal damage via activation of NK activity and macrophages\(^{[32]}\), both of which in their activated states have been shown to have deleterious effects on the embryo. Thus, studies demonstrating an association between high levels of these Th1 cytokines and unexplained RSM indicate a potential benefit in down-regulating their production.

We found that IL-4 and IL-6, both Th2 cytokines, are upregulated in the presence of dydrogesterone\(^{[22,24]}\). Increased production of IL-4 has been shown to favor further Th2 bias which would affect the eventual outcome of Th1/Th2 dichotomy\(^{[34]}\). It is relevant to note that progesterone has been shown to promote the differentiation of T cells into Th2 effectors and is
proposed to be responsible for a Th2 switch at the maternal-fetal interface during normal, successful gestation[20]. In our studies, progesterone was tested at a concentration (10^{-5} \text{ mol/l}) that is similar to that achieved at maternal-fetal tissues. Thus the increased production of the Th2 cytokine IL-4 and the decreased production of the Th1 cytokines IFN-\gamma and TNF-\alpha together could well result in a substantial swing in Th1/Th2 reactivity towards the pregnancy-conducive Th2 profile and away from the potentially harmful Th1 profile.

In summary, considering that inflammatory cytokines such as IFN-\gamma and TNF-\alpha may have effects that are detrimental to pregnancy and may lead to miscarriage, a shift in cytokine production patterns away from a predominance of these cytokines may well lead to the prevention of pregnancy loss due to miscarriage.

As far as PTD is concerned, a number of recent publications by several investigators supports the idea that progesterone should be considered for preventive therapy in women with a history of spontaneous preterm delivery. Several promising clinical studies have been reported on the use of 17\alpha-hydroxyprogesterone caproate (17P), a progestogen that is structurally related to progesterone and dydrogesterone and that has been used for recurrent miscarriage and various menstrual disorders in women presenting with a history of spontaneous PTD. Administration of 17P in a clinical trial resulted in a significantly lower occurrence of PTD as well as a reduction in the risk of low birth weight[63]. A recent double-blind, placebo-controlled trial in women with a history of spontaneous PTD showed that weekly injections of 17P led to a substantial decrease in the rate of recurrent PTD as well as a reduction in the likelihood of perinatal mortality and very low birth weight infants[63]. Further analysis of this data revealed that the use of 17P not only reduces the overall risk of preterm delivery but also reduces the risk of preterm birth in women with a history of PTD[64].

A recent meta-analysis of randomized controlled trials concluded that patients treated with 17P had lower rates of PTD. Administration of 17P or progesterone suppositories in a clinical trial led to a significant protective effect against PTD in six out of seven published clinical trials[65]. Taken together, these results suggest that patients who have had a prior spontaneous preterm birth may benefit from progesterone therapy.

Progesterone reduces intracellular calcium levels and reducing uterine contractility, and promotes myometrial relaxation, thus sustaining uterine quiescence. This relaxant effect of progesterone on the uterus in addition to its ability to inhibit the oxytocin effect of prostaglandin and stimulation of alpha-adrenergic receptors may explain its ability to prevent preterm labor and delivery; our studies described here suggest a possible additional, non-mutually-exclusive mechanism to elucidate the protective effect of progestogens in preterm delivery. Based on evidence that indicates an inflammatory bias in preterm labour and delivery, shifting the cytokine production profile away from an inflammatory bias in the uterus may well lead to the prevention of preterm labour and delivery.

Hill et al propose that potentially immunosuppressive doses of progesterone which has been termed “nature’s immunosuppressant” may benefit individuals in whom the etiology of RSM related to maternal Th1 cytokine predominance[22]. However, progesterone administered orally is poorly absorbed, is subject to first-pass mechanism, has a short biologic half life[65], loses much of its bioactivity[67] and is rapidly cleared[68]. Therefore the orally-active progestogen dydrogesterone is quite attractive from this perspective.

Our data indicates that dydrogesterone, a progestogen currently indicated for progesterone-related pregnancy disorders, has an immunomodulatory capability – in vitro it appears to be able to induce a maternal cytokine shift from Th1 cytokine dominance towards a Th2 bias, which has been described as being conducive to successful pregnancy[23,24,69]. Our observations are based on in vitro studies; if clinical trials confirm the immunomodulatory, cytokine-redirecting capability of dydrogesterone, then this molecule may well serve as an effective, safe and orally-administered therapeutic intervention in unexplained RSM and PTD.

In summary, it appears that there are very strong interactions between the immune system and the reproductive system; cross-talk between these two systems seem to be both positive and negative. The maternal immune system seems to contribute to the success of pregnancy, by stimulating responses that protect, rather than harm, the fetus. However, inappropriate maternal immune responses seem to be capable of compromising pregnancy. Much of this cross-talk between the immune system and the conceptus is done by cytokines, with the Th2 type of cytokine pattern nurturing the conceptus and the Th1 type of pattern having deleterious effects on the conceptus. Future studies will no doubt focus on the development of modalities for preventing and treating such cytokine-mediated complications.

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Comparative Efficacy of Two Methods of Skin Preparation of the Perineal and Genital Skin of Male Urological Patients

Elijah O Kehinde¹, Wafaa Jamal², Yousif Ali¹, Fathima Khodakhast³, Mohammed Sahsah¹, Vincent O Rotimi²
¹Departments of Surgery (Division of Urology) and ²Microbiology, Faculty of Medicine, Kuwait University, Kuwait

ABSTRACT

Objective: To compare the efficacy of two methods of skin antiseptic preparations of the genitalia and perineum in male urological patients

Design: Prospective study

Setting: Mubarak Hospital, Kuwait

Subjects: Adult male patients of two study groups numbering 114 (group-1) and 117 (group-2) admitted for cystoscopic procedures

Intervention: The perineum and genitalia of patients in both groups were prepared by applying chlorhexidine-cetrimide mixture (CCM) and CCM plus povidone-iodine solution respectively.

Main Outcome Measures: Swab specimens were obtained from the perineum and genitalia, before cleaning and disinfection (specimen A), after disinfection and draping (specimen B) and after the completion of the operative procedure (specimen C). Specimens were cultured on appropriate media and representative colonies identified by standard methods.

Results: In groups 1 and 2, the A specimen yielded bacterial growth in 35.1 and 63% of patients, respectively. The commonest isolates in both groups were Gram-positive bacteria (89.2%) while Gram-negative bacteria accounted for only 10.8%. The B and C specimens in group-1 yielded positive bacterial culture in 7.1 and 11.4% patients respectively. In group-2, specimens B and C yielded bacterial growth in 5.1 and 2.6% patients respectively. In both groups, there was a significant reduction of patients with culture-positive B specimens after skin disinfection (p < 0.001). The isolation rate of bacteria in specimen C in group-2 was significantly lower than group-1 patients (p < 0.001).

Conclusion: The addition of povidone-iodine to the CCM based regimen of perineal skin antiseptic preparation is associated with longer and more effective skin disinfection in male urological patients.

INTRODUCTION

The perineal route is increasingly being used in male urological patients for diagnostic and therapeutic procedures. These procedures range from transperineal prostate biopsy to insertion of artificial urinary sphincter (AUS) for stress urinary incontinence. Although it is generally assumed that the perineum is often heavily contaminated with microorganisms derived from the rectum, there is paucity of information in the literature regarding infectious complications after performing diagnostic or therapeutic maneuvers via the percutaneous transperineal route. With increasing use of the perineal route in urological practice, it is essential to study the resident bacterial flora of the male perineum so as to provide baseline data that could help in predicting the causative agents in post-operative perineal infections and possibly help in developing a policy for appropriate antibiotic prophylaxis or empirical treatment of such infections. This is extremely important when implants like AUS are inserted in the perineum. It has been shown that infection of the prosthetic implant is almost always due to organisms that are part of the patient’s skin flora, with Staphylococcus spp. being the commonest infecting organisms. The prevention of surgical site infections (SSIs) has helped to revolutionize surgery from a practice plagued by frequent infections and death into one that is more acceptable in the discipline today. However, infections related to surgery continue to remain a problem.

Data from the USA Center for Disease Control and Prevention (CDC), through the National Nosocomial Infections Surveillance (NNIS) System, indicate that...
SSIs are the third most common infection reported, accounting for 14-16% of all nosocomial infections[3-6]. Evidence also shows that SSIs are the most common nosocomial infection in surgical patients[7]. In addition, apart from costlier hospital stay, patients who develop SSIs carry an overall two-fold higher mortality rate, which is independent of their initial surgical risk and other survival predictors[8-10]. Thus, the prevention of this surgical complication represents a significant impact not only on cost and quality of life but also on mortality.

The goal of surgical prophylaxis is not to sterilize a patient but rather to decrease the bacterial burden at the surgical site. Prophylaxis augments the host’s natural immune defense mechanisms by increasing the magnitude of a bacterial inoculum needed to cause an infection[11]. Since the cause of post-operative infection can be multifactorial, so the prevention efforts should be multifactorial as well. Apart from the appropriate use of prophylactic antibiotics to reduce SSIs, other factors that are important include understanding the microbiology of the surgical site and resultant infections[12]. In most instances of SSIs, the patient’s endogenous flora is largely the main etiological factor[13]. Theoretically, the combination of surgical site disinfection and appropriate systemic prophylactic antibiotics should decrease the chances of wound infection. However, the use of pre-operative cleansing with an antibacterial solution, such as chlorhexidine or other antiseptics has been shown to decrease the bacterial burden of normal skin flora[14,15]. Unfortunately, this has not been definitely shown to translate into decreased rates of SSIs[16]. Hence, the aim of this study was to compare the efficacy of a single antiseptic versus combined antiseptic on perineal skin preparation.

SUBJECTS AND METHODS

The Patients

Between 2003 and 2007, all consecutive male patients undergoing in-patient cystoscopic procedures including transurethral resection of the prostate (TURP) and transurethral resection of bladder tumor (TURBT) in the urology unit of our hospital were included in this study. Informed consent was obtained from each patient after careful explanation of the scope of the study. In addition, approval was obtained from the local Ethics Committee. The biodata of each patient, including age and underlying diseases, were carefully recorded in a protocol sheet. Adult male in-patients undergoing cystoscopic procedures were randomized into two groups for perineal and genital skin disinfection. Patients were anesthetized and then placed in the lithotomy position on the operating table. In group-1 patients, the perineal and genital areas were scrubbed three times using savlon® (chlorhexidine cetrimide mixture [CCM]) only. In group-2 patients, the area was scrubbed twice initially using savlon® and a third time with betadine® (povidone-iodine solution).

The Specimens

Before scrubbing the perineum and genitals with antiseptics described above, specimens were taken from a midline perineum between the root of the penis and 5 cm from the anal orifice using commercially available albumin-coated sterile cotton wool swab (Medical Wire and Equipment Co Ltd, Corsham, Wilshire, England). The perineum was rubbed up and down 4-6 times with the same swab and then dipped into semisolid Amies’ transport medium. This first swab specimen was labeled specimen A. After application of the antiseptic regimen and draping of the patient, a second specimen labeled B was obtained. On completion of the surgical procedure and after removal of all drapes, but before the patients limbs were removed from the stirrups, a third swab specimen labeled C was taken. All swab specimens were taken in duplicates. Time taken to complete the surgical procedure was carefully recorded. All patients received antibiotic prophylaxis prior to undergoing surgical procedures. This consisted of intravenous amikacin 500 mg in patients with normal renal function or ceftriaxone 2 g in those with significant renal impairment (serum creatinine > 250 µmol/l). All specimens were transported immediately to the Anerobic Reference and Hospital Infection Laboratory (Department of Microbiology, Kuwait University, Kuwait) and processed within one hour of collection.

The temperature chart of patients was checked 24 and 48 hours after the surgical procedures. Patients with temperature > 38 °C had urine and blood cultures taken and were treated using appropriate antibiotics.

Microbiologic Investigation

In the laboratory one of the duplicate swabs was placed in 2 ml sterile thioglycolate broth (Oxoid, Basingstoke, Hampshire, UK) in sterile universal bottles and vigorously vortexed. Viable count was performed on the eluted suspension by serial 10-fold dilutions of 100 µl samples in sterile Eppendorf tubes containing 900 µl of appropriate sterile broth with a final dilution ranging from 10¹ to 10⁶. Dried agar plates were marked into six sectors, and three 10 µl aliquots of each dilution were plated onto the three sectors. Once dry, plates were incubated in appropriate incubators overnight at 37 °C. Colonies were counted in sectors containing a measurable number in triplicate and the number of colony forming units per millilitre (cfu/ml) calculated. The other swab was routinely streaked onto a set of selective and non-selective media including MacConkey agar (Oxoid, Basingstoke, UK), Brucella agar (Oxoid) supplemented with 5% horse blood,
Blood agar (Oxoid) supplemented with gentamicin 75 µg/ml, vitamin K1 1 µg/ml, haemin 1 µg/ml and L-cysteine HCL 5 µg/ml and plain Blood agar (Blood agar base, [Oxoid], plus 5% blood). The second swab was inoculated onto the surface of similar media. A set of inoculated Brucella agar, gentamicin Blood agar and plain Blood agar plates were incubated for 2-5 days anaerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile agar base, [Oxoid], plus 5% blood). The second swab for 2-5 days anerobically in jars rendered anerobic using the Anoxomat WS 8000 machine (MART Microbiology, Holland). The MacConkey and another Blood agar were incubated in air at 37 °C for 24 hour. Representative colonies were gram-stained and identified by the automated VITEK ID system (BioMerieux Inc., Hazelwood, MO, USA) and API 20A (BioMerieux Inc., Marcy Etiole, France). Sterile
DISCUSSION

The ideal antiseptic is one that is rapidly lethal to all forms of bacteria and their spores, capable of bactericidal activity for a prolonged period, has no injurious effects on wound tissues or skin, delineates the operation site and is easily applied and removed[15]. The antiseptic used in skin preparations and the sequence in which they are used is a matter of individual surgeon’s preference with variations from unit to unit and even within the same surgical unit as shown by McGrath and McCorry[16] in a survey of pre-operative skin preparation practices in surgical units and among surgeons in Northern Ireland. The outcome of our current study seems to justify our unit’s policy of using Savlon® as the antiseptic of choice for perineal skin disinfection prior to endoscopic procedures as its use was associated with significant reduction in the number of patients with culture-positive bacteria as well as in the quantity of the bacterial isolates as demonstrated in specimens B compared with samples A in both groups of patients. Our study further demonstrated that instead of using Savlon® alone to clean three times, the addition of an iodine-based antiseptic agent like Betadine® appears to guarantee better and more efficient disinfection. The use of Savlon® twice followed by further disinfection with Betadine reduced positive microbiological culture rate to 2.6% in swab C of group-2 patients, compared to 11.4% in group-1 (p < 0.001). This is because iodine containing antiseptics like Betadine®, in addition to having strong bacteriostatic activity are also known to kill bacteria, fungi, viruses and spores[17,18]. The bacteria isolation rates from the specimens of the two patient groups were significantly reduced (p < 0.001) post-disinfection of the perineum and genitalia.

In this study, our patients had about six-fold higher isolation rates of Gram-positive bacteria than the Gram-negative bacteria, a finding that was statistically significant (p < 0.001). Of these Gram-positive bacteria, S.haemolyticus was by far the isolate most frequently present in the perineum of these patients, including those with or without underlying co-morbidity. This finding is essentially similar to the reports of Magera et al[11] and Larson et a[19] which also found S.haemolyticus as the commonest isolate in their patients compared with S.hominis in controls. The next fairly common isolate was S.epidermidis but we did not isolate S. hominis or S.aureus from any of the patients which essentially also supports earlier report[19]. Of particular concern are the high isolation rates of the coagulase-negative staphylococci (CoNS) which are increasingly becoming important in nosocomial infections and infections in immunosuppressed individuals.

Post-operative septicemia occurred in more patients in group-1 compared to group-2, although the difference was not statistically significant. All the patients had endoscopic procedures; therefore they would be expected to have a low septicemia rate. Importantly, the efficacy of adding Betadine to the perineal skin antiseptic preparation regimen is demonstrated by the drastic reduction in the number of patients with positive perineal culture and the bacterial load post-disinfection as well as the significant lower septicemia rate in group B patients.
CONCLUSION

Both methods of perineal and genital skin disinfection resulted in a significant reduction of bacterial isolates in specimen B compared to specimen A (p < 0.001). Group-2 patients had lower bacteria isolation rate from specimen C compared to group-1 patients (p < 0.01). These data demonstrate that the addition of Betadine® to the regimen of perineal and genital skin antiseptic preparation promotes longer and a more effective perineal and genital skin disinfection in adult male urological patients.

REFERENCES

The Results of Thoracoscopic Surgery for Secondary Spontaneous Pneumothorax

Adel K Ayed¹, Chazian Chandrasekran²
¹Department of Surgery, Faculty of Medicine, Kuwait University, and ²Chest Diseases Hospital, Kuwait

ABSTRACT

Objective: To review our experience of video-assisted thoracoscopic surgery for the treatment of secondary spontaneous pneumothorax caused by bullous emphysema

Design: Prospective study

Setting: Chest Diseases Hospital, Kuwait

Subjects: Forty-six consecutive patients who underwent thoracoscopy for secondary spontaneous pneumothorax by a single surgeon during a five year period

Intervention: Video-assisted thoracoscopic bulllectomy and pleural sympysis procedure

Main Outcome Measure: Resolution of pneumothorax

Results: Mean age of patients was 49.3 years (range: 38 - 70 years), and 44 were men (96%). All patients had bullous emphysema; their mean preoperative forced expiratory volume in one second (FEV1) was 54.4% of predicted and mean forced vital capacity (FVC), 66.9% of predicted. Persistent pneumothorax was the most frequent indication for surgery, occurring in 35 patients (76%). The most common method of management was stapling of an identified bulla, which was done in all patients. Pleurodesis was achieved by gauze abrasion (n = 23) and apical pleurectomy (n = 23). Postoperative prolonged air leak occurred in seven patients (15%), six in the pleural abrasion group and one in the apical pleurectomy group (p = 0.04). The mean (± SD) postoperative hospital stay was 5.7 ± 4.5 days. Mean follow-up is 42 months (range = 36 - 54 months) for all patients. Pneumothorax recurred in three patients (6.5%) in whom pleural abrasion was done. The recurrences occurred in the first six months of follow-up.

Conclusions: Video-assisted thoracoscopic surgery is a safe procedure in the treatment of select secondary spontaneous pneumothorax caused by bullous emphysema. Apical pleurectomy is a more effective way of producing pleural sympysis.

INTRODUCTION

Spontaneous pneumothorax (SP) can be divided into primary SP resulting from rupture of subpleural blebs, and secondary SP, which is related to the presence of an underlying lung disease (e.g., bullous emphysema, tuberculosis). The indications for surgical treatment include persistent air leak, recurrent SP, contralateral SP, and SP in a high-risk occupation, such as pilot or diver. The aims of surgical treatment are to close the site of the air leak, to allow full re-expansion of the lung, and to prevent future recurrence. The use of video-assisted thoracoscopic surgery (VATS) has been advocated and used as an alternative to thoracotomy in the treatment of primary SP. The standard surgical treatment for secondary SP is through a thoracotomy approach, with very low recurrence rate. Surgical intervention by means of thoracotomy in the setting of secondary SP is associated with a much higher morbidity than in the setting of primary SP. The role of VATS and its long-term results for patients with secondary SP is still unclear. The aim of this study was to describe our experience in Kuwait and to report on the long-term follow-up of 46 consecutive patients with secondary SP caused by bullous emphysema.

SUBJECTS AND METHODS

The study was conducted at the Chest Diseases Hospital, which is the only center for the surgical treatment of chest disorders in Kuwait. From January 2002 to December 2005, 46 patients with persistent or recurrent secondary SP were treated by VATS; these patients comprise the study subjects. Preoperative investigations included a chest radiograph, a computed tomography of the chest, complete blood count, serum
electrolytes, renal function tests, and spirometry. The study was approved by the local ethical committee.

Operative Technique of VATS
The patient was administered general anesthesia using a double-lumen endotracheal tube to allow single lung ventilation. The patient was placed in a posterolateral thoracotomy position. A 10-mm trocar was introduced through 1.5-cm skin incision in the eighth intercostal space at midaxillary line for insertion of a 0° videothoracoscope (Karl Storz; Tuttingen, Germany). Two additional ports were then inserted under direct vision: a 12-mm trocar through the fifth intercostal space on the anterior axillary line, and a 12-mm posterior trocar through the fifth intercostal space near the tip of the scapula. The bulla was identified and grasped with empty sponge stick. The excision was done by using an ENDO-GIA stapling device (Auto Suture Company; United States Surgical Corp; Northwalk, CT). Then, a parietal pleural abrasion by gauze or apical pleurectomy was performed. Pleurectomy was performed with a hook electrocautery; the longitudinal limit of the resection ran in an apical direction along the sympathetic trunk to the height of the left subclavian artery or the brachiocephalic trunk on the right side. The pleura was incised at least one cm away from the sympathetic trunk. Then it was grasped with the endograsper, raised, and divided with the dissector. Once the plane was developed pleural stripping was achieved by lifting the pleural flap with the aid of a gauze pledged. The area of pleurectomy requires precise hemostasis. None of the patients had bleeding complications. A 28 F chest tube was inserted through the inferior incision in the eighth intercostal space and connected to underwater seal suction with a negative pressure of 20 cm H₂O.

Postoperative Care
All patients were extubated in the operating room and transferred to the thoracic surgery ward. Antibiotic in the form of cefoxitin was give to all patients. An analgesic, pethidine, was administered IM every 4 to 6 h according to patient request, and an oral analgesic (acetaminophen) was given as needed. The intercostal drain was removed when the underlying lung was fully expanded with no air leakage and < 100 ml pleural fluid drained through the tube for 24 h. All patients were discharged the day after removal of the chest tube.

Postoperative Assessment
Data recorded for all patients included the number of episodes of pneumothorax, and the operative time. The output and the duration of the pleural drainage after operation, the amount of analgesia given in the first 24 h after the operation, length of hospital stay, postoperative air leak, and recurrences were also recorded. The follow-up chest radiograph was done at intervals of one week, one month, and three months, and then the patients were followed up with a telephone communication for this study. The recurrence was proved by chest radiography during follow-up period.

Statistical Analysis
Data were expressed as mean ± SD. Data analyses were made using SPSS software windows version-8 package (SPSS, Chicago, IL). The cut-off level for statistical significance was a p-value of less than 0.05. The unpaired Student’s t test was used to assess the significance between means of variables in the groups. The Pearson $\chi^2$ test was used to ascertain the significance of association between two categorical variables. The $\chi^2$ test was replaced by Fisher’s exact test if the cell frequencies of any of the 2 $\times$ 2 contingency tables went below five.

RESULTS
This series included 44 male and two female patients (mean age, 49.3 ± 10.3 years, range 38 to 70 years). In all cases, secondary SP was diagnosed on the basis of the existence of emphysematous bullous disease confirmed by chest radiographic appearance, computed tomography of the chest, and preoperative spirometry; the mean forced expiratory volume at one second (FEV1) was 54.4% of predicted (range, 43 - 110%); and the forced vital capacity (FVC) was 66.9% of predicted (range, 47 - 110%).

Thirty-five patients (76%) were operated upon when they had persistent air leak more than seven days. In eleven patients (24%), VATS was done because of a recurrent episode of SP. VATS was unilateral in all cases and all procedures were performed by the same surgeon, on the right side in 35 cases (76%) and on the left side in 11 cases (24%). Extension of the trocar incisions was necessary in three patients because of adhesions.

The operative time was 62.3 ± 9.8 minutes (range, 40 - 90 minutes). Pleural procedures performed included gauze abrasion in 23 cases (50%) and apical pleurectomy in 23 cases (50%). The clinical data on these procedures are shown in Table 1.

All patients were extubated at the end of the operation, and no patient required mechanical ventilation during the postoperative period.

The mean amount of postoperative analgesia in the form of pethidine was 101.9 ± 26.8 mg in the first 24 h.

The duration of chest tube drainage was 4.8 ± 4.5 days (range, 2-22 days). Seven patients (15%) had an air leak lasting more than five days. These patients
required prolonged pleural drainage for 7 to 22 days, and none required a re-operation. Air leak occurred in six patients after pleural abrasion procedures and one occurred after apical pleurectomy. The difference is statistically significant (p = 0.04). Air leak occurred in six out of 22 patients in whom multiple bullous disease was identified. Air leak occurred in one out of 24 patients with single bulla. The difference is statistically significant (p = 0.02).

The postoperative hospital stay ranged from three to 23 days (mean, 5.7 ± 4.5 days). There were no deaths in this series, and no patients required monitoring in the ICU.

All patients in this study were followed regularly (mean follow-up time, 42 months; range from 36 - 54 months). Recurrent ipsilateral pneumothorax occurred after three of the 46 procedures (6.5%). These occurred at four, 16, and 24 weeks after the original procedure. All these recurrences had occurred after pleural abrasion procedures and in patients with multiple bullous disease. Two patients underwent a re-operation by thoracotomy; excision of the air leak site and partial pleurectomy was performed. One patient who had recurrence at 24 weeks after the original procedure healed by chest drainage and chemical pleurodesis.

**DISCUSSION**

Videothoracoscopy is a rapidly developing technique that allows many surgical procedures to be performed without the need for thoracotomy. VATS allows inspection of the entire lung, identification of bullae, and resection of bullous disease. Previous reports of the use of VATS have concentrated on its use in the treatment of primary SP. VATS has become the surgical approach of choice in the management of select primary SP[4,19]. VATS bullectomy and mechanical pleurodesis carry long-term results that are comparable with those of thoracotomy[4]. For secondary SP, because patients are generally older and ill, the role of VATS approach is still unclear[7-9,10]. VATS for secondary SP has been shown to be associated with a higher morbidity[9]. Therefore, careful patient selection and improvement of the surgical technique are important factors for ensuring optimal outcome. In this series, we have successfully treated 46 patients with secondary SP caused by bullous emphysema using VATS procedure. This group represents a population with minimal co-morbidity who can tolerate selective one-lung ventilation and general anesthesia. However, problems with intraoperative desaturation were encountered. Two-lung ventilation was then necessary, but, to enable the procedure to continue, low-tidal-volume manual ventilation was employed while dissection or manipulation was performed. VATS causes less respiratory dysfunction than thoracotomy, thus improving postoperative recovery.

Short term results from this series were comparable with those reported in the literature[11-13]. The duration of postoperative chest tube drainage is determined by the presence of complete expansion of the lung and the absence of air leak. In the literature, the duration of postoperative drainage is variable. Waller et al[10] reported a mean duration of 6.3 days, Andres et al[12] reported 5.4 days, and Passlick et al[10] reported five days. We report a mean of 4.8 days (range, 2 - 21 days).

The postoperative hospital stay is determined mainly by the duration of pleural drain. Other factors of importance are postoperative pain and early mobilization. We have reported a mean hospital stay of 5.7 days (range, 3 - 23 days). The use of small incisions of VATS procedure has shown a trend toward shorter hospital stay. Passlick et al[10] have reported a mean hospital stay of 12.5 days, Andres et al reported 7.7 days, and Waller et al[10] reported nine days.

There were no intraoperative or postoperative deaths in this series. The most frequent postoperative complications was prolonged air leak lasting more than five days[10,12]. Seven patients (15%) in this series had prolonged air leak. Andres et al[12] have reported 25% incidence. Passlick and colleagues[10] found that 16.6% had prolonged air leak and all required a second intervention by lateral thoracotomy. The cause of the air leak problem is either an air leak on the raw surface of staples or missed bullous areas. Thus, the resection of the bullous area has to be done with care, and the entire lung should be inspected for other bullae. Passlick et al[10] have reported that incomplete pleurodesis without an obvious air leak is another factor for prolonged air leak. We have encountered the problem of postoperative air leak after bullectomy in emphysematous lung with patients who have multiple bullae, particularly on more than one lobe and in a position which are not easily dealt with using

**Table 1**: Results in patients with secondary SP treated by pleural abrasion or apical pleurectomy

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pleural Abrasion* (n = 23)</th>
<th>Apical Pleurectomy* (n = 23)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative time (min)</td>
<td>58.8 ± 9.2</td>
<td>66.7 ± 8.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Postoperative pleural drainage (ml)</td>
<td>338.2 ± 106.4</td>
<td>370.4 ± 98.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Analgesia requirement (mg)</td>
<td>94.3 ± 22.3</td>
<td>109.5 ± 29.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Chest tube duration (days)</td>
<td>5.3 ± 5.5</td>
<td>3.8 ± 2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>6.3 ± 5.5</td>
<td>4.7 ± 2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Postoperative air leak: n (%)</td>
<td>6 (26)</td>
<td>1 (4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Recurrence: n (%)</td>
<td>3 (13)</td>
<td>0</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Data presented as mean ± SD or n (%)
endoscopic stapling. Many surgical techniques have been described for resecting the bullae. One way is wedge resection using an endoscopic stapler.[3,14] Other methods, such as endoscopic stapling device that does not excise the bulla,[8,9], electrocoagulation, laser coagulation of bulla, or a combination of different methods, are favored by different authors.[8,14] Ogawa et al and colleagues have devised a method of spraying the staple line with aerosolized fibrin glue to seal the air leak sites.[15]

Our long term recurrence rate is three out of 46 patients (6.5%). All these recurrences occurred within six months after the intervention and two required a re-operation. One reason for recurrence is failure to recognize the site of the leak in the absence of bullous disease. Unrecognized bullae or inadequate resection of the diseased portion of the lung may also contribute. Another factor is inadequate pleurodesis, especially in between the trocar sites. These failures suggest that gauze pleural abrasion is probably less effective than apical pleurectomy. Like Tanaka et al, we found that the recurrences were more frequent in patients in whom multiple bullous disease was identified.[3]. It is for such patients that apical pleurectomy may be indicated, and this will probably provide more pleural adhesion with a decreased subsequent recurrence rate. It is not time-consuming, difficult to achieve or a source of postoperative bleeding as others have suggested, and is, therefore, preferred to pleural abrasion which has been associated with a higher recurrence rate.[16]. Our recurrence rate is comparable to that reported in the literature after thoracoscopy, which varies from four to 8.6%.[11,12,17]

CONCLUSIONS

VATS procedure can be done safely in the treatment of selected group of patients with secondary SP who are in good general condition. The procedure is well tolerated and allows early discharge usually within five days. It is now our procedure of choice and has become the routine approach for the treatment of bullous disease of the lung. Because of its less invasiveness, reduced morbidity and shortened postoperative stay, we tended to intervene slightly earlier in patients with persistent air leaks and even during the first episode of pneumothorax. VATS wedge excision and apical pleurectomy represent a satisfactory treatment modality in patients with bullous emphysema and secondary SP.

REFERENCES

INTRODUCTION

Natural cell-mediated cytotoxicity (NCMC) is a major component of innate cellular immunity against cancer and infection. NCMC is mediated primarily by leucocytes which perform natural killing (NK) and natural cytotoxicity (NC) without the requirement for prior sensitization and there is no immunological memory associated with this type of responses. This mechanism is characteristically and functionally different from the cell-mediated cytotoxicity of cytotoxic T lymphocytes. Cells mediating NK and NC are two distinct and probably related NCMC effector mechanisms which are distinguishable from each other by the time differences required to mediate their killing, as well as the kinetics of their appearance and decline in mice[1-3].

Natural killer (NK) cells belong to an important lymphocyte population that eliminates transformed cells and invading viral pathogens without any prior sensitization. These cells possess not only natural killing activity against non-self and altered-self cells but also exhibit cytokine production and antibody-dependent cell-mediated cytotoxicity (ADCC)[4]. It has been shown that NK cells might not serve merely as cytotoxic lymphocytes combating viral pathogens and malignant tumors, but must also be considered as important immunoregulatory cells with a significant influence on adaptive immunity[5].

The effector’s functions of NK cells are regulated by integrated signals across the array of stimulatory and inhibitory receptors engaged upon interaction with target cell surface ligands[6]. Intensive research during the 1990s has defined a large number of activating and inhibitory receptors[7-9]. NK cells are heterogeneous in their receptor repertoire, in the sense that different cells express different combinations of activating and inhibitory receptors. In addition, a functional heterogeneity is emerging, at least in the human. The majority of blood NK cells express moderate levels of CD56 in combination with various molecules of killer cell immunoglobulin receptor (KIR) family[10]. In addition, there is a small subpopulation of blood NK cells that express high levels of CD56 in combination with the inhibitory receptor NKG2A, and no receptors of the KIR family. This subset has low perforin levels and seems to be specialized for high cytokine secretion rather than...
direct killing. The latter subset may be the only NK cells present in the lymph nodes, an organ that was initially thought to be completely devoid of NK cells[11]. Human NK cells can be divided into two functional subsets based on their surface expression of CD56; CD56 (bright) immunoregulatory cells and CD56 (dim) cytotoxic cells. The importance of early hematopoietic growth factors, such as c-kit ligand and flt-3 ligand, and their synergy with IL-15 in the development of human NK cells in the bone marrow has permitted the investigation of novel cytokine combinations for optimizing in vivo expansion of NK cell in the clinic. The importance of lymph nodes as a site for NK cell development has recently been elucidated[12].

The major differences between NK and NC are the receptors and mechanisms involved in cytotoxicity[13-15]. The initial step in the mechanism of cytotoxicity of NK and NC is the recognition and binding of effector cells to tumor cells via receptor-ligand interactions. A number of these receptors have been identified on human and rodent leucocytes mediating NK cytotoxicity. To date, however, only two such receptors, NC-1.1 and NC-2 were recognized[16,17]. NC-1.1 was identified by a mouse anti-mouse monoclonal antibody (mAb) 1C4 (anti-NC-1.1). It is a monomeric phosphoprotein of 45,000 MW expressed on the surface of predominantly large and granular leucocytes of different hemopoietic cell lineages[16]. Flow cytometric analysis showed that NC-1.1 is expressed on less than 5% of fresh CBA mouse spleen cells and 20-50% of CBA- interleukin-3 cells. In vitro treatment of spleen cells from a number of inbred mouse strains with anti-NC-1.1 (1C4), markedly decreased NC activity of spleen cells against 51Cr-labeled WEHI-164 targets[16]. Administration of a single dose of 1C4 in a number of mouse strains depletes NC activity from the spleens of mice for at least one week, with the maximal effect occurring 24 hours after treatment[18]. NC-2 was identified by a rat anti-mouse monoclonal antibody D9 (anti-NC-2). It is a 50,000 MW molecule expressed on the surface of mainly large and granular leucocytes in mice. This receptor is expressed on < 6% of splenic leucocytes of different inbred mouse strains. Pretreatment of (CBA × C57BL/6) F1 mouse spleen cells with different doses of mAb D9 in vitro blocked NC against WEHI-164, whereas NK activity against YAC-1 was not affected[19]. By culturing CBA mouse spleen cells in interleukin-3 conditioned media, a stable cell line called mast cell line (MCL) was generated[19]. It has been shown that this cell line has high natural cytotoxicity against WEHI-164 tumor target cells and was used as a NC like cell line to characterize natural cytotoxic cells. These cells expressed NC-1.1 receptor but not characteristic cell surface markers of T, B lymphocytes, macrophages, or NK cell. Pretreatment of MCL cells with anti-NC1.1 antibody blocked the NC activity of these cells by approximately 70%[19,20].

This article reports the expression of NC-2 on MCL cells, and the investigation of the activity of this receptor.

MATERIAL AND METHODS

Tissue culture medium (TCM)

The study was conducted in the cellular and molecular research center at Sharekord University of Medical Sciences, Iran. Approval of the ethical committee was obtained. Dulbecco’s modification of eagles medium (CSL, Melbourne, Victoria, Australia) was supplemented with 20 mm HEPES, 2 mM L- glutamine, 50 μM 2-mercaptoethanol, 0.15% sodium bicarbonate, 50 μg/ml gentamicin and 10% fetal calf serum (FCS). The supplemented TCM was then further conditioned either with IL-3 for growth of MCL or with IL-2 for C57BL/6 IL-2 dependent cell lines. Cell lines and monoclonal antibodies.

NK-like MCL and NK-like C57BL/6-interleukin-2 (IL-2) dependent cell lines were generated[19,20].WEHI-164 (a BALB/c fibrosarcoma) was provided by Walter and Elisa Hall Institute, Melbourne, Australia. The mAbs used were anti-NC-2 mAb D9 (rat IgG2a) and anti-CD32/CD16 (rat IgG2b; clone 2.4G2, anti-Fc gamma receptor RII/RIII).

Flow cytometric analysis

MCL (NC like) and C57BL/6-IL2 (NK like) cells were harvested and washed in PBS containing 1% FCS at 5x10^7 cells / ml. One hundred micro liter of each cell suspension were aliquoted into each staining tubes. After blocking Fc receptors with 2.4G2 mAb, the cells were incubated either with 100 μl biotinylated D9 mAb and rat IgG2a isotype control mAb for 30 min on ice, followed by a further incubation with streptavidin–fluorescein isothiocyanate (FITC) complex (Amersham, Buckinghamshire, UK). After two washes, the cells were re-suspended in 250μl PBS and analyzed in the FACScan automated flow cytometer using the CELL QUEST software (Becton Dickinson Immunocytometry Systems, San Jose, CA). Five thousand cells were acquired for analysis in flow cytometer[20].

Immunoperoxidase Staining techniques

Fresh MCL cells were cytocentrifuged onto gelatin coated slides. The specimens were air dried and fixed in acetone for 10 minutes. The fixed cells were incubated with 100 μl of pretitred anti-Fc gamma receptor (2.4G2) for 30 minutes.
The slides were washed with PBS pH 9.6 and incubated with 100 µl of pretitered mAb D9 in a humid box at room temperature for 30 minutes. Endogenous peroxidase activity was quenched in a 2% w/v hydrogen peroxidase/methanol bath for 10 minutes. The specimen were then incubated with 100 µl strepavidin-conjugated Horse-Radish-Peroxidase conjugate (Amersham, Buckinghamshire, UK), followed by reaction with 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma, St Louis, MO,USA) for not more than 10 minutes. The cells were washed with tap water and counter stained with Carazzi's hematoxylin (Histo-Lab Fronine, NSW, Australia) with replacement of slides into Carazzi's hematoxylin for two minutes, followed by rinsing in tap water. The slides were then dipped in 1% acid alcohol for five seconds and then transferred into Scott's tap water for two minutes. The slides were then dehydrated through three lots of 70% and two of 100% ethanol and then mounted with natural mounting medium (Ajax Chemicals, Australia) for microscopic analysis.

Cytotoxicity assay
NC activity was assayed by in vitro lysis of 51Cr-labelled WEHI-164, as previously described[19]. The experiment was conducted in quadruplicate in 200 µl TCM in 96-well micro-titer trays. Briefly, MCL cells were incubated with 51Cr-labelled targets at effector / target (E : T) ratios in the range of 100:1-12.5:1. The cells were incubated at 37 °C / 5% CO₂ for 18 hr. Supernatants (100 µl) from each well were harvested and the level of radioactivity was measured in a COBRA gamma-counter (Packard Instrument CO., Downer’s Grove, Illinois). The results were first calculated as percentage specific lysis:

$$\text{Percent specific lysis} = \frac{\text{CPM (Sample)} - \text{CPM (Background)}}{\text{CPM (Total Release)} - \text{CPM (Background)}} \times 100$$

Background count per million (CPM) was obtained from wells containing target cells alone and total release was determined by counting 51Cr-labelled target cells. Lytic units (LU) were calculated from the linear portion of a graph of percentage specific lysis versus E:T ratios. One LU is herein defined as the number of effector cells which mediate 20% specific lysis of the target cells. Results are expressed either as LU per 10⁸ MCL or percentage reductions in LU according to the following formula:

$$\text{Percent reduction in LU} = \frac{\text{LU (control)} - \text{LU (D9 treated)}}{\text{LU (control)}} \times 100$$

For antibody blocking studies, the MCL cells were pretreated with mAb D9 or isotype matched mAb, washed and resuspended to the original volume prior to use in the cytotoxicity assay[21].

RESULTS
To show the expression of NC-2 on MCL and NK-like C57BL/6-IL2 cells, flow cytometric analysis was performed. Results showed that mAb D9 (anti NC-2 receptor) bound to more than
95% of NC-like MCL cells, but not to the NK-like C57BL/6-IL2 NK-like cells (Fig. 1). To confirm the flowcytomery finding, immunoperoxidase staining technique was carried out on fresh MCL cells; results approved the flow cytometric finding (Fig. 2).

Natural cytotoxic activity of MCL cells was assayed by in vitro lysis of $^{51}$Cr-labelled WEHI-164 cells (NC tumor target). Results demonstrated the maximal lysis of WEHI-164 target at 18 hours in the cytotoxicity assay. The lytic units of MCL cells were reduced by approximately 63% following pretreatment of cells with mAb D9 compared to the control cells treated with IgG2a isotype matched mAb (Fig. 3).

DISCUSSION

Our flow cytometry results showed that NC-2 receptor is expressed on about 95% of MCL cells, this was confirmed by immunoperoxidase staining technique. Furthermore, in a $^{51}$Cr-release assay, anti-NC-2 antibody (D9) blocked 63% of NC activity in vitro. Previous study showed that NC-2 is expressed on about 6% of (C57BL/6 × CBA) F1 and BALB/c cells and about 4% of C57BL/6 and CBA mice spleen cells. Anti-NC2 antibody blocked 60% of NC activity of splenic leukocytes in a cytotoxicity assay [17]. It has been previously reported that NC-1.1 is a molecule expressed on spleen cells of different mouse strains [14,16] and as reported in our previous publication the NC-1.1 receptor is also present on MCL cell line [19]. Further studies showed additional effects of anti-NC-2 (D9) and anti-NC-1.1 (IC4) on cytotoxicity of mice spleen cells and suggested that NC-1.1 and NC-2 are two different receptors on mouse spleen cells. Western blot analysis of affinity purified NC-1.1 and NC-2 indicated that the receptor identified by mAb D9 (anti-NC-2) is not the previously described NC-1.1 [17]. Co-expression of NC-1.1 and NC-2 on granular splenic leukocytes of (C57BL/6 × CBA) F1 mice was examined by dual color flow cytometry. Results showed that all NC-2 positive granular leukocytes co-expressed NC-1.1 whereas the converse was not true [17]. Our present finding suggests that, similar to previous reports on different inbred mouse strains, NC-1.1 and NC-2 are two distinct receptors which are expressed on MCL cells. Furthermore, it is possible that in MCL cells population there is a group of cells which may have both the receptors, which in turn probably abrogates the cytotoxicity of each individual receptor [22].

Recent studies on NK cells have identified multiple receptors which recognize ligands on the surface of target cells. These receptors belong to two multi-gene families, NKR-P1 and Ly-46, which display either activation or inhibition effects on natural killing [13]. Upon ligation to its specific ligand the receptor either transduces a positive signal to the effectors to kill the target cell or a negative signal to turn off the killing thus providing a delicate regulatory mechanism to control cytotoxicity [14].

Phosphorylation studies of NC-1.1 showed that key intracellular signaling pathway involving protein kinase C (PKC) and protein kinase G (PKG) interact to effect a coordinated control of NC. The fact that increase in phosphorylation up-regulates NC argues for NC-1.1 to be an activated receptor [14]. Biochemical studies to determine whether NC-2 is...
also a signaling receptor are in progress, and data
to date suggest that NC-2, like NC-1.1, may also
be an activation receptor. In order to achieve a
balanced control in the regulation of NC, it is likely
that inhibitory receptors also exist on leukocytes
mediating natural cytotoxicity.

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Phenotype and morphology of murine NC-1.1
Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Techniques

Ali A Dashti1, Mehrez M Jadaon1, Abdulsamad M Abdulsamad2, Hussein M Dashti3

1Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, Health Science Center, Kuwait University, Kuwait
2Department of Surgery, Amiri Hospital, Kuwait
3Department of Surgery, Faculty of Medicine, Health Science Center, Kuwait University, Kuwait

ABSTRACT

Objective: To evaluate the efficacy of two simple methods involving use of heat for extraction of bacterial deoxyribonucleic acid (DNA) be used in molecular techniques like polymerase chain reaction (PCR), restriction fragments length polymorphism (RFLP) and DNA sequencing and compare them with DNA extraction using commercial kits.

Design: DNA extraction by improved alternative methods and commercial kit.

Setting: Microbiology Research Laboratory, Faculty of Allied Health Sciences, Kuwait University, Kuwait

Material: Forty isolates of Klebsiella pneumoniae

Intervention: DNA was extracted from isolates by either boiling for 10 minutes or microwave irradiation for 10 seconds. For comparison, DNA was also extracted using a commercial kit. All extracted DNA samples were analyzed by PCR, RFLP and / or DNA sequencing of TEM and SHV genes of the bacteria.

Main Outcome Measures: Successful extraction of DNA

Results: PCR, RFLP and DNA sequencing gave the expected results in all the DNA samples extracted by all the three methods (boiling, microwave irradiation and the commercial kit). The results were qualitatively equivalent in all methods.

Conclusion: Heat may be used to extract DNA from K. pneumoniae which can be utilized successfully in performing PCR, RFL and DNA sequencing.

INTRODUCTION

The science of molecular biology has become an integral part of all medical research fields including bacteriology. Techniques including polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), hybridization techniques and DNA sequencing are being extensively used in identification and classification of different bacterial species and subspecies. In fact, many bacterial strains are now classified based solely on molecular characteristics[1-2]. Molecular techniques in bacteriology usually start with bacterial DNA extraction and purification. A large number of DNA extraction methods (performed manually or by automation) have been and are still being developed, each of which has its own advantages and disadvantages. Many of these methods are based on the traditional phenol-chloroform extraction method, which needs a variable number of reagents and equipment[3-4]. Moreover, several trials have been made to simplify the procedure for bacterial DNA extraction and purification. These methods tried to break the cells and release the DNA using certain lysing agents containing different chemicals like lysosyme, proteinase K, TWEEN20, sodium hydroxide/sodium dodecyl sulfate, guanidine isothiocyanate, and Triton X-100[5-14]. In addition to chemical agents, physical factors have also been attempted including heating, cooling, freezing, microwave irradiation, beads beating, magnetic field capturing, binding to glass beads, the use of ultrasound waves and passing through heat-exchanger coils and nylon filters[15-18].

Address correspondence to:
Prof. Hussein M Dashti, Department of Surgery, Faculty of Medicine, PO Box 24923, 13110, Safat, Kuwait. Tel: (965)5319475, Fax: 965-2533098, E-mail: aad@hsc.edu.kw
Many have also used combinations of chemical and physical methods\cite{5-19}. Still, most of these methods are laborious, time consuming and costly. In the last two decades, many commercial kits have been developed to extract bacterial DNA using simpler steps and a shorter time frame. Although they made the DNA extraction process quicker, such methods are costly and require several steps and reagents, and sometimes special equipment, to obtain the target DNA\cite{14,20,25}.

In this study, the authors have tried two very simple methods that may be used to extract bacterial DNA using heat only in a very simple manner. Using heat for bacterial DNA extraction is not new. High temperature exposure is known to cause damage to cell membranes and cell walls\cite{14,16,20-22}. Jose and Brahmadathan reported that heating at 94 °C for two minutes was enough to denature cell walls\cite{16}. Low temperatures were also observed to destroy cell walls and membranes. Freezing induces crystallization of water inside cells which leads to destruction of cytoplasmic structures\cite{12,16,20}. In fact, Tell et al used cycles of freezing and thawing to obtain bacterial DNA\cite{12}. In practice, heating bacterial material for DNA extraction purposes was performed by boiling in a water bath or on hot blocks, or using microwave ovens\cite{5-16}. Microwaves can cause many different biological effects; these are mainly due to the heating process (thermal effects) but there are also athermal effects on cellular material, which were thought to be due to acceleration and collision of ions with other molecules, partitioning of ions, or altering the polarity of molecules in alternating electric fields\cite{52-24}. In this study, the use of heat has been improved in two simplified ways to extract DNA from bacteria. To assess the suitability of the extracted DNA for performing molecular biology techniques, the extracted bacterial DNA was processed by polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP) and DNA sequencing. For comparison, a commercial DNA extraction kit was also used. These two methods, as well as the commercial kit, were tried on Klebsiella pneumoniae isolates harboring extended spectrum β-lactamase (ESBL). ESBLs are mainly derived from TEM, SHV or CTX-M β-lactamases that have mutated to expand their spectrum of activity to include third generation cephalosporins\cite{12,25}. Although they were first reported in Klebsiella species\cite{26}, ESBLs are now also commonly found in Escherichia coli and they have also been found in other species of Enterobacteriaceae\cite{6}. To date, more than 130 TEM and more than 104 SHV derivatives have been found\cite{1,2,25}.

**MATERIAL AND METHODS**

The study was conducted in the microbiology research laboratory of the faculty of Allied Health Sciences, Kuwait University. Approval of the local ethical committee was obtained.

**Samples**

Forty strains of Klebsiella pneumoniae were included in this study. These strains were isolated from a variety of clinical specimens submitted to the clinical bacteriology laboratories in Al-Amiri Hospital. They were flagged as ESBL-positive by the Vitek 2 GNI and AST-N020 cards (Bio Merieux, Marcy L’Etoile, France). Samples were grown at 37 °C on Luria Bertani (LB) media (from GIBCO, BRI, Life Technologies, UK), before extracting their DNA.

**Methods for DNA extraction**

In the first method, two colonies of overnight growth bacteria were used. The colonies were put in a test tube containing one ml of distilled water and boiled for 10 minutes in a water bath, and then were centrifuged for five minutes at 1000 rpm. Five microliters of the supernatant were used for the PCR. The second method was based on using a National microwave oven (Matsushita Electric Industrial Company, Japan) to heat the bacterial colonies (two colonies dissolved in 500 μl distilled water) for 10 seconds, followed by centrifugation for two minutes at 1000 rpm. Similarly, 5 μl of the supernatant were used for the PCR.

**Commercial Kits for DNA extraction**

Genomic DNA from the same bacterial isolates was extracted for PCR by using Gentra Puregene DNA isolation kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer’s instructions. DNA samples were tested by spectrophotometry at dual UV light (260/280) and the ratio was 1.7-1.9 for all samples.

**PCR**

PCR was performed on all the DNA samples extracted using the two methods and the commercial kit. Five microliters of the DNA were mixed with 45 μl of pre-aliquoted Reddy-Load PCR Mix (from ABgene, UK) containing 1.25 units of Taq DNA polymerase, 75 mM Tris-HCl (pH 8.8), 20 mM (NH₄)₂SO₄, 1.5 mM MgCl₂, 0.01% (v/v) Tween 20, 0.2 mM of each of the four deoxynucleotide triphosphates (dATP, dCTP, dGTP and dTTP) and 100 pmol of each of the primers indicated in Table 1. The expected sizes of PCR products for the two sets of primers were 308 and 858 base-pairs (bp), respectively (Table 1). For SHV primers, the PCR mixture was incubated for five minutes at 95 °C as an initial denaturation step, followed
by 32 cycles of successive alternating temperatures as follows: denaturation step at 94 °C for one minute, annealing step at 57 °C for one minute, and extension step at 72 °C for one minute. A final extension step at 72 °C for 10 minutes was allowed. On the other hand, and for the TEM primers, the PCR mixture was incubated for five minute at 95 °C as an initial denaturation step, followed by 30 cycles of successive alternating temperatures as follows: denaturation step at 94 °C for 30 seconds, annealing step at 55 °C for one minute, and extension step at 70 °C for one minute. A final extension step at 75 °C for 10 minutes was also allowed. The PCR reaction for both sets of primers was performed in a programmable PCR Thermal Cycler (Perkin Elmer, Wellesley, MA, USA).

**RFLP**

TEM-specific PCR products were digested by *Sau3AI* endonuclease using 10 μl of the PCR product without purification, according to the recommendation of the restriction endonuclease suppliers (Promega, Ltd, UK). The following amounts were used: 5 μl of restriction buffer (10 mM Tris-HCL, pH 7.5, 60 mM NaCl, 7 mM MgCl2), 1 μl of BSA (0.1mg/l), 1 μl of restriction enzyme and 4 μl of sterile distilled water. Digestion was carried out for four hours at 37 °C. For SHV, PCR products were digested with 10 U/μl of *NheI* restriction enzyme (Promega, Ltd, UK), 5 μl of restriction buffer (10 mM Tris-HCL, pH 7.5, 60 mM NaCl, 7 mM MgCl2), 1 μl of BSA (0.1mg/l), 4 μl of sterile distilled water and 40 μl of the amplified PCR product. Digestion was carried out for a maximum of four hours at 37 °C. Restriction pattern of PCR products for both sets of primers were analyzed by agarose gel electrophoresis, using 2% agarose in 1X Tris acetate EDTA (TAE) buffer, which were then stained with ethidium bromide and visualized by exposure to UV light in a gel documentation system (UVP Company, Upland, CA, USA). A DNA marker from Sigma (Sigma-Aldrich, Inc., Saint Louis, MI, USA) was run on the gel along with the PCR amplicons to identify the sizes of these amplicons.

**DNA sequencing**

DNA sequencing was performed on 10 randomly selected bacterial isolates out of the 40 isolates included in this project as representatives of the whole group PCR products for the SHV gene, obtained from the PCR step above and were taken for sequencing. These products were first cleaned by ethanol precipitation; 25 μl of template suppression reagent (TSR) was added to the pellet, mixed, and finally heated for two minutes at 95 °C. For sequencing PCR, one microliter of each PCR product from the previous step was mixed with 3.2 picomol of either a forward (5’-CTG GGA AAC GGA ACT GAA TG-3’) or a reverse primer (5’-GGG GTA TCC CGC AGA TAA AT-3’), and 8 μl of a dye terminator ready sequence reaction mix (Prism TM Ready Reaction Dye-Deoxy TM Terminator Cycle Sequencing Kit, Perkin Elmer, Wellesley, MA, USA). The sequencing PCR reaction was then carried out in the Thermal Cycler programmed to 30 cycles of 96 °C for 20 seconds, 50 °C for 20 second, and 60 °C for four minutes. The products were cleaned again as mentioned above, and the products were kept on ice till the sequencing was run on an automated DNA sequencer (ABI3100, Applied Biosystem, Foster City, CA, USA). Sequences results were analysed by the BLAST online search engine (http://www.ncbi.nih.gov/cgi-bin/BLAST), with the susceptible strains sequences in the database.

**RESULTS**

PCR amplicons were produced successfully in all DNA samples included in this project. The amplified products obtained with primers specific for both *blaTEM* and *blaSHV* were 858 bp and 308 bp, respectively, which were the expected product sizes of the amplified gene with the set of primers used. That was true whether the DNA was extracted by the two simple methods described here, or using the commercial kit. Figure 1 shows a photograph of agarose gel electrophoresis of these PCR amplicons. In the RFLP step, *NheI* restriction endonuclease was used to cleave the SHV-specific PCR product, while *Sau3AI* restriction endonuclease was used to cleave the TEM-specific PCR. The results of all the restricted PCR products (SHV or/and TEM) were as expected for each restriction enzyme. The patterns of cutting were similar whether the DNA was extracted by the two methods introduced by the authors, or using the commercial kit (Fig. 2 and 3).
The automated analysis of the sequenced SHV PCR products showed the expected nucleotide sequences in all the 10 representative bacterial isolates. Moreover, four out of the 10 isolates were found to have a Gly238Ser mutation that is characteristic of SHV-2 ESBL; while the rest of the isolates harboured the Gly238Ser mutation as well as a Glu240Lys mutation; presence of both is characteristic of SHV-5 ESBL (Fig. 4). That was true in all the three DNA extraction methods used in this project.

**DISCUSSION**

Molecular biology techniques to study bacterial DNA (like PCR, RFLP, and DNA sequencing) usually need DNA extraction and purification from the bacteria with a high quality for perfect performance. However, present DNA purification procedures, especially the commercial ones, are costly, laborious and need a large number of reagents and equipment. Several researchers have tried to liberate DNA from bacterial cells by breaking bacterial cell walls using certain reagents, especially by enzymatic treatment with lysosymes and proteases[5,7,9,10-14]. However, Agersborg reported that lysozyme and proteinase K treatment, was not always sufficient to hemolyse certain cells[7]. On the other hand, Merk et al found proteinase K to be superior to other methods in extractingDNA[14]. Other researchers have tried other synthetic lysing solutions like SDS (sodium dodecyl sulfate), TWEEN20[8], Triton X-100[7] and guanidine isothiocyanate (GITC)[5,10,14]. GITC was reported to be able to damage cells with hard walls like fungi. Besides chemical methods, several researchers have successfully extracted bacterial DNA using physical power; for example, forceful rupture of cells was achieved by vortexing suspensions of cells[8], or beating cells with beads[12] or ultrasound waves[27]. Moreover, certain glass or iron beads were used to capture DNA molecules, which could later be eluted and separated[9,17-18]. Other physical powers were also used, like high or low temperatures. Heating
cells, such as boiling or microwave irradiation, was widely used to extract DNA molecules\[5,7,9,10-13\].

Still, many of the previous methods were either followed or preceded by enzymatic or detergent treatment to obtain DNA for molecular techniques. Many companies have utilized the previous concepts in producing commercial kits that could be used in extracting DNA from a variety of cellular material\[14,19\]. Although providing simpler DNA extraction methods, such kits added extra costs to experiments needing DNA extraction.

In this study, simplified DNA extraction methods to produce bacterial DNA samples were evaluated. The aim was to minimize the time and the need for reagents, while still not affecting the quality of DNA extracted and the productivity of the subsequent molecular techniques. The methods were based on using heat without adding any reagent. Heating bacterial material (suspended in distilled water without any other additions) was achieved by boiling for 10 minutes or microwave irradiation for 10 seconds. It was shown that these two methods have provided enough DNA molecules to perform subsequent molecular biology techniques. The methods were tried on ESBL genes of Klebsiella pneumoniae. The ESBL genes were detected by PCR amplification of the DNA sequences coding for blaTEM and blaSHV ESBL genes. PCR was successful in all cases, giving the expected PCR amplicons. That was additionally verified by performing the same PCR protocol on DNA samples extracted from the same bacterial samples using a commercial DNA extraction kit. PCR amplicons were qualitatively equivalent in all experiments. Furthermore, and in the RFLP experiment, digestion of TEM and SHV PCR products with Sau3AI and NheI endonucleases, respectively, showed the same fragments and results in all the tested samples whether the DNA used for PCR was extracted by the two methods introduced by the authors or using the commercial kit. Finally, DNA sequencing was also successful in all DNA extraction methods used in this project, giving the expected sequences.

To compare with the work of this study, a limited number of researchers have also used boiling and/or microwave irradiation to extract DNA without any reagents added. However, most of these researchers have boiled their samples or exposed them to microwave irradiation for a time longer than the presented method in this paper\[14,19\] or have subjected their samples to multiple microwave irradiation\[8,13\]. To the best of the authors’ knowledge, the report by Merk et al was the only one in which the samples were boiled for 10 minutes like in our study\[14\]. Unlike this paper, their samples were blood and lung tissue which were artificially infected with Burkholderia cepacia. In addition, their extracted DNA was processed by PCR only. The present paper may be the first to report using a 10-minute boiling, or as short as 10-second microwave irradiation to extract bacterial DNA suitable to perform three essential molecular biology techniques; namely PCR, RFLP and DNA sequencing.

CONCLUSION
In conclusion, the presented methods (heat-treatment of bacteria) are very simple, cheap, quick and successful methods for DNA extraction from bacteria in order to be used directly in molecular techniques, yielding excellent results as other more complicated methods for DNA extraction and purification. The findings of this study may probably encourage trying the procedure on other types of biological specimens such as whole blood, culture cells, body fluids and hair.

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REFERENCES
Evaluation of Problem Based Learning by Tutors and Students in a Medical Faculty of Turkey

Erol Gurpinar, Yesim Senol, Mehmet R Aktekin
Department of Medical Education, Akdeniz University Faculty of Medicine, Antalya, Turkey

ABSTRACT

Objectives: To determine the opinion of tutors and students in charge of problem-based learning (PBL) courses during the academic year of 2006-2007 about the extent of contribution of PBL to certain skills in comparison with conventional education and to clarify whether or not they are content with PBL. Design: Cross sectional research Setting: Akdeniz University Faculty of Medicine, Antalya, Turkey Subjects and Methods: One hundred and fifty three tutors in charge of PBL courses during the academic year of 2006-2007 and all of the first year medical students (n = 170) were included. A questionnaire was sent to the study population in June 2007. Intervention: Analysis of completed questionnaire

Main Outcome Measure: Independent sample t-test analysis was used to determine whether mean scores were different in two groups.

Results: Majority of the tutors (87.5%) and students (97.1%) responded to the questionnaire. The question “Is PBL an application that is in general beneficial to the student?” was answered as “yes” by 66.9% of the tutors. The question “Are you content with PBL?” was answered as “yes” by 54.9% of the tutors. On the other hand, 74.5% of the students answered “yes” to this second question.

Conclusion: Our results show that PBL is well received by tutors and students and they think that PBL offers significant contribution to the students in areas that are considered to be superior aspects of PBL when compared to conventional education.

INTRODUCTION

Problem based learning (PBL) was introduced into medical school curricula by McMaster University in 1969 and has since been adopted by many medical schools worldwide[1,2]. PBL is both a method and philosophy involving problem-first learning via work in small groups and independent study[3].

In a PBL program, the students use a seven-step procedure to structure their activities. This procedure consists of clarifying vague phrases and concepts in the problem, defining the problem, analysing the problem on the basis of prior knowledge, arranging the proposed explanations, formulating learning objectives, trying to fill in the knowledge gaps by means of self study and finally reporting the finding in the groups[4-13]. Such learning is based on adult learning model, with emphasis on self-directed learning.

The first applications of PBL began in 1997 in Turkey. Until today, four medical schools have been performing their education programs completely based on PBL while most of the remaining medical schools in Turkey have adopted the hybrid education model[14]. We, Akdeniz University Faculty of Medicine (AUFM), are one of the faculties that have been implementing the hybrid education model since the academic year of 2002-2003.

The faculty members (tutors) and students constitute the most crucial points in PBL[15,16]. We believe that soliciting the opinion of both the tutors and the students may be extremely beneficial for evaluation of PBL. Moreover, it is also important for the detection and the subsequent improvement of the shortages of the education program. When we reviewed the literature, it was seen that there are many studies about PBL. However, only a limited number of studies have been performed for investigating the opinion and thoughts of the tutors and the students together[17-20].

Thus, the aim of the present study was to determine the opinion of tutors in charge of PBL...
courses during the academic year of 2006-2007 and their students about the extent of the contribution of PBL to learning certain skills in comparison with conventional education and clarify whether or not they are content with PBL.

SUBJECTS AND METHODS

This is a cross-sectional and descriptive study.

PBL Setting in Curriculum

Basic medical sciences are being taught in an integrated program composed of five thematic blocks during first two years in AUFM. The courses of different disciplines are integrated on the organ system based themes in these blocks. The duration of each block is eight weeks and the first week is allocated to PBL modules. A case-based scenario is used in PBL sessions. Our students are expected to achieve relevant learning objectives while trying to solve the problems they face in the scenario. Problem solving is only a part of the PBL module. PBL week is entirely devoted to PBL activities and free of other traditional classes. Throughout this week, in three half-day time period, PBL small group discussion sessions, laboratory and field studies, clinical skill practices, and supportive theoretical conferences take place.

Participants and Collection of the data

The research population consisted of 153 tutors and all the students enrolled in first years (n = 170). Before taking charge in a PBL module, the tutors of AUFM had participated in the PBL courses supported by the faculty developmental program. PBL course is a part of faculty developmental program which is obligatory for faculty members participating in teaching and tutoring activities. This PBL course takes three days. In the first two days theoretical information is provided to the participants. After that, two sample PBL sessions among participants are carried out and all participant tutors take part in these sessions as both students and tutors. After the course, all participants observe a real PBL module (three full discussion sessions) and are certified to be a tutor for PBL module.

In this study, a questionnaire was sent to the study population in June 2007, which is the final month of the term. Questions included in the questionnaire aimed to determine the department of the tutor, whether he / she thinks that PBL is a beneficial application for students, and finally whether he / she is content with PBL. Additionally, a literature review was performed to determine areas which are accepted as better improved by PBL rather than by conventional education[2-7]. Twelve subject headings were determined and questions related to these subject headings were included in the questionnaire. In both questionnaires for tutors and for students, the participants were asked to answer the questions using a 5-point Likert scale (1 - it did not contribute at all, 5 - it contributed well enough).

Statistical Analysis

Data analysis was carried out using SPSS package version 13.0. Independent sample t-test analysis was used. P-values less than 0.5 were considered statistically significant.

Table 1: Answers of tutors to the question “Is PBL a beneficial practice for the students in general?” according to their departments

<table>
<thead>
<tr>
<th>Departments of tutors</th>
<th>Is PBL a beneficial practice for the students in general?</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>%</td>
<td>I am indecisive</td>
<td>No</td>
<td>%</td>
<td>Total</td>
<td>%</td>
</tr>
<tr>
<td>Basic sciences</td>
<td>25</td>
<td>80.6</td>
<td>6</td>
<td>19.4</td>
<td>0</td>
<td>0.0</td>
<td>31</td>
</tr>
<tr>
<td>Medical sciences</td>
<td>37</td>
<td>64.9</td>
<td>12</td>
<td>21.1</td>
<td>8</td>
<td>14.0</td>
<td>57</td>
</tr>
<tr>
<td>Surgical sciences</td>
<td>27</td>
<td>60.0</td>
<td>7</td>
<td>15.6</td>
<td>11</td>
<td>24.4</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>66.9</td>
<td>25</td>
<td>18.8</td>
<td>19</td>
<td>14.3</td>
<td>133</td>
</tr>
</tbody>
</table>

PBL = Problem based learning

Table 2: Answers of tutors to the question “Are you content with PBL?” according to their departments

<table>
<thead>
<tr>
<th>Departments of tutors</th>
<th>Are you content with PBL?</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>%</td>
<td>I am indecisive</td>
<td>No</td>
<td>%</td>
<td>Total</td>
<td>%</td>
</tr>
<tr>
<td>Basic sciences</td>
<td>23</td>
<td>74.2</td>
<td>5</td>
<td>16.1</td>
<td>3</td>
<td>9.7</td>
<td>31</td>
</tr>
<tr>
<td>Medical sciences</td>
<td>30</td>
<td>52.6</td>
<td>11</td>
<td>19.3</td>
<td>16</td>
<td>28.1</td>
<td>57</td>
</tr>
<tr>
<td>Surgical sciences</td>
<td>20</td>
<td>44.4</td>
<td>10</td>
<td>22.2</td>
<td>15</td>
<td>33.3</td>
<td>45</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>54.9</td>
<td>26</td>
<td>19.5</td>
<td>34</td>
<td>25.6</td>
<td>133</td>
</tr>
</tbody>
</table>

PBL = Problem based learning
RESULTS

Majority of the tutors (87.5%) and students (97.1%) responded to the questionnaire. 66.9% tutors answered “yes” to the question, “Is PBL an application that is in general beneficial to the student?”. When the answer was evaluated according to the departments, it was determined that this answer was mostly given by the tutors employed in departments of basic sciences (80.6%, Table 1).

The question, “Are you content with PBL?” was answered as “yes” by 54.9% tutors. When the answer was evaluated with regards to the tutors’ departments, it was determined, once again, that the answer “yes” was mostly given by tutors employed in the departments of basic sciences (74.2%, Table 2). 74.5% students also answered “yes” to this question (Table 3).

Among the close-ended questions those aimed at determining the opinion of tutors and students about the extent of PBL’s contribution to certain subject headings, “it contributed” response was ticked by most of the tutors and students for statements, “interpersonal relationships, adaptation to teamwork” and “developing communicational skills”, respectively (Table 4).

When the mean scores obtained from the responses to the above-mentioned questions were compared, a significant difference was observed between the mean scores given by the tutors and the students. It was determined that the students gave higher scores to the six items given in Table 5 (p < 0.05).

DISCUSSION

It was observed that similar results have been obtained with studies in the literature which aim to determine opinion of tutors and students about PBL application in medical education[17-21]. Our study revealed that the majority of the tutors in charge of PBL sessions felt that PBL was a beneficial application for the students and that they were content with PBL. Additionally, it was found that a great majority of tutors felt that PBL offers significant contribution to students in areas, which were considered as the superior aspects of PBL when compared to conventional education.

It was a striking finding that the number of tutors who were content with PBL and thought that PBL was beneficial to the students were higher in the departments of basic sciences when compared to the other departments. Since PBL is an education model that is used in basic sciences in medical education, the answers given by the tutors employed in these departments become more important. Additionally, the high rate of students (74.5%) who are content with PBL is an eminently important finding. When the mean scores of the study groups given to items aimed to determine skills that are better acquired through PBL than through conventional education were compared, it was found that the mean scores given by the students were higher for eight items. This finding shows that the students feel that PBL makes positive contributions in terms of skills mentioned in those items. In the light of these findings, it may be

<table>
<thead>
<tr>
<th>Subject headings</th>
<th>Tutors</th>
<th>%</th>
<th>Students</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpersonal relationships, adaptation to teamwork</td>
<td>95</td>
<td>71.4</td>
<td>105</td>
<td>61.8</td>
</tr>
<tr>
<td>Skill of telling the information gained to others</td>
<td>95</td>
<td>71.4</td>
<td>116</td>
<td>68.2</td>
</tr>
<tr>
<td>Developing the skill of communication</td>
<td>88</td>
<td>66.7</td>
<td>123</td>
<td>72.4</td>
</tr>
<tr>
<td>Self-directed learning and use of resource</td>
<td>86</td>
<td>64.7</td>
<td>106</td>
<td>62.4</td>
</tr>
<tr>
<td>Developing the skill logical thinking</td>
<td>81</td>
<td>61.4</td>
<td>123</td>
<td>72.4</td>
</tr>
<tr>
<td>Using the information sources like library and internet for access to information</td>
<td>80</td>
<td>60.6</td>
<td>101</td>
<td>60.1</td>
</tr>
<tr>
<td>Problem solving skill</td>
<td>80</td>
<td>60.6</td>
<td>121</td>
<td>71.2</td>
</tr>
<tr>
<td>Developing the skill of decision-taking</td>
<td>70</td>
<td>52.6</td>
<td>122</td>
<td>71.8</td>
</tr>
<tr>
<td>Integrating obtained knowledge</td>
<td>66</td>
<td>50.0</td>
<td>111</td>
<td>65.3</td>
</tr>
<tr>
<td>Skill of selecting useful information among information sources</td>
<td>64</td>
<td>48.5</td>
<td>81</td>
<td>48.2</td>
</tr>
<tr>
<td>Increasing the motivation for learning</td>
<td>63</td>
<td>47.7</td>
<td>114</td>
<td>68.3</td>
</tr>
<tr>
<td>The development of the skill of approaching the patient as a biopsychosocial whole</td>
<td>57</td>
<td>42.9</td>
<td>117</td>
<td>68.8</td>
</tr>
</tbody>
</table>

Table 3: Responses of both tutors and students to the question “Are you content with PBL?”

Table 4: The percentage of tutors and students who said “it contributed” and “it contributed well enough” with PBL’s contribution in certain subject headings
Table 5: Mean scores obtained from responses of both tutors and students to the suggestions of PBL’s contribution in certain subject headings

<table>
<thead>
<tr>
<th>Subject headings</th>
<th>Tutors Mean</th>
<th>Tutors SD</th>
<th>Students Mean</th>
<th>Students SD</th>
<th>Statistical analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpersonal relationships, adaptation to teamwork</td>
<td>3.81</td>
<td>1.08</td>
<td>3.70</td>
<td>1.05</td>
<td>0.34</td>
</tr>
<tr>
<td>Skill of telling the information gained to the others</td>
<td>3.79</td>
<td>0.93</td>
<td>3.74</td>
<td>1.05</td>
<td>0.65</td>
</tr>
<tr>
<td>Developing the skill of communication</td>
<td>3.72</td>
<td>0.94</td>
<td>3.95</td>
<td>1.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Self-directed learning and use of resource tutors</td>
<td>3.61</td>
<td>0.91</td>
<td>3.63</td>
<td>1.18</td>
<td>0.89</td>
</tr>
<tr>
<td>Developing the skill logical thinking</td>
<td>3.57</td>
<td>0.95</td>
<td>3.85</td>
<td>1.06</td>
<td>0.01</td>
</tr>
<tr>
<td>Using the information sources like library and internet for access to information</td>
<td>3.60</td>
<td>0.99</td>
<td>3.57</td>
<td>1.13</td>
<td>0.81</td>
</tr>
<tr>
<td>Problem solving skill</td>
<td>3.61</td>
<td>0.97</td>
<td>3.88</td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td>Developing the skill of decision-taking</td>
<td>3.43</td>
<td>0.94</td>
<td>3.87</td>
<td>0.97</td>
<td>0.00</td>
</tr>
<tr>
<td>Integrating obtained knowledge</td>
<td>3.33</td>
<td>1.01</td>
<td>3.71</td>
<td>1.10</td>
<td>0.00</td>
</tr>
<tr>
<td>Skill of selecting useful information among information sources</td>
<td>3.34</td>
<td>0.94</td>
<td>3.19</td>
<td>1.28</td>
<td>0.243</td>
</tr>
<tr>
<td>Increasing the motivation for learning</td>
<td>3.40</td>
<td>1.01</td>
<td>3.81</td>
<td>1.08</td>
<td>0.00</td>
</tr>
<tr>
<td>The development of the skill of approaching the patient as a biopsychosocial whole</td>
<td>3.29</td>
<td>1.02</td>
<td>3.83</td>
<td>1.08</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Independent sample t-test

concluded that PBL is useful in teaching especially of basic sciences in medical education and it helps the students to acquire skills which are crucial components of education.

On the other hand, it is an interesting finding that the tutors employed in surgical sciences were those who were least content with PBL. When the responses of this group to the open-ended questions aimed at determining the opinion and suggestions about PBL were examined, it was determined that some problems were mentioned in their answers. These problems were the time-consuming nature of PBL, the difficulty of allocating time for PBL while being busy with the routine tasks, the burden of acting as orientators for a topic in which they are not competent, and the lack of their faith in PBL. In order to solve these problems, attempts were made to assign the tutors to PBL sessions once in every term and particularly to modules that are closely relevant to their branches.

The part of the questionnaire entitled “Your opinion and suggestions about PBL” helped determine points on which the tutors and students would like to see the greatest improvement. According to this part of the questionnaire, the tutors asked for the selection of topics that are susceptible to self-directed learning and convenient to be discussed comprehensively and demanded better construction of scenarios. The students, on the other hand, requested tutors to attend the PBL modules well-prepared and have a participation standard. Moreover, they have requested better selection of PBL topics. All these requests are and will be taken into consideration in the process of planning and developing educational and instructional activities.

CONCLUSION

Our results show that PBL is well received by the tutors and students in AUFM and PBL is acceptable in a Turkish setting of undergraduate medical education in our faculty.

ACKNOWLEDGMENTS

The authors thank the Akdeniz University Research Fund for financial support.

REFERENCES

8. Hill J, Rolfe IE, Pearson SA, Heathcote A. Do junior doctors feel they are prepared for hospital practice?
Impaired Holter-Derived Variables of Parasympathetic Activity in Diabetic Patients with Daily-Life Silent Myocardial Ischemia

Ahmad Ali Al-Dousary¹, Saleh El-Enezy¹, Mousa AJ Akbar², Ali Mohamad Hegazy¹
¹Department of Medicine, Farwania Hospital, Kuwait
²Department of Medicine, Sabah Hospital, Kuwait

Kuwait Medical Journal 2009; 41 (2): 128-133

ABSTRACT

Objective: To evaluate Holter-derived variables of impaired parasympathetic activity in diabetic patients with silent myocardial ischemia

Design: Cross sectional nature cohort study

Setting: Department of Medicine, Farwania Hospital, Kuwait

Subjects: One hundred and sixty patients with diabetes mellitus

Interventions: 24-hour Holter electrocardiography (ECG) monitoring was used for heart rate variability and silent myocardial ischemia

Results: Predictive indices revealed that Holter derived variables of parasympathetic activity (p-NN50, rMMD, SDANN-i) are considered as indicators for prediction of likelihood of daily life silent myocardial ischemia in diabetic patients. Sensitivity was 86, 85, 82%, specificity = 88, 89, 81%, accuracy = 87, 86, 87%, positive predictive value = 89, 90, 86% and negative predictive value = 84, 83, 81% respectively. Multivariate analysis revealed that duration of diabetes status and serum level of HbA1c, as independent variables were associated with likelihood of daily life silent myocardial ischemia (p < 0.05). Receiver operating characteristic (ROC) curve data revealed that the best cut-off value of p-NN50 was 8% with sensitivity = 86% and false positive = 16%, (rMMD) = 26 msec with sensitivity = 85% and false positive =15% and (SDANN-i) = 96 msec with sensitivity = 81% and false positive = 21% for prediction of likelihood of daily life silent myocardial ischemia in diabetic patients.

Conclusion: Silent myocardial ischemia in diabetic patients provides statistically significantly association with impaired parasympathetic activity.

KEY WORDS: diabetes mellitus, parasympathetic nervous system

INTRODUCTION

Coronary artery disease is the ultimate cause of death in more than half of diabetic patients and frequently manifests itself silently and prematurely¹,². Previous studies among patients with known coronary artery disease indicate that those with diabetes have more frequent silent myocardial infarction and silent myocardial ischemia during exercise testing and ambulatory electrocardiographic monitoring than non diabetic patients³.

Because diabetics are predisposed to a higher incidence of coronary artery disease and autonomic neuropathy, they present a unique opportunity to study the pathophysiology of silent myocardial ischemia⁴. Diabetic autonomic neuropathy may involve the cardiac afferent sympathetic system which is a part of the pain perception pathway from myocardial pain receptors to the cerebral cortex. It is possible that the development of myocardial ischemia in patients with such a neuropathy may be associated with more frequent silent myocardial ischemia⁵-⁹.

Parasympathetic and sympathetic afferents are thought to convey sensory data from the heart to modulate homeostasis and regulate cardiac function through cardiocardiac reflexes¹⁰. In humans sympathetic afferent nerves are also thought to convey the symptom of angina in patients with ischemic heart disease and evidence for this is provided by findings that surgical rhizotomy (cervical sympathectomy) abolished angina in patients with ischemic heart disease¹¹,¹².

At present, several simple tests are available such as the heart rate response to the Valsalva maneuver, deep breathing and standing. Individual results are

Address correspondence to:
Dr Alhamad Al-Dousary, Department of Medicine, Farwania Hospital, P.O. Box 18373, Kuwait - 81004, Tel: +965 24888000 / 5497, E-mail: aldousai@hotmail.com
sometimes difficult to interpret as measurements are
made only over very short spans and a single test may
give an atypical result owing to natural variability\textsuperscript{[13]}.

The aim of this study was to evaluate Holter-
derived variables of impaired parasympathetic activity
in diabetic patients with silent myocardial ischemia.

**SUBJECTS AND METHODS**

One hundred and sixty diabetic patients were
included in the study. All patients were evaluated
clinically by looking at history, physical examination,
12-leads ECG, plain chest X-ray and routine laboratory
investigations.

Exclusion criteria included patients with hypertension, renal failure, acute myocardial
infarction, and left ventricular failure. Exclusion was
based on medical history, physical examination and
12-lead electrocardiogram.

This study was approved by the hospital ethical
committee.

**Transthoracic echocardiography**

It was performed for all patients in the study with
the use of Toshiba Power Vision or GE vivid 7 and a
3.5 MHZ phased array transducer to exclude patients
with segmental wall motion abnormalities and to
assess left ventricular systolic and diastolic function.

This study was approved by the hospital ethical
committee.

**Cardiac autonomic function tests\textsuperscript{[7]}**

1. Resting heart rate: A heart rate greater than 100
   beats/minute was considered abnormal.

2. Postural hypotension: It was as defined as a fall
   in systolic blood pressure greater than or equal
to 30 mmHg immediately upon standing from
   supine position.

3. Abnormal heart rate variation with deep
   breathing: It was defined as a change in heart
   rate of less than 10 beats/minute in a supine patient
   breathing at six breaths/minute.

4. Abnormal heart rate variation with Valsalva
   maneuver: The patient blew into a manometer
   hose and maintained a pressure of 40 mmHg
   for 15 seconds. The longest R-R interval after
   the Valsalva strain divided by the shortest RR
   interval during the maneuver was defined as the
   Valsalva ratio. An abnormal ratio is less than or
   equal 1.10.

5. Sustained (isometric) hand grip testing: The
difference in diastolic blood pressure was
measured after the subject was asked to maintain
a hand grip at 50\% of maximal voluntary
contraction with a hand grip sphygmomanometer
for a maximum of five minutes. The normal
response is an increase of 16 mmHg.

24-hour Holter monitoring and heart rate
variability

During analysis, only cycles in which beats
had normal morphologic characteristics and were
within 25\% of the preceding cycle length, were
selected for calculation for heart rate variability.
Time domain measurements were obtained from
normal to normal sinus beats including the mean
RR interval and its SD (SDNN), the average value
of the five minute standard deviations for selected
intervals (SDNN-i), the percentage of successive
RR interval that deviated by > 50\% from the prior
RR interval (p-NN50), the root mean square of
successive RR interval differences (rMMD) and
SD of the average of RR intervals in 5-minute segments of the 24 hour recording (SDANN-i)\textsuperscript{[14]}.

24-hour Holter monitoring and daily-life silent
myocardial ischemia

1. Ischemic episodes: ST segment depression
   1 mm or more occurred 80 msec after J point
   lasting one minute or more and separated
   from other significant episodes by one
   minute or more. For each patient, total
   number of ischemic episodes/24 hours and
total duration of ischemic episodes (msec)
   were calculated.

2. Non-ischemic episodes: were defined as no
   ischemic ST segment depression while, the
   patients having the same heart rate with onset
   of ischemic episodes and presented within
   1 - 2 hours before or after onset of ischemic
   episodes in the same patients\textsuperscript{[15]}

**Study Design**

There were two main groups in the study.

- Control group: included 50 non diabetic
  subjects.
- Patient sample: included 160 diabetic patients
  who were stratified into:
  - Group I: included 87 diabetic patients with
    silent myocardial ischemia.
  - Group II: included 73 diabetic patients without
    silent myocardial ischemia.

**Statistical analysis**

Continuous variables are summarized as a
mean ± standard deviation (SD). Comparison
between two groups was performed with t-test
for continuous variables and chi-square test for
categorical variables. A p-value < 0.05 was
considered statistically significant and a p-value <
0.01 was considered statistically highly significant.
A stepwise multivariate regression model was
used to identify possible independent variables
associated with daily life silent myocardial
ischemia. The strength of the association with impaired Holter derived parasympathetic activity is presented as 95% confidence intervals. Potential confounding of clinical variables was entered as independent variables.

The validity of Holter derived parasympathetic activity variables to predict the likelihood of daily life silent myocardial ischemia was assessed by estimating the predictive indices. Predictive indices: True positive (TP), true negative (TN), false positive (FP), false negative (FN), sensitivity, specificity, accuracy, positive predictive value and negative predictive value were calculated.

Receiver operating characteristic (ROC) curve (grade of sensitivity versus false positive) was used to identify the sensitivity and false positive of certain value of the variable with area under curve and probability of error with sensitivity 100% to detect usefulness of Holter derived variables of parasympathetic activity in the diabetic patients for prediction of likelihood of daily life silent myocardial ischemia. ROC was calculated using likelihood ratio method. Likelihood ratio +ve = sensitivity / 1-specificity and likelihood ratio -ve = 1-sensitivity / specificity. The best cut off point should be close to the top left hand corner of the graph: high detection rate with low false positive rate.

RESULTS

Clinical characteristics

With regards to the age and gender of the patients, there was no significant difference between group I and II (52.42 ± 4.11 versus 47.5 ± 4.03 years, (p = NS), 77 (88.5%) versus 66 (90.4%) male, (p = NS) and 10 (11.5%) versus 7 (9.6%) female, (p = NS), respectively). There was no significant difference between group I and II regarding a percentage of patients with history of smoking [43 (49.4%) versus 40 (54.6%) patients, (p = NS)]. There was no significant difference in the heart rate, systolic and diastolic blood pressure between patients from the control group and study sample (78.15 ± 5.64 versus 80.52 ± 6.82 beat/minute, p = NS, 119.61 ± 6.62 versus 122.19 ± 5.91 mmHg, p = NS and 76.41 ± 5.30 versus 78.43 ± 4.54 mmHg, respectively, p = NS). There was a significantly more increased duration of diabetes and level of HbA1c in patients from group I than those of group II (8.94 ± 1.62 versus 4.11 ± 2.43 years and 9.34 ± 1.23 versus 5.91 ± 1.16 %, respectively, p < 0.05).

Echocardiography and Doppler study

In the control group, the median value of (E velocity) = 9 cm/sec, median value of (A velocity) = 8.3 cm/sec and median value of Em / Am ratio = 1.56.

There was a significantly more decreased (E velocity) and Em / Am ratio in patients of group I than those from group II (7.2 ± 1.6 versus 9.8 ± 2.4 cm/sec and 0.59 ± 0.23 versus 0.93 ± 0.16, respectively, p < 0.05), but there was a significantly more increased (A velocity) in patients of group I than those of group II (9.2 ± 1.62 versus 4.11 ± 2.43 years and 9.34 ± 1.23 versus 5.91 ± 1.16 %, respectively, p < 0.05).

---

Table 1: Mean and standard deviation of time domain heart rate variability Holter derived variables in all groups of the study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Group</th>
<th>Group I</th>
<th>Group II</th>
<th>F Value</th>
<th>CG Vs G I</th>
<th>CG Vs G II</th>
<th>G I Vs G II</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (msec)</td>
<td>134.84 ± 9.52</td>
<td>112.47 ± 5.27</td>
<td>140.31 ± 6.83</td>
<td>4.963 &lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>SDNN-I (msec)</td>
<td>71.40 ± 5.31</td>
<td>54.72 ± 6.41</td>
<td>67.52 ± 3.66</td>
<td>3.719 &lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>SDANN-I (msec)</td>
<td>130.34 ± 7.73</td>
<td>97.2 ± 5.71</td>
<td>121.26 ± 4.15</td>
<td>3.873 &lt; 0.05</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>r-MSSD (msec)</td>
<td>42.79 ± 4.67</td>
<td>25.13 ± 4.67</td>
<td>37.11 ± 2.72</td>
<td>5.165 &lt; 0.01</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>14.93 ± 5.71</td>
<td>8.16 ± 5.76</td>
<td>13.91 ± 3.61</td>
<td>4.619 &lt; 0.01</td>
<td>NS</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

CG: control group; G I: group I; G II: group II; SDNN: standard deviation of all selected RR intervals; SDNN-I: the average value of the 5 minute standard deviations for selected intervals (SDNN index); SDANN-I: the standard deviation of the 5 minute average for selected intervals (SDNN index); r-MSSD: square root of the mean of the squared successive differences in RR intervals; pNN50: the percentage of intervals that are at least 50 msec different from the previous interval.

---

Table 2: Predictive indices of values from Holter derived variables of parasympathetic activity for prediction of likelihood of Holter-derived daily life silent myocardial ischemia

<table>
<thead>
<tr>
<th>Variables</th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>Sen %</th>
<th>Spec %</th>
<th>Acc %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN (msec)</td>
<td>70</td>
<td>66</td>
<td>12</td>
<td>12</td>
<td>85</td>
<td>84</td>
<td>85</td>
<td>85</td>
<td>84</td>
</tr>
<tr>
<td>SDNN-I (msec)</td>
<td>69</td>
<td>65</td>
<td>13</td>
<td>13</td>
<td>84</td>
<td>83</td>
<td>87</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>SDANN-I (msec)</td>
<td>71</td>
<td>63</td>
<td>11</td>
<td>15</td>
<td>82</td>
<td>81</td>
<td>87</td>
<td>86</td>
<td>81</td>
</tr>
<tr>
<td>r-MSSD (msec)</td>
<td>74</td>
<td>65</td>
<td>8</td>
<td>13</td>
<td>85</td>
<td>89</td>
<td>86</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td>pNN50 (%)</td>
<td>73</td>
<td>66</td>
<td>9</td>
<td>12</td>
<td>86</td>
<td>88</td>
<td>87</td>
<td>89</td>
<td>84</td>
</tr>
</tbody>
</table>

The ideal cut-off value = high detection rate (sensitivity) with low false positive, false positive = 1- specificity

24-hour Holter monitoring and heart rate variability

Table 1 shows that there was a significantly more decreased 24-hour Holter monitoring derived time domain variables (SDNN, SDNN-i, SDANN-i, p < 0.05) and a highly significantly more decreased (r-MSSD, pNN50, p < 0.01) in patients of group I than control group and those of group II, but there was a non significant difference between control group and those of group II (p = NS).

Validity and reliability

The predictive indices showed that Holter derived variables of parasympathetic activity (SDNN, SDNN-i, SDANN-i, r-MSSD, pNN50), with positive and negative likelihood ratio. The best cut-off value of (SDANN-i) to predict diabetic patients with likelihood of Holter derived daily life silent myocardial ischemia (Table 2).

Receiver operating characteristic (ROC) curve

Table 3 shows the ideal cut off value (high detection rate (sensitivity) with low false positive) of the Holter derived variables of parasympathetic activity (SDANN-i, r-MSSD, pNN50), with positive and negative likelihood ratio. The best cut-off value of (SDANN-i) to predict diabetic patients with likelihood of daily life silent myocardial ischemia was 96 msec at 81% sensitivity and 79% specificity (the maximum sensitivity and maximum specificity near to the left diagonal), with 29% probability of error at 100% sensitivity and the area under curve was 0.897. The ideal cut-off value of (r-MSSD) to predict diabetic patients with likelihood of daily life silent myocardial ischemia was 26 msec at 85% sensitivity and 85% specificity with 32% probability of error at 100% sensitivity and the area under curve was 0.778. The ideal cut-off value of (pNN50) to predict diabetic patients with likelihood of daily life silent myocardial ischemia was 8% at 86% sensitivity and 84% specificity, with 27% probability of error at 100% sensitivity and the area under curve was 0.814.

Forward stepwise logistic analysis

Multivariate analysis revealed that duration of diabetes status (OR = 2.789, 95% CI = 1.541 - 4.196),

<table>
<thead>
<tr>
<th>Variables</th>
<th>Ideal cut-off values</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>False +ve %</th>
<th>Likelihood ratio +ve</th>
<th>Likelihood ratio -ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>110 msec</td>
<td>81</td>
<td>82</td>
<td>18</td>
<td>3.83</td>
<td>0.158</td>
</tr>
<tr>
<td>SDNN-i</td>
<td>51 msec</td>
<td>83</td>
<td>81</td>
<td>19</td>
<td>2.92</td>
<td>0.197</td>
</tr>
<tr>
<td>SDANN-i</td>
<td>96 msec</td>
<td>81</td>
<td>79</td>
<td>21</td>
<td>3.61</td>
<td>0.163</td>
</tr>
<tr>
<td>r-MSSD</td>
<td>26 msec</td>
<td>85</td>
<td>85</td>
<td>15</td>
<td>4.84</td>
<td>0.178</td>
</tr>
<tr>
<td>pNN50</td>
<td>8 %</td>
<td>86</td>
<td>84</td>
<td>16</td>
<td>4.72</td>
<td>0.189</td>
</tr>
</tbody>
</table>

24-hour Holter monitoring and heart rate variability

Table 1 shows that there was a significantly more decreased 24-hour Holter monitoring derived time domain variables (SDNN, SDNN-i, SDANN-i, p < 0.05) and a highly significantly more decreased (r-MSSD, pNN50, p < 0.01) in patients of group I than control group and those of group II, but there was a non significant difference between control group and those of group II (p = NS).

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Forward stepwise logistic analysis

Multivariate analysis revealed that duration of diabetes status (OR = 2.789, 95% CI = 1.541 - 4.196),

<table>
<thead>
<tr>
<th>Variables</th>
<th>Regression coefficient</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.0383</td>
<td>NS</td>
<td>0.835</td>
<td>0.234 - 1.778</td>
</tr>
<tr>
<td>Gender</td>
<td>0.0629</td>
<td>NS</td>
<td>0.627</td>
<td>0.474 - 1.431</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.0418</td>
<td>NS</td>
<td>0.751</td>
<td>0.325 - 1.214</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>0.0763</td>
<td>NS</td>
<td>0.728</td>
<td>0.078 - 1.637</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>0.0629</td>
<td>NS</td>
<td>0.629</td>
<td>0.174 - 1.131</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>0.0018</td>
<td>NS</td>
<td>0.784</td>
<td>0.325 - 1.914</td>
</tr>
<tr>
<td>Duration of diabetes status</td>
<td>0.1919</td>
<td>&lt; 0.05</td>
<td>2.789</td>
<td>1.541 - 4.196</td>
</tr>
<tr>
<td>Level of HbA1c</td>
<td>0.1954</td>
<td>&lt; 0.05</td>
<td>1.794</td>
<td>1.326 - 3.416</td>
</tr>
</tbody>
</table>

DISCUSSION

Ambulatory ECG monitoring was used to detect daily life silent ischemic episodes as well as heart rate variability in patients from our study. Zharov et al[16] used 24 hours monitoring and reported its usefulness to detect silent myocardial ischemia as compared with echocardiography segmental wall motion abnormalities. Hikita et al[17] found that ambulatory ECG monitoring is more sensitive for detection of silent myocardial ischemia than treadmill exercise ECG testing. Goseki et al[18] used ambulatory ECG monitoring to detect silent myocardial ischemia and heart rate variability before the occurrence of silent ischemia as a non-invasive parameter of autonomic nervous system activity.
Table 5: Stepwise logistic multivariate analysis of patients with versus without Holter-derived daily life silent myocardial ischemia with regard to independent variables of cardiac autonomic function tests

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Regression coefficient</th>
<th>p-value</th>
<th>Odds ratio</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting heart rate &gt; 100 beat/minute</td>
<td>0.0284</td>
<td>NS</td>
<td>0.891</td>
<td>0.234 - 1.778</td>
</tr>
<tr>
<td>Postural hypotension &gt; 30 mmHg</td>
<td>0.1527</td>
<td>&lt;0.05</td>
<td>1.861</td>
<td>1.374 - 2.931</td>
</tr>
<tr>
<td>Abnormal heart rate variation with deep breath</td>
<td>0.1814</td>
<td>&lt;0.05</td>
<td>2.297</td>
<td>1.541 - 3.196</td>
</tr>
<tr>
<td>Abnormal heart rate variation with Valsalva maneuver</td>
<td>0.0665</td>
<td>NS</td>
<td>0.821</td>
<td>0.078 - 1.637</td>
</tr>
<tr>
<td>Sustained (isometric) hand grip testing</td>
<td>0.1724</td>
<td>&lt;0.05</td>
<td>1.729</td>
<td>1.674 - 3.131</td>
</tr>
</tbody>
</table>

We found that 74 patients (85%) with silent myocardial ischemia had autonomic nervous dysfunction and a similar proportion has been detected by O’Brien et al[19], Langer et al[7], Quek et al[17], Hiketa et al[18] and Gupta et al[6]. Previous studies reported that diabetic autonomic neuropathy is not only a frequent complication of diabetes mellitus but also its presence was associated with grave prognosis[20]. Quek et al[19] and Jermendy et al[4] found that cardiovascular autonomic dysfunction occurs frequently in diabetic patients and postulated that autonomic neuropathy could be a possible explanation for lack of symptoms from abnormal ST segment depression in diabetic patients. Gupta et al[3] reported that diabetic neuropathy affects autonomic pain fibers that innervate the heart and these may be involved in the mechanism of silent myocardial ischemia in diabetics.

We found that the patients with cardiac autonomic dysfunction and silent myocardial ischemia had a predominant parasympathetic dysfunction. Lund et al[29] studied a group of diabetic rats with chemically-induced diabetes. After 12 weeks of induction of diabetes, they found that choline acetyl transferase (CAT) as a biochemical marker of parasympathetic innervation of the heart was reduced. Treatment of rats with insulin protected them against these changes as observed in the group of rats treated with insulin after induction of diabetes. Our patients with autonomic neuropathy and silent myocardial ischemia had more uncontrolled diabetes than those without silent ischemia as they had significant increased percentage of HbA1c.

We found that values of Holter derived variables of parasympathetic activity were significantly decreased in patients with silent myocardial ischemia. Malliani et al[21] and Risk et al[22] found that R-R interval difference provided a useful index of cardiac parasympathetic activity as the diabetic patients with evidence of vagal damage from the cardiovascular reflex had almost no variation in R-R intervals. Goseki et al[15] reported that decreased parasympathetic activity with background of high sympathetic activity can explain the appearance of ischemia during daily life. We found patients in our study had a non-significant change in heart rate before the occurrence of ischemic episodes than non-ischemic patients and this indicated that the ischemic episodes were due to decreased blood supply and not due to increased oxygen consumption. The silent myocardial ischemia is due to decreased coronary blood flow rather than increased demand as there was lower heart rate and lower blood pressure during silent ischemic episodes compared with exercise induced ischemia[23].

Shakespeare et al[24] reported that ischemic episodes became symptomatic if the ischemic stimulus exceeded the initial time period of vasodilatation. So, the silent myocardial ischemic episodes would be expected to be shorter in duration than symptomatic episodes and represent a less severe form of myocardial ischemia. Such a stimulation takes no account of other factors such as the intensity of myocardial ischemic stimulus but a greater initial propensity to vasodilate may also limit the extent of more intense ischemic stimulus[25].

CONCLUSION

Inspite of limitations and confounders of our study, we propose through multivariate analysis and ROC investigation that impaired parasympathetic activity heart rate variability provides statistically significantly better independent prediction accuracy to identify diabetic patients with likelihood of daily life silent myocardial ischemia.

REFERENCES


Preliminary Report

Effects of Visual Feedback Balance Training by Using Computerized Dynamic Posturography in Patients with Multiple Sclerosis

Maria Kondeva Ivanova, Mohieldin M Ahmed, Doahoo Mosalem M, Waleed Ahmed Al-Busairi
Physical Medicine and Rehabilitation Hospital, Ministry of Health, Kuwait

Kuwait Medical Journal 2009; 41 (2): 134-139

ABSTRACT

**Objectives:** To study the effects of the visual feedback balance training on the Berg Balance Scale (BBS) under static and dynamic conditions, the somatosensory, visual and vestibular systems by dynamic posturography before and after training program in patients with multiple sclerosis (MS)

**Design:** Retrospective case training study

**Setting:** Physical Medicine and Rehabilitation Hospital, Ministry of Health, Kuwait

**Subjects and Methods:** A total of 23 patients with MS were recruited.

**Intervention:** All patients were evaluated using both the BBS and the sensory organization test (SOT) using computerized dynamic posturography (CDP) before and three months after the training program. The SOT consists of six conditions and composite equilibrium score (CES).

**Main Outcome Measures:** CES (%) and SOT were calculated

**Results:** Before training, all patients had a reduction of BBSS and parameters of the SOT. After training, a significant increase of BBS (p < 0.05), all parameters of the SOT (p < 0.05) and CES (p < 0.001) were observed.

**Conclusion:** Improvement in all parameters of the SOT after training program could be explained by change in the somatosensory system of the posture control system. Also, there is impairment of the somatosensory system, rather than a specific lesion of vestibular and / or visual systems.

**KEY WORDS:** CDP, computerized dynamic posturography, multiple sclerosis, visual feedback balance training

INTRODUCTION

Multiple sclerosis (MS) is a chronic neurological disease characterized by multiple areas of focal demyelination that develop throughout the white matter of the central nervous system at varying times[1]. Balance impairments are common in persons with MS[2]. Human balance control system is the result of complex interactions between musculoskeletal and neuromuscular systems, including sensory, motor, and integrative components[3]. It relies on feedback from the somatosensory, vestibular, and visual systems. Diminished somatosensation is associated with increased postural instability during quiet standing with eyes closed[4]. Somatosensory stimulation may thus prove to be an effective way to improve balance control in these patients[5]. Because these sensory, motor, and integrative components are frequently affected by MS, many persons with MS have balance deficits[6].

Clinical balance tests, e.g., the Romberg test may not detect subtle deficits in adults with MS who are not yet experiencing functional limitations or disability[7]. Dynamic posturography has become an important tool for understanding standing balance in clinical settings. A key test in dynamic posturography system, the sensory organization test (SOT), provides information about the integration of multiple components of balance. The SOT leads to an outcome measure called the “equilibrium score” (ES), which reflects the overall coordination of the visual, proprioceptive, and vestibular systems for maintaining standing posture[8, 9].

Thus, the aim of this study was to 1) study the effects of visual feedback balance training on the Berg Balance Scale (BBS) as balance functional impairment and 2) study effects of visual feedback
balance training on static and dynamic conditions and the somatosensory, visual and / or vestibular systems by computerized dynamic posturography before and after rehabilitation program in moderately disabled persons with MS in Kuwait.

SUBJECTS AND METHODS

A total of 23 female Kuwaiti patients with MS were recruited for this study and selected from the out-patient clinic in the Physical Medicine and Rehabilitation Hospital, Kuwait. All patients were evaluated clinically with a brief neurological examination. All patients underwent image studies such as brain computed tomography or magnetic resonance imaging to confirm their diagnosis. The diagnosis of MS is generally made by revised diagnostic criteria for multiple sclerosis[9]. The assessment of neurological impairment of MS was done using the Kurtzke Expanded Disability Status Scale (EDSS)[11]. Formal consent was taken from all the patients.

Inclusion criteria were the following: (1) ambulatory patients with ability to ambulate 25 ft independently; and (2) mild or moderate MS deficits defined by an EDSS of ≤ 2.3. Those with severe MS, severe spasticity or cognitive deficit, peripheral neuropathy or significant visual field problems were excluded. The patients gave informed voluntary consent to participate in the study according to the protocol approved by the local ethics committee and in accordance with the ethical standards of the Helsinki declaration.

Balance functional impairment was done by using the BBS. The maximum score for this assessment is 56. Based on clinical experience, Berg et al contend that scores below 45 indicate that someone is impaired, with an increased risk for falls[12].

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases n (%)</th>
<th>Mean ± (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.2 ± 7.6</td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>16.84 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>EDSS</td>
<td>2.3 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Therapy with interferon</td>
<td>21 (91.3)</td>
<td></td>
</tr>
<tr>
<td>Clinical course of MS:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relapsing-remitting</td>
<td>21 (91.3)</td>
<td></td>
</tr>
<tr>
<td>Secondary progressive</td>
<td>2 (8.7)</td>
<td></td>
</tr>
<tr>
<td>Primary progressive</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Progressive relapsing</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Dysarthria</td>
<td>16 (69.7)</td>
<td></td>
</tr>
<tr>
<td>Cerebellar lesion</td>
<td>14 (60.87)</td>
<td></td>
</tr>
<tr>
<td>Brainstem lesion</td>
<td>3 (13.1)</td>
<td></td>
</tr>
<tr>
<td>Pyramidal lesion</td>
<td>17 (79.9)</td>
<td></td>
</tr>
<tr>
<td>Sensory lesion</td>
<td>11 (73.9)</td>
<td></td>
</tr>
<tr>
<td>Bladder lesion</td>
<td>2 (8.9)</td>
<td></td>
</tr>
<tr>
<td>Mental lesion</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

EDSS: Expanded Disability Status Scale

The SOT was used for balance assessment. It included six test conditions. The first three conditions include SOT 1 (eyes open), SOT 2 (eyes closed), SOT 3 (sway-referenced vision with standing on a fixed platform called static posturography. The second three conditions include SOT 4 (eyes open), SOT 5 (eyes closed), SOT 6 (sway-referenced vision with standing on a moving platform called dynamic posturography. Composite Equilibrium Score (CES %) was calculated that describes the overall level of performance under the six conditions. Scores range from 0 to 100, with 0 representing a fall and 100 representing perfect stability[9].

The sensory analysis scores represent the influence of each sensory system on the individual’s stability, and quantify the relative difference in scores between conditions. The somatosensory ratio compares condition 2 to condition 1 and measures postural stability when vision is removed. The visual ratio compares condition 4 to condition 1 and measures the ability of the visual system to function when somatosensory input is altered by sway-referencing.

Table 1: Demographic data and principal characteristics of 23 patients with multiple sclerosis

Table 2: Mean ± SD of Berge Balance Scale and parameters of sensory organization test (SOT) before and after training in MS patients

Table 3:  Mean ± SD of Berge Balance Scale and parameters of sensory organization test (SOT) before and after training in MS patients

The paired-sample Student’s t test, * = Significant, ** = Highly significant, # = non-Significant.

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The vestibular ratio compares condition 5 to condition 1, assessing the stability of the individual when both somatosensory and visual input have been altered by sway-referencing or eye closure, respectively. A vision preference score, an indication of whether the participant is overly reliant on visual information, is also calculated using the ratio of the sum of conditions 3 and 6 to the sum of conditions 2 and 5[9].

All patients received complex training course for three months including visual feedback balance training (once weekly) by using computerized dynamic posturography and conventional physical therapy (three times weekly). All patients were evaluated in a study of postural stability by the BBS.
and computerized dynamic posturography (CDP) before and three months after the training program.

**Statistical analysis**

Study data were analyzed using the SPSS statistical package. The paired-sample Student’s t test indicates the magnitudes of the differences of means between the BBS, ES and SOT scores before and after treatment in MS patients. A p-value of < 0.05 was considered as significant. This study was approved by the hospital ethical committee.

**RESULTS**

Table 1 represents characteristics of patients with multiple sclerosis. Table 2 shows results of BBS, CES and six tests conditions and sensory analysis scores before as compared to results after training of MS patients. Fig. 1 and 2 represent CDP before and after treatment in MS patients.

There was statistically significant increase of mean (SD) of BBS after treatment (51.4 ± 3.9) as compared with results before training (42.3 ± 4.8) (p < 0.05). There was an increase of mean (SD) of CES after treatment (68.3 ± 4.2) as compared to results before training (49.5 ± 3.8, p < 0.001). Moreover, a statistically significant increase was observed for the static and dynamic balance after treatment as compared to results before training (p < 0.05).

According to the sensory analysis scores, a significant increase was observed for the somatosensory ratio score after treatment (93.1 ± 2.6) as compared to results before training (72.3 ± 1.8, p < 0.05). However, there was a statistically non-significant difference in visual ratio score, vestibular ratio score and preference score before as compared with results after training (p > 0.05). The data suggest that there is an impairment of the somatosensory system, rather than a specific lesion of vestibular and/or visual systems.
DISCUSSION

Balance problems and falls are common in people with MS but their cause and nature are not well understood. It is known that MS affects many areas of the central nervous system that can impact postural responses to maintain balance, including the cerebellum and the spinal cord\textsuperscript{[13]}. Ambulatory patients with MS frequently present with poor balance\textsuperscript{[14]}

Our data demonstrate that there was a statistically significant increase of BBS and CES after treatment as compared to results before treatment. Also, a statistically significant increase in static and dynamic balance functions was observed after treatment as compared to results before treatment. These data suggest that good balance control after treatment could be explained by changes of the somatosensory system rather than vestibular and/or visual systems and the CDP can provide a useful tool for quantitative detection of imbalance in patients with MS.

This study agrees with other studies. Cha et al reported that BBS showed statistically significant improvement in functional status in patients with MS\textsuperscript{[15]}. Dalla et al studied treatment that was based on postural feedback on standing balance platform. After rehabilitative treatment the clinical and functional finding and standing balance was improved\textsuperscript{[16]}, CDP facilitates measurement of standing balance and permits a quantification of the role of proprioception, vision and the vestibular system in the maintenance of standing balance\textsuperscript{[17]}.

Jackson et al studied twenty-seven patients with mild MS by CDP. They found poor performance of balance which probably indicates a disruption of the integration of visual, vestibular, and somatosensory information in MS. Although patients with early MS and patients with purely vestibular disorders often have similar complaints, they have quite different profile of abnormalities in posturography testing\textsuperscript{[18]}.
Balance deficits in people with MS appear to be caused by somatosensory and not by cerebellar involvement in some MS patients\textsuperscript{[13]}. Some MS patients had a vestibular dysfunction pattern or a combined visual-vestibular or somatosensory-vestibular impairment. Posturography might serve as one method to evaluate the functional consequences of a vestibular deficit in patients with MS\textsuperscript{[19]}.

In our study, we found impaired balance in patients with relapsing remitting multiple sclerosis (RRMS). But other authors found that balance in MS patients is impaired in RRMS, secondary progressive MS (SPMS) and primary progressive MS (PPMS). There were some differences of balance between previous groups in all the balance tests\textsuperscript{[20]}.

Some authors found balance impairments including minimal balance deficits of the BBS and impaired center of pressure displacement during standing in MS\textsuperscript{[7]}. Achiemeere suggests that dynamic posturography can provide useful diagnostic information in patients with balance disturbances in MS patients\textsuperscript{[21]}.

In contrast to our results, Frzovic et al. reported that there were no differences between MS and control groups on the ability to maintain standing balance. There was little change in balance from morning to afternoon in participants with MS, despite an increase in self-rated fatigue\textsuperscript{[6]}. Some authors found the MS patients had a vestibular dysfunction pattern or a combined visual-vestibular or somatosensory-vestibular impairment\textsuperscript{[19]}. There is a disruption of the integration of visual, vestibular, and somatosensory information in patients with early MS and patients with purely vestibular disorders in posturography testing in MS\textsuperscript{[18]}.

One possible explanation for our findings is that the CNS damage, caused by MS is supposed to reflect some change in the structure of the posture control system such as the impairment of the somatosensory system, rather than a specific lesion of vestibular and / or visual modalities\textsuperscript{[22]}. Jackson et al found poor performance of balance and abnormalities in posturography testing which indicates a disruption of the integration of somatosensory, visual or vestibular information in MS\textsuperscript{[19]}.

This study briefly describes the results of our experience in visual feedback training by using the dynamic posturography for MS patients. This study also represents the first attempt in Kuwait to use the dynamic posturography equipment as an assessment tool to study the effect of visual feedback training in MS patients. Further research in use of the posturography equipment is needed to study various mechanisms of balance dysfunction in patients with MS.

**Limitations**

The small number of the study subjects may have some influence on the outcome and the authors are continuing the study by adding more subjects.

**ACKNOWLEDGMENTS**

We acknowledge the help of our colleagues in the Physical Medicine and Rehabilitation Hospital, Kuwait for their assistance in this study.

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INTRODUCTION

Reports of illnesses resembling meningococcal disease date back to the 16th century. The description reported by Viesseux in 1805 is generally thought to be the first definitive identification of the disease. The causative organism; *Neisseria meningitidis* was first isolated in 1887[1]. It is one of the most feared infections in children due to its possible rapidly fatal course and relatively high incidence of sequelae in survivors. Despite treatment with the appropriate antimicrobial agents and optimal care, the overall case fatality rates have remained relatively stable over the past 20 years reaching a rate of up to 40% amongst the patients with meningococcemia[3]. Between 11-19% of survivors of meningococcal disease have sequelae such as hearing loss, neurological disability or loss of a limb[4,5].

Most of the research that has been done in recent years was directed at the pathophysiological mechanisms in the acute phase. However, relatively little is known about the complications occurring in the sub-acute phase (4-10 days after the initial antibiotic treatment); the so-called type 3 immune complex hypersensitivity reactions according to Gell and Coombs[2].

In meningococcal disease type 3 reaction, further called immune associated complication (IAC), can occur as arthritis, vasculitis, episcleritis, pericarditis or rarely nephritis[2]. More than one complication can occur concurrently in some patients. We describe two siblings in this case study. One developed arthritis and very rarely nephritis. We report two siblings with meningococcal disease. The first developed arthritis and vasculitis while the younger sister developed only arthritis of the right ankle. To the best of our knowledge this is the first case report to be published in Kuwait.

CASE PRESENTATION

Case One

A nine-year old girl, second in order of siblings of six children, was admitted with complaints of fever, headache, vomiting and a skin rash. On examination she looked ill with altered level of consciousness, had signs of meningeal irritation as well as purpuric and ecchymotic skin rash all over her body. Intravenous ceftriaxone (100 mg/kg/day) was commenced following admission with a clinical diagnosis of meningococcemia. Complete blood count showed white blood cell of 5.2 x 10⁹/l, neutrophils 86%, hemoglobin 117 g/l, platelet 267 x 10⁹/l. Erythrocyte sedimentation rate (ESR) was 38 mm/h and C-reactive protein (CRP) was 259 mg/l. Latex agglutination test for blood and urine as well as blood cultures were all positive for *Neisseria meningitidis* serogroup W 135. Complement 3 and 4 levels (C3, C4) were 0.241 g/l (0.88-2.01) and 0.179 g/l (0.16-0.4) respectively. Parents declined lumbar puncture. She improved clinically over the first three days. She became more conscious on the second day, afebrile after about 36 hours and was moving about freely in her room. On the fourth day she developed a secondary rise of temperature which was associated with arthritis of the right shoulder and left knee. Tender subcutaneous nodular lesions consistent with vasculitis were seen on both thighs. ESR rose to 80
mm/h on the fifth day. CRP dropped to 60 mg/l on day three and showed secondary rise to 76.2 mg/l on the fifth day. Intravenous dexamethasone in a dose of 0.4 mg/kg/day for three days and 0.2 mg/kg/day for the next four days as well as oral ibuprofen (40 mg/kg/day) were added to her treatment. She showed remarkable improvement 24 hours later. She was discharged after completing 10 days of antibiotic treatment and remained well on follow up after 9 and 12 months. Her C3 and C4 levels on follow up after nine months were less than 0.0583 g/l (0.88-2.01) and 0.162 g/l (0.16-0.4) respectively. All her contacts except the youngest sister (case 2) received oral antibiotic prophylaxis (rifampicin) and the quadrivalent meningococcal vaccine.

Case two

This eight month-old girl, the youngest sister of case one received neither prophylactic antibiotic nor meningococcal vaccine. This infant developed fever, vomiting and skin rash four days following the onset of her sister’s illness. It was proven to be meningococcal disease with positive blood culture of \textit{N.meningitidis} serogroup W135. Complete blood count showed white cells of 7.5 x 10\(^9\)/l, neutrophils 60\%, platelets 230 x 10\(^9\)/l and ESR 25 mm/h. Her C3 and C4 were 0.3 (0.88-2) and 0.152 (0.16-0.4) respectively. She was treated with ceftriaxone intravenously at a dose of 100 mg/kg/day for 10 days. The infant showed initial improvement but on the fifth day she developed arthritis of the right ankle associated with secondary rise of temperature and raised ESR of 70 mm/h. This was managed by ibuprofen with a clinical diagnosis of an immune-associated arthritis.

She was discharged home after 10 days and remained well on follow up after 9 and 12 months. Her C3 and C4 were 0.06 g/l and 0.166 g/l after nine months.

DISCUSSION

A recent large study of meningococcal disease in children showed that 15.3\% of the patients had IAC\(^2\). However, it is surprising that only 16 cases were reported in the period between 1960 and 2003. The authors concluded that the incidence of IAC has not declined over the past 20 years\(^2\).

IAC can present as arthritis, vasculitis, episcleritis, pericarditis or rarely nephritis. Risk factors for the development of IAC are severe disease, age of the patient (being more common in adolescents or adults)\(^3\), serogroup W135\(^6\) and group C\(^7\).

The two patients in our report had more than one of these risk factors. Both of them had severe disease (meningococcemia), the organism was serogroup W135 and the older patient is a pre-adolescent.

IAC usually develops 4-10 days after the onset of meningococcal infection and presents with local manifestations and a secondary rise in temperature associated with secondary increase in ESR and CRP\(^2\). This was well documented in our two patients.

Differential diagnosis should include secondary infection, subdural effusion, persistent infection, primary meningococcal arthritis and allergic reaction to medications.

Careful and thorough physical examination will give clues that aid in the diagnosis of the disease without the necessity of extensive additional investigations\(^2\).

Case one developed arthritis of the right shoulder and the left knee as well as vasculitis involving both thighs on day four with secondary rise of temperature. There was no evidence of subdural effusion such as convulsions, headache or vomiting. There were no itchy skin rashes, no edema or puffiness of eyelids. A second blood culture and sensitivity on the fifth day showed no growth.

Case two developed meningococcemia most probably because she did not receive chemoprophylaxis. In fact both patients appeared to be more prone to develop meningococcemia in view of their hypocomplementemic state.

Meningococcal vaccine was not given to case two considering that it is not immunogenic below the age of two years.

Treatment of IAC depends on the site involved. Specific treatment for arthritis is generally not indicated except for pain relief and the prognosis is excellent. Pericarditis has a significant mortality related to cardiac tamponade. The effusion often requires treatment but occasionally it may resolve spontaneously. Therapy consists of salicylates, steroids, pericardiocentesis, or a combination of these\(^2\). Cutaneous digital vasculitis associated with ischemia can be treated with prostacycline analogue to obviate the need for amputation\(^6\).

Both our cases were treated with nonsteroidal anti-inflammatory drugs (ibuprofen) for arthritis. However, dexamethasone was added to case one, as she had cutaneous vasculitis in addition to arthritis. Both of them improved without complications when reviewed at nine months and one year follow-up.

CONCLUSION

Despite the lack of recent publications, IAC remains a well-defined complication of meningococcal infection. Arthritis is the most common manifestation of IAC. In addition to local clinical manifestations, IAC presents with secondary fever and secondary increased ESR and CRP. Thorough physical examination with minimal
additional investigations will give clues to the diagnosis.

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Case Report

Pneumatosis Intestinalis of Small Bowel in an Adult: A Case Report

Rajan Arora¹, Amany Abd El-Hameed¹, Obaid Al Harbi²
¹Department of Pathology and ²Department of Surgery, Al Farwaniya Hospital, Kuwait

ABSTRACT

Pneumatosis Intestinalis (PI) is rare in adults although it can be seen in the pediatric population as a complication of necrotizing enterocolitis. We report a case of PI affecting the small bowel in a 27-year-old patient who presented with signs and symptoms of acute abdomen due to perforated duodenal ulcer. Histopathologic findings are demonstrated and the pathogenesis is discussed with the objective of highlighting that PI is not a diagnosis but a finding which needs further evaluation and management in view of the underlying etiology.

KEY WORDS: gastrointestinal, necrotizing enterocolitis, small bowel

INTRODUCTION

Pneumatosis intestinalis (PI) defined as gas within the wall of gastrointestinal (GI) tract, is not a diagnosis but a physical or radiological finding. It is secondary to an underlying disease process in 85% of cases which include obstructive pulmonary disease¹, cystic fibrosis², obstructive GI disease (e.g., volvulus³ and intussusception⁴), gastroduodenal ulcer, ulcerative colitis, necrotizing enterocolitis⁵, acquired immunodeficiency syndrome, and trauma⁶. Secondary form typically involves the small bowel but may occur throughout the GI tract. Since the etiology is so varied, the course and outcome varies from benign self resolving to fatal depending on the underlying disease process.

The primary form which accounts for 15% of cases is a benign idiopathic condition usually affecting the colon⁷. Histopathologic findings in both are usually the same but pathogenesis is complex depending on interaction of many factors like mucosal integrity⁸, intraluminal pressure⁷, bacterial flora⁹, and intraluminal gas¹⁰. Management depends on treating the underlying cause and surgery is indicated when acute complications appear such as perforation and peritonitis.

CASE REPORT

Clinical findings

A 27-year-old male was admitted to surgical casualty with signs and symptoms of acute abdomen. Radiological investigations revealed gas under the diaphragm (indicative of perforation) as well as air in relation to bowel wall on plain radiograph. Computerized tomographic scan could not be done due to the nature of the emergency, and laparotomy was performed.

Operative findings

A perforated duodenal ulcer was found which was closed by patch omentoplasty. The terminal 180 cm of the small bowel all the way to the ileocecal junction showed multiple thin walled, tense, air filled cysts on the serosal surface (few were perforated and collapsed). The cecum and colon were not involved. The small bowel proximal to the involved portion was distended. A limited right colectomy and resection of the involved small bowel was done. After resection, the patient was managed in the intensive care unit and was discharged from the hospital after 10 days. He is on follow up and is in good health till date.

Pathological findings

Gross features: The specimen (comprising of terminal ileum, cecum with appendix, and part of ascending colon) was received in 10% neutral buffered formalin. It was floating in the container due to buoyancy of the air. Outer surface of whole length of small bowel (180 cm) showed congestion and multiple cysts in grape like manner (Fig. 1). On cut section, the mucosa showed cobblestone

Address correspondence to:
Dr. Rajan Arora, Senior Registrar, Department of Pathology, Farwaniya Hospital, PO Box : 18373, Farwaniya 81004, Kuwait. (F) 00965-4893078, E-mail: arorarajan73@rediffmail.com, drrajarora@yahoo.com
appearance (Fig. 2) with numerous air filled cysts (pneumocysts) in the wall. The cysts varied in size from 0.2 to 2 cm in diameter. No mass, polyp, diverticula, volvulus, or intussusception was identified.

Microscopic features: The cysts identified grossly in small bowel were located in the submucosal (Fig. 4), and subserosal region. They were lined by mostly multinucleated giant cells. No true lining epithelium was seen. There were no features of any granulomatous inflammation, necrosis, inflammatory bowel disease, or malignancy. Moderate to severe serositis was observed. Special stains (Periodic acid-Schiff, Grocott’s methamine silver, and Gram’s stain) for micro-organisms were negative.

DISCUSSION
PI is rare and preliminary diagnosis depends on clinical and radiological findings. Computerized tomographic scan is the best imaging modality although plain radiograph also shows characteristic findings. The pathogenesis has been debated for years and various explanations have been suggested by various authors.[5,7,9,10]. However the spectrum of diseases which underlie the development of these cysts point toward a multifaceted phenomenon. Two most crucial considerations are: (a) from where the gas came, and (b) how it got into the bowel wall. Three possible sources of bowel gas are intraluminal gas, bacterial production of gas, and pulmonary gas. Intraluminal gas can leak to the bowel wall due to increased intraluminal pressure and mucosal injury, either of them occurring singly[5,6], or together in various conditions, e.g., GI obstruction, and ulcerative colitis[3-5,10]. Bacterial production of gas has been suggested as an inciting factor for PI and is supported by the fact that gas disappears after antimicrobial drug treatment[9]. The original theory of pulmonary gas leaking as a result of alveolar rupture leading to dissection along vascular planes to mediastinum and then tracing caudally to retroperitoneum
appears logical\cite{1}, but lack of interstitial emphysema within lungs or mesentry in many of these patients seems to contradict the original explanation\cite{2,9,10}, and association of pulmonary disease with PI may be simply due to fluctuations in intra-abdominal pressure by pulmonary obstruction\cite{5}.

Duodenal perforation leading to extravasation of its content including air causing peritonitis and dissection of air along mesenteric vessels appears to be the most logical explanation underlying formation of pneumocysts in our case, since serositis and subserosal cysts were predominant findings.

CONCLUSION
This case demonstrates the classical histopathological findings of PI and stresses on the fact that PI is not a disease but a finding which needs further evaluation to discover the etiology. Treatment of the underlying disease process should be the focus of treatment. Surgery is indicated in patients with signs and symptoms of perforation, peritonitis (like the present case), or abdominal sepsis. When PI is associated with conditions in which surgical treatment has no role or no other definitive treatment exists, use of inspired oxygen may be beneficial.

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Case Report

Idiopathic Pulmonary Hemosiderosis

Abdullah Almutairi¹, Nasser Behbehani¹, Tareq MMA Mohammed²
¹Department of Medicine, Mubarak Alkabeer Hospital, Kuwait
²Department of Pathology, Mubarak Alkabeer Hospital, Kuwait

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ABSTRACT

Idiopathic pulmonary hemosiderosis (IPH) is a rare disorder that is usually characterized by the triad of bilateral pulmonary infiltrates, hemoptysis and iron deficiency anemia. The disease is well known to affect the pediatric age group with conflicting treatment trials. We report a case of a 19 year-old girl with IPH and respiratory failure who had excellent initial response to systemic steroids. To the best of our knowledge this is the first reported case in this age group in the region.

INTRODUCTION

Idiopathic pulmonary hemosiderosis (IPH) is a rare disorder, which affects children in the majority of cases. There are several reported cases in the adult population. Typically, intensive search for aetiology ends up negative. Patients usually present with cough and hemoptysis, but iron deficiency anemia could be the sole presentation. This presentation in an adult makes the diagnosis more difficult as other causes of alveolar hemorrhage need to be carefully excluded. We report the case of a 19 year-old girl who presented to Mubarak Hospital with hemoptysis and respiratory failure followed by a review of relevant literature.

CASE REPORT

Ms. F is a 19-year-old girl, who was transferred to Mubarak hospital from another institution with respiratory failure. Her illness started five days prior to her presentation with fever, cough and shortness of breath. She described episodes of hemoptysis during her illness with few blood clots occasionally. On further questioning, she described similar attack three years ago, where she had been coughing streaks of blood and was febrile. This was followed by a protracted course of mild hemoptysis for almost one year followed by spontaneous resolution. Since then she continued to feel short of breath on exertion, but attributed it to the iron deficiency anemia that she suffered from.

Her anemia was severe enough to require blood transfusion twice. She denied any skin rash, joint pain or swelling, mouth or genital ulcers.

On admission to the referring hospital, she was febrile, tired and short of breath. Her initial chest X-ray showed bilateral alveolar infiltrates (Fig. 1). She was initially managed as community acquired pneumonia. Soon after admission, her condition deteriorated with marked hypoxemia requiring 70% inspired oxygen. CT chest revealed bilateral diffuse infiltrates, mixed alveolar opacities and interstitial infiltrates.

She was then transferred to our facility for further workup and management. On arrival she was significantly hypoxic, but hemodynamically stable. On a FIO₂ of 0.7 her O₂ saturation was only 91%. She was admitted to the intensive care unit and started on broad spectrum antibiotics (maxipime and intravenous erythromycin). Bronchoscopy was performed and transbronchial biopsies were taken. Her bronchoalveolar lavage done prior to the biopsy was consistently bloody. Full immunological investigations were sent to exclude any pulmonary vasculitis including: ANA, C and P ANCA, Anti-GBM, complements and rheumatoid factor. These were all negative except for a non-specific weak positive C ANCA and ANA. The transbronchial biopsy showed extensive hemosiderin in the tissue as well as hemosiderin laden macrophages (Fig. 2). There was also marked interstitial fibrosis with no evidence of vasculitis or capillaritis.

Address correspondence to:
Dr Abdullah Almutairi, Department of Medicine, Mubarak Alkabeer Hospital, Al-Jabriyah, Kuwait. E-mail: doctorq8@gmail.com
During her admission she had a 2D echo which was normal. On reviewing her old chest X-Ray done in 2003 (Fig. 3), it did show a large air space lesion on the right lower lung zone (which in retrospect could represent alveolar hemorrhage). She was started on systemic steroid at a dose of prednisolone 40 mg daily with complete resolution from a radiological as well as clinical aspect. Her maintenance treatment consisted of steroids with chloroquine.

When her steroids were tapered down to 10 mg over four months, she started to experience multiple episodes of minor hemoptysis, none of which was clinically significant. Currently, azathioprine has been added as steroid sparing agent.

**DISCUSSION**

IPH is a rare disease with an estimated incidence of 0.24 -1.23 cases per million in the pediatric population\[^4\]. It is usually diagnosed after combining clinical and radiological parameters with exclusion of more common disorders that leads to pulmonary hemorrhage. Clinically IPH manifest with a triad of pulmonary infiltrates, iron deficiency anemia, and hemoptysis. An absolute requirement for the diagnosis is identification of hemosiderin laden macrophages in sputum, bronchoalveolar lavage, lung biopsy specimens and gastric washings\[^5\]. Multiple blood transfusion for severe anemia in IPH patients has been reported in the literatures\[^6\]. Our patient was labeled as iron deficiency anemia and required blood transfusion twice. The exact etiology of IPH is unknown, although most therapeutic attempts used immunosuppressive agents for treatment\[^7\].

Treatment is based on case series, but in the initial phase of presentation, steroids in high dose (e.g., prednisone 2 - 5 mg/kg/d or equivalent) are considered the treatment of choice\[^4,8\]. This has proven to control the acute bleeding episode and decrease the frequency of pulmonary hemorrhage. However, the long term benefit is still controversial. A retrospective review of 23 children diagnosed with IPH in whom steroids in low-dose have been tried after initial high-dose on presentation, showed prevention of crises and milder disease course\[^6\]. However, there are some patients who fail to respond to steroids alone and in whom another form of immunosuppression has been tried.

Such therapies include azathioprine, chloroquine, or cyclophosphamide. There are several case reports and retrospective studies that showed beneficial effect of azathioprine in long
term control of symptoms and as a steroid sparing agent\textsuperscript{[5,9,10]}. However, mortality benefits are difficult to prove in such types of publications. The usual starting dose varies from 1 to 2 mg/kg daily or on alternative days. Chloroquine has long been the favored immunosuppressive therapy of choice due to acceptable side effects profile. There are several case reports of the effectiveness of chloroquine, where it has been used for long term control of pulmonary hemorrhage\textsuperscript{[11,12]}. The reported dosage ranged from 200 to 400 mg daily, but retinal changes were detected in one patient on 400 mg of chloroquine, which resolved after discontinuation of the drug. Cyclophosphamide, on the other hand, is the least used immunosuppressive therapy reported\textsuperscript{[7]}. The range of dosage in these reports was from 1.5 to 2.5 mg/kg/d.

Given all these data, the main concern in choosing between the different regimen of immunosuppression would be the side effect profile of the drug and patient tolerance. The five years survival of IPH has been recently reported to be 86\%, in contrast to previous reports of 2.5 years average survival\textsuperscript{[10]}. There are different reports of favorable survival factors but with conflicting results.

Lastly, the presence of c-ANCA in low titre in the serum of our patient, although not associated with systemic or pathological manifestation of vasculitis, has been reported in the literature\textsuperscript{[13]}. It has been linked to classifying IPH according to severity, and was thought to represent a prognostic value. This, though has not been consistent through the literature.

REFERENCES

Case Report

Scrub Typhus Associated with Systemic Lupus Erythematosus: A Case Report

Mohsen Nasr¹, Mohamed Mostafa Abdelwahab Mostafa¹, Soondal Koomar Surrun²

¹Department of Internal Medicine, Al-Jahra Hospital, Kuwait
²Department of Internal Medicine, Singapore General Hospital, Singapore

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ABSTRACT

The etiology of systemic lupus erythematosus (SLE) is largely unknown and there is a probable contribution of genetic, hormonal, immunological and environmental factors for its manifestation. The widespread immunological destruction of many organs in SLE and the associated decreased immunity increase the risk of infections. Steroids and other immunosuppressant are important in the treatment of SLE, but they further increase the risk of infections, and sometimes with rare organisms. We present a case of an adolescent girl with prolonged fever, joint pains and without skin rashes. The initial diagnosis was SLE. She was treated with steroids with improvement in her general condition and relief of joint pains. However the fever persisted and subsequent investigations revealed an associated scrub typhus. The fever subsided after treatment with oral tetracycline. There were no complications of scrub typhus. Since scrub typhus infection is not common in the Arabian Peninsula the disease was not initially thought of. In the investigation of prolonged fever in SLE, viral, bacterial, protozoal as well as rickettsial diseases should be borne in mind.

KEY WORDS: pyrexia of unknown origin, scrub typhus, systemic lupus erythematosus

INTRODUCTION

Systemic lupus erythematosus (SLE) may present clinically in many subtle ways. Initial presentation as prolonged fever is common. The course of SLE is sometimes punctuated by infections, sometimes with rare organisms[1]. Infections remain a major cause of morbidity and mortality in SLE. When a patient with SLE becomes infected with scrub typhus, the associated infection may not be entertained initially, especially in non-endemic regions leading to delay in investigations and diagnosis. The typical eschar may be absent, and other skin lesions of scrub typhus may be mistaken for other febrile illnesses including SLE[2]. Scrub typhus infection is endemic in Asia, the Pacific islands including northern Australia and New Zealand. It is a zoonosis that is transmitted by mites and before the advent of antibiotics the disease was occasionally fatal. As with SLE, fever is a common presenting symptom in scrub typhus. Additionally, lymphadenopathy, an eschar or skin rashes may also be present. During the Second World War scrub typhus was a dreaded disease causing substantial morbidity and occasional deaths. Nowadays, it occasionally occurs outside the endemic areas and rarely in the Arabian Peninsula.

CASE HISTORY

A 17-year-old girl was admitted with easy fatigue, recurrent bouts of fever, painful wrists and elbows and loss of hair of two months’ duration. The pain occurred simultaneously in the affected joints, was continuous and partially relieved by paracetamol. She did not notice any loss of weight or dryness of the mouth, and there was no morning stiffness. She was previously in good health and had no major medical or surgical problems.

On examination she was pale, without jaundice or cyanosis. There were no skin rashes or nail changes. There was no discoid alopecia. Her temperature was 38.8 °C, pulse 80 beats per minute and blood pressure 110/80 mm Hg. There was no generalized lymphadenopathy. Both elbows and wrists were swollen and red, with impaired extension of the elbows. Movement of the wrists though painful was full. There was no wasting of the interossei, thenar or hypothenar eminences. Examination of the eyes was normal. Heart

Address correspondence to:
Dr. Mohsen Nasr, PhD, Department of Internal Medicine, Al-Jahra Hospital, PO Box 3038, Kuwait 01032. E-mail: mohsennasr@hotmail.com
sounds were normal and there were no murmurs. The chest examination was normal. There was no hepatosplenomegaly and neurological examination was normal.

Hematological tests showed hemoglobin of 8.4 gm/dl, white blood cell count of 2.2 x 10⁹/ml and a platelet count of 230 x 10⁹/ml. ESR was 118 mm/hr, CRP negative, coagulation profile normal and Coomb’s test negative. Serum iron was 5 µmol/l, bilirubin was normal with an AST of 151 iu/l, ALT of 87 iu/l and ALP of 423 iu/l. Urine microscopy was normal, the pH 5 and the 24-hour urine protein was 0.19 g/day. Complement tests showed a C3 of 78 (normal range 75-135) mg/dl and C4 of 16 (normal range 12-72) mg/dl. Hepatitis profile, monospot test, brucella antigen test and Widal test were negative. Chest radiograph, the abdomen and pelvic ultrasound were normal. The rheumatoid factor (RF) IgM titer was 26 µmol/ml (n < 20), RF IgG 14 µmol/ml (n < 20), RF IgA 10 µmol/ml (n < 15), ANA 1/320 (speckled pattern), and anti-dsDNA antibody 76 IU/L. A clinical diagnosis of SLE was made and prednisolone was started at a dose of 40 mg per day. The patient felt well, the pain in the joints improved but the fever persisted. White blood cell count rose to 14.4 x 10⁹ /ml, liver enzymes remained high and the CRP became positive on repeated examination. An associated infective process was then considered and a diagnosis of pyrexia of unknown origin was entertained.

Repeated chest radiographs and blood cultures were normal. Further blood investigations were negative for HIV, toxoplasma, parvovirus and fungus. Gallium scan was negative. However, Weil-Felix reaction was positive for OX-K proteus antigen with a titer of 1/320, rising to 1/640 after three days.

A definitive diagnosis of scrub typhus complicating SLE was made. The patient was prescribed 500 mg of oral tetracycline every six hours by the medical on-call team. The fever abated within two days and the patient felt much better. On clinical grounds the treating team preferred not to change tetracycline to doxycycline. The general condition of the patient continued to improve and the white blood cell and liver function tests became normal. She was discharged on oral prednisolone 40 mg daily and tetracycline 500 mg qds for a total of two weeks. On review the patient was asymptomatic and well.

DISCUSSION

SLE presenting as prolonged fever is a well-known clinical entity. When classical clinical symptoms and signs are present and the appropriate laboratory investigations are positive the diagnosis is usually straightforward. However borderline cases may take some time as the laboratory investigations, ANA and anti-dsDNA antibody may not be positive at the beginning of the disease[3,4]. When the diagnosis of SLE is definitive and the disease is treated with adequate dose of steroids and other immunosuppressant the fever settles quickly and skin rashes gradually fade or disappear altogether.

If fever does not settle quickly or if prolonged fever occurs, the diagnosis of an associated infection should be entertained. In our patient, when the diagnosis of SLE was made and oral steroids prescribed, joint pains were relieved but fever persisted. As the ESR remained elevated, the CRP became positive and the liver enzymes remained high, we investigated for a possible infective cause. Serological tests revealed an associated infection with scrub typhus. Isolation of Orientia tsutsugamushi (O. tsutsugamushi) was not attempted. With the appropriate dose of tetracycline the patient promptly responded and the fever abated.

Many major infections complicate the course of SLE, and scrub typhus is rarely incriminated. Scrub typhus is a mite-borne infectious disease caused by O. tsutsugamushi. The organism is a gram-negative coccobacillus that is antigenically distinct from the typhus group rickettsiae, which is distributed throughout the Asia Pacific rim. Scrub typhus is endemic in South East Asia, Australia but found on rare occasions in the Arabian Peninsula.

Patients with scrub typhus develop high fever, generalized headache, diffuse myalgia with the presence of a skin rash or a typical necrotic lesion called an eschar. Sometimes there is also generalized lymphadenopathy and splenomegaly[5]. Laboratory findings include leucopenia or leucocytosis, elevation of hepatic enzymes and bilirubin[5]. The pathological hallmark of scrub typhus is a lymphohistiocytic vasculitis seen on biopsy of the eschar or skin rashes[6]. It is difficult to culture O. tsutsugamushi and this is done only in specialized laboratories. Scrub typhus lasts for 14 to 21 days without treatment. Severe infections may be complicated by interstitial pneumonia, pulmonary edema, congestive heart failure, circulatory collapse, and a wide array of signs and symptoms of central nervous system dysfunction, including delirium, confusion, and seizures. These complications may lead to death, usually late in the second week of the illness. Fortunately none of these complications were noted in our patient who responded quickly to tetracycline.

As scrub typhus is not endemic in the Arabian Peninsula and the patient did not travel abroad, the diagnosis of scrub typhus was not considered initially. Furthermore, she did not remember being bitten by a mite and there were no skin
lesions. It is known however, that patients with scrub typhus may not show the typical eschar or skin rashes. In our patient, even if there were skin lesions, they could have been attributed to the skin manifestations of SLE, but the presence of an eschar would have pointed to an earlier diagnosis. The raised liver enzymes associated with scrub typhus were also present in this patient. Though the drug of choice in the treatment of scrub typhus is doxycycline 100 mg orally or intravenously twice daily, tetracycline 500 mg four times daily has also been used with success, and chloramphenicol is still commonly used in some endemic areas. Tetracycline was initially prescribed for the patient. Changing tetracycline to doxycycline was considered but eventually not done as she had improved markedly within 48 hours. The drug was prescribed for two weeks to reduce the risk of relapse. No vaccine is presently available and chemoprophylaxis with a weekly dose of 200 mg of doxycycline is highly effective when used by non-immune individuals visiting or working in endemic areas. This case report highlights the fact that scrub typhus may rarely occur in non-endemic areas and that prolonged fever in SLE should be fully investigated for associated viral, bacterial, protozoal, fungal and rickettsial diseases.

**CONCLUSION**

SLE sometimes presents as unexplained prolonged fever. But, if fever persists in spite of adequate dose of steroids and other immunosuppressants in a patient with SLE, the possibility of an associated infection should be seriously considered and detailed investigations performed. Scrub typhus may rarely complicate the course of SLE. In our patient, the diagnosis of the associated scrub typhus infection was slightly delayed but the infection was quickly controlled with tetracycline and there were no complications.

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Case Report

A Case of Severe Primary Hyperthyroidism, Secondary Hyperparathyroidism, Adrenal Insufficiency and Osteoporosis with Multiple Fractures

Ramen C Basak, Manas Chatterjee, Mahmoud W Rassem
Department of Internal Medicine, King Khaled General Hospital, Hafr Al Batin, Kingdom of Saudi Arabia

INTRODUCTION

Bone loss and relevant pathological fractures are major serious adverse effects of long-term corticosteroid therapy[1]. It has been well documented that trabecular bone injury predominates, especially in this entity[1] and few studies have focused on the cortical bone too[2]. Alendronate, one of the bisphosphonates, has been reported to be effective in prevention and treatment of corticosteroid-induced osteoporosis[3]. The active vitamin D metabolites (alfacalcidol, 1-hydroxyvitamin D3, calcitriol, 1, 25 dihydroxy vitamin D3) have also been reported to be beneficial but these are inferior to bisphosphonates[4]. Furthermore, the combined use of both drugs was shown to be more effective than either alone[5]. Parathormone (PTH) analogue (teriparatide) is superior to bisphosphonates not only in terms of increasing bone density and reducing fracture risk in vertebrae but also effective at non-vertebral sites[6]. Recently it is approved by FDA though long term safety is yet to be established. Osteoporosis induced fractures frequently involve the spine, hip and wrist. The best screening test is dual energy X-ray absorptiometry (DEXA) which is quick, simple and yields accurate result. It measures the density of bones in these areas and accurately follows the changes over time.

Osteoporosis is generally known to be one of the most serious adverse effects of long-term corticosteroid administration. Recently it was discovered that corticosteroid-induced osteoporosis occurs not only in trabecular bone but also in cortical bone, leading to the reduction in the strength of bones and subsequent fracture. We report a case of severe hyperthyroidism, secondary hyperparathyroidism, adrenal insufficiency and osteoporosis with multiple fractures (most likely collectively due to chronic steroid intake because of steroid dependant bronchial asthma, hyperparathyroidism and hyperthyroidism) which was treated appropriately and made an uneventful recovery.

KEY WORDS: corticosteroid therapy, hyperparathyroidism, hyperthyroidism, osteoporosis

CASE HISTORY

This 26-year-old female, married, having regular menstruation, was a known asthmatic on prednisolone 10 mg daily for about 10 years. She was admitted in the medical department of King Khaled General Hospital, KSA with suspected deep vein thrombosis (DVT). In the course of her investigation DVT was ruled out but she was found to have severe hyperthyroidism though clinically mildly symptomatic. She had severe osteopenia with multiple pathological fractures with sign of healing over the shaft of both fibulae and femur bilaterally, detected on plain X-ray (Fig. 1 and Fig. 2).

Examination revealed a conscious and oriented patient. Pulse and BP were 120/min and 90/60 mmHg respectively. She had mild diffuse goiter without bruit, fine tremor and no ophthalmopathy or pre-tibial myxedema. The proximal muscles were weak with tenderness in thigh muscles on both sides. She could barely stand with support and hence postural drop of blood pressure could not be assessed. Other systemic examination revealed no significant abnormalities. Laboratory evaluation depicted that she had primary hyperthyroidism as her FT3 was 30.19 pmol/l (N = 2.8 – 7.1 pmol/l), FT4 57.70 pmol/L (N = 12 – 22 pmol/l), and TSH 0.006 µU/ml (N = 0.27 - 4.2 µU/ml). Secondary hyperparathyroidism was diagnosed.

Address correspondence to:
Dr Ramen Chandra Basak, MD, Endocrinologist, King Khaled General Hospital, PO Box. 591, Hafr-Al-Batin, KSA. Tel: 037228179, Mobile: 0551106087, E-mail: basakrc@yahoo.com
as her Ca was 7.4 mg\% (N = 8 - 10.5 mg\%) and PTH 152 pg/ml (upper limit 62 pg/ml) with PO$_4$ 2.3 mg\% (N = 2.5 - 5 mg\%), alkaline phosphatase 378 U/l (N = 40 - 230 U/l) and normal albumin. Secondary adrenal insufficiency was diagnosed as her cortisol was low, 165.17 nmol/l (N ≥ 550 nmol/l) one hour after ACTH stimulation with normal electrolytes. Level of 25-hydroxy vitamin D and 1, 25-dihydroxy vitamin D were also normal. She had severe osteoporosis as revealed by DEXA (T score = -3). Other investigations showed normal CBC, mild microcytic hypochromic anemia with normal ESR, renal and hepatic functions. Doppler sonography of the left leg was normal. The ultrasonography of thyroid showed right lobe 6 x 1.4 x 3 mm and left lobe 6.9 x 1.7 x 2.2 mm with no focal lesion. The plain X-ray of bilateral fibulae and femur revealed multiple healing fractures. ECG showed sinus tachycardia.

The patient was managed conservatively by the orthopedic team. She was started on alendronate 70 mg weekly, alfacalcidol 1 µg daily and calcium carbonate (CaCO$_3$) 600 mg thrice a day. Her fractures healed completely within four months. Initially, her heart rate was controlled by propranolol without compromising her asthmatic state under the coverage of steroid which was discontinued eventually. She was also subjected to propylthiouracil therapy for hyperthyroidism. The follow up after six months revealed her to be in euthyroid state clinically and biochemically with controlled asthma.

**DISCUSSION**

Osteoporosis represents a major and emerging public health problem with the aging population. Major clinical consequences and economic burden of the disease pertain to the ensuing fractures. Many risk factors are associated with these fractures including low bone mass, hormonal disorders namely hyperparathyroidism and hyperthyroidism, thin built, use of certain drugs (e.g., glucocorticoids), cigarette smoking, excessive intake of alcholol, low physical activity, vitamin D insufficiency and low intake of calcium[7]. Osteoporosis and thyroid dysfunction are both common in older women. Eight to 13% of women older than 50 years of age have biochemical evidence of thyroid dysfunction[8] and 30% are osteoporotic according to the bone density criteria[9]. The osteoporotic fractures have long been associated with florid hyperthyroidism[10] although the relationship between biochemical evidence of excess thyroid hormone and fracture risk is not known[11]. The risk for hip fracture is more than threefold and that of nonspine fracture is twofold higher among women with low TSH levels than those with normal TSH levels[12]. Biochemical markers of bone turnover are elevated in women with low TSH levels supporting the view that low TSH levels reflect excessive thyroid hormone, which in turn increases skeletal remodeling[12]. All patients with hyperparathyroid disease will eventually develop osteoporosis regardless of their age or sex. Women tend to develop osteoporosis from parathyroid disease faster than men[13]. Osteoporosis is a well-recognized
adverse effect of corticosteroid therapy. The bone loss is most marked during the first six to 12 months of treatment. Corticosteroids affect both bone formation and bone resorption. A decrease in bone formation has been attributed to a decrease in osteoblast activity, number and life span (apoptosis). Corticosteroids alter gonadal sex steroid production through straight action and inhibition of gonadotrophin secretion and suppress adrenal androgen production, resulting in decreased bone formation[14]. They also increase the rate of bone resorption by stimulating the formation and action of osteoclasts[15]. The increase in bone resorption also may be explained, in part, by increased parathyroid hormone (PTH) mediated activation of osteoclasts having PTH receptors which osteoclasts are lacking. PTH-mediated stimulation of osteoclasts is believed to be indirect, acting in part through cytokines released from osteoclasts to activate them[16]. The secondary hyperparathyroidism results from reduced intestinal as well as renal tubular calcium absorption by steroid[17].

Patient receiving glucocorticoids chronically may have depressed circulating level of 1, 25 dihydroxy vitamin D; the mechanism being unknown[18]. However, other studies show no consistent abnormalities in vitamin D, PTH, or calcitonin levels in glucocorticoid-treated patients[19]. Common osteoporotic fracture sites include the vertebrae, the hip, the distal radius of the forearm with an incidence of 32, 16 and 15 percent respectively[20]. Osteoporotic bones are ten times more susceptible to fracture than normal[21] which often does not become clinically apparent until a fracture occurs. The best screening test is dual energy X-ray absorptiometry (DEXA) which is quick, simple and precise. It measures the density of bones in the areas most likely to be affected and accurately follows the changes in these bones over time[22]. Recently, many studies showed the need to administer vitamin D 800 IU or alfalcacidol 1 µg or calcitriol 0.5 µg/day in treatment of osteoporosis. Active vitamin D analogues, such as calcitriol and alfalcacidol, stimulate the formation and action of osteoclasts[23] leading to increased bone formation[24]. Effects of vitamin D resulted in lower risk of fractures and falls, as well as improvement of neuromuscular performances. In more than ten years of practice and several and short term clinical studies, alendronate 70 mg/week lowered the risk of vertebral and extra-vertebral fractures and improved bone mineral density (BMD) of all measured sites in both sexes with osteoporosis. The positive results of alendronate were demonstrated in different entities like persons of various ages and grades of lower BMD or patients with glucocorticoid-induced osteoporosis. Combination of vitamin D with efficacious antiresorptive drug like alendronate maintains all pharmacological features and demonstrates the clinical effects of weekly alendronate[25]. PTH analogue seems superior in prevention of vertebral fractures although their long term safety needs to be established.

We report a case of severe primary hyperthyroidism, secondary hyperparathyroidism, adrenal insufficiency, and osteoporosis with multiple fractures, most likely due to chronic steroid intake because of steroid dependent bronchial asthma. Secondary hyperparathyroidism in our patient may be explained by hypocalcemia resulting from decreased intestinal absorption and increased renal excretion of calcium due to decreased tubular reabsorption by steroid. Adrenal insufficiency resulted from chronic steroid administration.

CONCLUSION

The atypical presentation (uncommon early age osteoporosis and unusual site of fracture) is probably multifactorial. The treatment was directed towards the etiology and consequences of the diseases, leading to uneventful recovery. In such a patient presenting with osteoporosis even with subtle symptoms of thyrotoxicosis, thyroid hormonal evaluation is warranted. Furthermore, parathyroid hormone assay is indeed one of the most important biochemical markers in an osteoporotic subject.

REFERENCES


Case Report

Chronic Inflammatory Demyelinating Polyradiculoneuropathy in Two Children

Maliha Askar Soud Al-Bloushi, Yousif Kassim Habeeb, Eman Sadiq Al-Jumah
Department of Pediatrics, Neurology Unit, Mubarak Al-Kabeer Hospital, Kuwait

ABSTRACT

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is relatively rare in children. We report two cases diagnosed over a thirteen year period. One patient had a monophasic course resulting in complete recovery while the other case had a slowly progressive relapsing course with significant morbidity.

KEY WORDS: CIDP, children, neuropathy

INTRODUCTION

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is relatively rare in children\cite{1,2}. It is a chronic, potentially treatable, paralytic disorder of the peripheral nervous system developing over at least two months\cite{3}. Muscle stretch reflexes are either absent or depressed. Cerebrospinal fluid shows cytoalbuminologic dissociation. Demyelination is shown in both electrophysiological and pathologic studies. Patients can be classified into two groups; those with complete recovery after a monophasic course and those with prolonged disability and morbidity after a protracted or relapsing course\cite{2,4,6}. Intravenous immunoglobulin (IVIg) and corticosteroids are the first line treatment\cite{7}. In progressive or treatment resistant cases other options should be considered like plasma exchange (PE) and various immunosuppressive drugs. We report two cases diagnosed over a thirteen year period; one with monophasic course resulting in complete recovery and one with slowly progressive relapsing course with significant morbidity.

CASE REPORT

A previously healthy 34-month-old girl presented with a two month history of progressive gait disturbance after achieving normal walking at the age of one year. She had frequent falls with difficulty in standing from sitting position and going up the stairs. There was no associated upper limb weakness. She had difficulties with chewing and swallowing but no visual or respiratory symptoms. There was no history of skin rash or joint pain. She had uneventful prenatal and perinatal periods. Her family history was unremarkable for neurological diseases.

Examination on presentation revealed a well child with wide based waddling gait and mild lumbar lordosis. She had atrophy of quadriceps muscle with normal sensation and tone. There was weakness in lower limbs (4/5) but no weakness in upper limbs, face or eye movement. Knee and ankle tendon reflexes were absent. Romberg sign was positive. Sensory examination was normal. Her other systems examination was normal. Over a period of three months the course was progressive with weakness of her lower limbs (3/5) and mild weakness of upper limbs (4/5).

Investigations were normal including: creatine kinase, complete blood count, ESR, renal profile, liver profile, lipid profile, calcium, lead, serum immunoglobulin, X-ray hips and spine, computed tomography of brain, magnetic resonance imaging (MRI) of brain and lower spine. Muscle biopsy showed non-specific atrophic changes with no inflammatory changes. Cerebrospinal fluid analysis showed increased protein (862 mg/l) with no cells.

Her electrodiagnostic study showed signs of denervation affecting lower limbs more than upper limbs. Motor conduction velocity (CV) in median nerve was 36 m/s (normal range for age is 53.7 ± 4.70 m/s)\cite{8} and peroneal motor CV was 39 m/s (normal range for age is 48.7 ± 4.86 m/s)\cite{8}.

CIDP was considered and the child received her first intravenous immunoglobulin (IVIg)
400 mg/kg/day for five days followed by infusions of IVIg (1 g/kg for two days) once per month for three months. Then oral prednisolone, 2 mg/kg/day, was introduced due to inadequate response to IVIg. She showed marked improvement clinically (independent ambulation, going up the stairs and normal chewing). Steroids were reduced to 25 mg every other day two months later. On this dose she had slight deterioration necessitating IVIg dose (1 g/kg for two days). She was back to normal and steroids were stopped after fifteen months of treatment. At four years and nine months of age her motor CV in median nerve was 40 m/s (normal range for age is 55.0 ± 5.20 m/s)\(^8\) and 40 m/s in tibial nerve (normal range for age: 48.6 ± 4.25 m/s)\(^8\). She had no more relapses and remained well over nine years of follow up.

The second case presented at the age of three and half years with a three month history of progressive lower limb weakness manifested initially as frequent falls, inability to go up the stairs and unsteady gait. Later she was unable to walk and was unable to carry objects. There was no history of limb pain or skin rash. She was born at full term by cesarian section due to prolonged labor. She had an uneventful neonatal period. Parents were neurologically normal. The family history was negative of neuromuscular disorders.

On examination she was hypotonic with absent deep tendon reflexes in all limbs. She had weakness in limbs and trunk. She was unable to sit up from lying position with positive Gower sign. Power grade was 3/5 in upper and lower limbs. She had no sensory or cranial nerve deficit.

Blood investigations were normal including; creatine kinase, liver function test, renal profile, complete blood count, lactate and ammonia. Motor CV was extremely low: 9 m/sec in the median nerve (normal range for age 55.0 ± 5.20 m/s) and 6.7 m/sec in tibial nerve (normal range for age 48.6 ± 4.25 m/s), prolonged motor terminal latencies and very low M wave amplitude. There was marked temporal dispersion with proximal conduction block and no sensory responses. The finding was of demyelinative peripheral neuropathy. Nerve conduction study for parents was normal.

Diagnosis of CIDP was made and treatment begun with IVIg, 2 g/kg over two days, with mild improvement. The second dose of IVIG was given (400 mg/kg for five days) two weeks later without noticeable effect. Due to failure of IVIg, oral prednisolone was introduced at a dose 1.5 mg/kg (25 mg per day). She showed impressive improvement with power grade of 4/5 after which she almost completely recovered. Her motor CV showed improvement with motor CV in median nerve of 29 m/s and 21 m/s in tibial nerve.

After four months of recovery gradual tapering of steroids was done with no deterioration in her motor power. Four weeks later she suffered her first relapse necessitating oral steroids. Again she responded well. After tapering the dose to 10 mg alternate day oral prednisolone, she suffered a second relapse and the dose was increased to 20 mg daily in addition to IVIg infusion. She had her third relapse four months later and regular IVIg infusions, 2 g/kg over five days, were given monthly for six months in addition to her steroids 30 mg on alternate days but only with partial improvement.

In an attempt to reduce the dose of prednisolone, azathioprine was introduced at a dose of 1-3 mg/kg/day. After three months she had a fourth relapse with worsening of NCS: motor CV in median nerve was 6 m/sec (normal range for age 57.2 ± 3.71 m/s)\(^8\) and 11 m/sec in tibial nerve (normal range for age 48.2 ± 2.76 m/s)\(^8\) and steroids were switched to daily regimen while azathioprine was discontinued after a trial period of three years. Cyclosporine was added initially 25 mg twice per day and dose was increased to 100 mg twice per day without noticeable effect and was discontinued after 16 months. High dose intravenous pulse methylprednisolone of 10 mg/kg/day for three consecutive days was given with partial improvement.

At the age of eleven years, due to her progressive relapsing course, the patient was wheel chair bound with severe weakness in her upper limbs. She was assessed in USA where she had nerve biopsy which showed severe demyelinating inflammatory process. She was placed on additional monthly cyclophosphamide for six months while on daily oral steroids of 12.5 mg. Due to failure of previous treatment options and the need for long term daily steroids, plasma exchange was arranged with mild improvement in her upper limb power (2/5). There was difficulty with maintaining her central line with repeated obstructions. Other treatment options being considered included methotrexate and interferon beta. This young girl is now severely disabled, totally dependent and requires constant attention.

**DISCUSSION**

Hereditary causes of childhood chronic neuropathies are more common than acquired ones\(^{2,9}\) and among the latter, acute neuropathies are by far the commonest presentation\(^{10,11}\). CIDP is relatively rare in children with an estimated prevalence of 1-1.9 per 100,000 persons in adults corresponding to childhood prevalence of 0.48 per 100,000\(^{11}\). Initial presentation may occur as early as infancy. Male predominance in both adults and children was noted though female predominance was also reported\(^{10}\). 93% of childhood cases
presented for neurologic evaluation within six months of onset (mean 3.4 months). Prodromal events were noted in 20-57% of patients\(^6,9\). There are no reported cases of an underlying medical condition in children with CIDP in contrast to adults\(^12\).

The diagnosis is according to the criteria of the Ad Hoc Subcommittee of the American Society of Neurology\(^13\) which have been revised for the clinical diagnosis by the members of the 88th European neuromuscular center international workshop in 2000 (appendix 1)\(^3\) with the major change being in defining the mandatory clinical feature as progression of weakness in proximal and distal muscles over at least four weeks or alternatively when rapid progression (GBS-like presentation) is followed by relapsing or protracted course (more than one year)\(^11\).

Cardinal clinical symptoms and signs of CIDP are progressive or relapsing motor and sensory dysfunction affecting both proximal and distal muscles of two months duration and reduced or absent muscle stretch reflexes\(^9\). Gait disturbance due to lower limb weakness is the universal presentation in children\(^1,3,9\). Initial presentation may mimic Gullain-Barre’ syndrome\(^2,9,14,15\) or rarely multiple sclerosis\(^16\).

Distal sensory loss is often difficult to elicit. Pain is more common than paraesthesiae. Infrequent neurologic findings occur including facial nerve involvement, diplopia and papilledema\(^6\). Rarely patients needed mechanical ventilation\(^6,17\).

Laboratory and Radiological Studies

An elevated CSF protein (more than 35 mg/dl) without pleocytosis (less than 10 cells/mm\(^3\)) i.e., albuminocytologic dissociation is observed in 90-100% of adult and childhood CIDP. Generally, blood laboratory studies are unremarkable but autoantibodies have been reported in subsets of adult but not pediatric CIDP patients\(^2\).

MRI documented enhancement and/or enlargement of spinal nerve roots\(^18\). This finding was not found to correlate with either disease activity or severity\(^13,16\).

Electrophysiologic studies play an important role in both detection and characterization of polyneuropathies\(^10\). It helps in differentiating children with CIDP from those with inherited neuropathies\(^2\). Polat et al reported five different electrophysiologic patterns of childhood polyneuropathies and found childhood CIDP in only 4% of 74 children studied. The predominant changes seen indicate demyelination but axonal injury may occur presumably secondary to demyelination\(^6\). Evidence of demyelination on NCS must demonstrate at least three out of four major abnormalities in motor nerve: Slowing of motor conduction velocities to < 60% of normal values, dispersion of the compound muscle action potential, prolonged distal latencies in more than two nerves and absent or prolonged F-wave minimal latencies. These do not distinguish acquired from inherited demyelinating polyneuropathies but the finding of non-uniform slowing, conduction block at sites not prone to entrapment which is the hallmark for focal demyelination\(^9\), or abnormal temporal dispersion favors an acquired rather than an inherited etiology\(^3\). Treatment should not be withheld, if a patient does not meet these criteria as many patients with clinical CIDP do not fulfill these criteria. Serial NCS cannot be used to guide therapy or determine prognosis\(^6\).

Nerve biopsy may be necessary to assist differentiating acquired from hereditary polyneuropathies but it has been replaced by molecular and genetic testing\(^10\). It is not a required mandatory criteria in definite CIDP cases\(^9,13\) as there is no additional diagnostic value of sural nerve biopsy in the diagnosis of CIDP\(^20\). The most important pathologic features are inflammatory infiltrate around epineural blood vessels, onion bulbs, which are concentric Schwann cell proliferation secondary to continued cycle of demyelination and remyelination\(^2,3\).

Pathogenesis

The understanding of molecular and cellular mechanisms resulting in inflammatory damage of the peripheral nervous system has been considerably studied. It is an autoimmune disease that targets the myelin sheath of peripheral nerves\(^11\). The target antigen and the role of humoral and cell mediated immunity remain unclear\(^27\). It is a heterogeneous syndrome with many parallels to multiple sclerosis.

Treatment

Although 20% of patients with CIDP are children, there are no control studies of the efficacy of different immunomodulating therapies. Several therapeutic options are used in immune neuropathies with similar efficacy including steroids, IVIg and PE which are the three most frequently used and well studied therapies for CIDP in a predominantly adult population\(^1,17,21\). The initial response to each of these therapies in adult CIDP is 60% compared to 80-100% initial response rates in children\(^1,17,22\). It is unclear which treatment, IVIg or oral steroids should be used as initial therapy\(^17,23\). PE is effective in adults but has little place in childhood CIDP and only considered as third option.

IVIg is an effective first line treatment in childhood CIDP\(^2,17,23\). It is safely used in children
with other autoimmune disorders[23]. There is clearly class I evidence for IVIg as standard therapy for CIDP but most studies have only been made for induction therapy rather than at long term therapy. Factors in favor of the use of IVIg over steroids includes safer long term profile and ease of use (it can be given at home but not in this country) while factors in favor of steroids include the lower cost, availability worldwide and the ease of administration whether intravenous or oral.

The main drawbacks of IVIg are higher cost and sometimes availability. Minor side effects such as rash, headache, fever and chills may be minimized by slowing infusion rate and using anti-inflammatory premedication. Serious side effects including allergic reactions, aseptic meningitis and thromboembolic events occur in less than 5% of infusions[9].

There are different therapeutic regimens as to dosage and duration of IVIg[24]. One gram/kg/day for two days may induce faster improvement than 0.4 gram/kg/day for five consecutive days[3]. A dose of 0.4 g/kg body weight was superior to 0.2 g/kg when given over five days. It can be given as 2 g/kg over 2-5 days or 0.4 g/kg weekly or every other week or every 28 days. The routine cycle of IVIg given over five days results in clinical improvement within 7-10 days in 56-79% of adult patients. The mean half life of IVIg is 18 - 32 days. Therefore, periodic maintenance infusions at intervals of four weeks are needed[22,23,24]. More than 40% of patients need at least 1 g/kg every 2-8 weeks to prevent further relapses. After 3-6 months consider beginning gradual wean off by decreasing frequency of treatment and lowering the dose and later trail off the IVIG.

Prednisolone is an effective alternative to IVIg as two thirds respond to either treatment. Family must be made aware of potential acute and chronic risks of corticosteroids. The dose of 1-2.5 mg/kg per day is usually used for at least four weeks followed by slow tapering. Relapses were common when dose was lowered. There are many potential adverse effects for corticosteroids therapy (both acute and chronic) including weight gain, osteopenia, altered growth (occurred in our 2nd case), hypertension, susceptibility to infections and cataracts. These side effects may be minimized by administering the medication on an alternate day basis or in pulse treatments.

Children like adults demonstrate initial improvement in two thirds of cases[17] after initiating treatment with prednisolone but children require continued high dose therapy as on tapering relapses are frequent. This was evident in our 2nd case. If steroids are needed for more than one year, complete withdrawal is often very difficult. Attempts to reduce the dose without addition of an immunosuppressive medication often lead to unnecessary relapses.

The use of high dose pulse methylprednisolone has been suggested with similar efficacy to IVIg and oral steroids[6,24] but physicians should be aware of possible significant clinical deterioration after high dose intravenous methylprednisolone as reported by Rostasy et al[25,26].

PE is considered as a third option[22] and trials comparing IVIg with PE did not find significant difference in short term effect of these two treatments[1,17,23]. Although 60% of patients respond to PE, it is less often used in children because of technical difficulties with installation of central catheters which may account for its limited use in young children[11]. In addition to technical difficulties, there are potential infectious, thrombotic and hemodynamic complications of the treatment itself. The effect lasts only few weeks with over two thirds having a relapse or end of dose effect after two weeks making it unsuitable for long term treatment. It is usually administered 2-3 times per week for 6-10 treatments and may be tapered slowly. Improvement may begin within days but it is temporary and relapses and even rebound phenomenon are common when treatment is terminated. PE is an effective temporary or adjuvant therapy for CIDP but immunosuppressive drug treatments are required for long term management.

The short term effects of initial treatment with IVIg and PE, in addition to the serious side effects of prolonged use of steroids necessitates the use of several immunosuppressive agents as adjunctive therapy to reduce the dose of steroids or the frequency of IVIg and PE courses[22,27]. In these treatment resistant cases, different immunosuppressant including azathioprine, methotrexate[27], cyclosporine A[28], cyclophosphamide[9], mycophenolate[29], interferon-α and β-Ia, can be used but the evidence is insufficient to recommend one over the other[11,14].

Prognosis

The prognosis in children is generally more favorable than adults with complete remission or minimal weakness in 70-100% cases[1,2,3,9]. In comparison, adults exhibit moderate to severe sequelae in 30-40% of cases. A small number of children respond poorly to all treatment modalities or develop significant side effects to protracted immunosuppression resulting in moderate to severe neurological disability. Pain at onset and infectious prodromal illness predicts a better outcome while axonal loss on electrodiagnostic studies predicts a poor prognosis[2].
CONCLUSION

Childhood CIDP is rare but potentially treatable and should be considered in any child with subacute neuropathy, where hereditary causes are excluded. Treatment should be initiated early in order to minimize demyelination and secondary axonal loss.

REFERENCES

**MANDATORY CLINICAL CRITERIA**
1. Progression of muscle weakness in proximal and distal muscles of upper and lower extremities over at least 4 weeks, or alternatively when rapid progression (GBS-like presentation) is followed by relapsing or protracted course (more than 1 year)
2. Areflexia or hyporeflexia

**Major laboratory features**

**A - Electrophysiological criteria**

Must demonstrate at least three of the following four major abnormalities in motor nerves (or 2 of the major plus 2 of the supportive criteria):

**A-1 Major**
1. Conduction block: at least 50% drop in negative peak area or peak-to-peak amplitude of proximal compound muscle action potential (CMAP) if duration of negative peak of proximal CMAP is < 130% of distal CMAP duration
2. Temporal dispersion: abnormal if duration of negative peak of proximal CMAP is > 130% of distal CMAP duration

Recommendations: (a) Conduction block and temporal dispersion can be assessed only in nerves where amplitude of distal CMAP is > 1 mV, (b) Supramaximal stimulation should always be used

2. Reduction in conduction velocity (CV) in two or more nerves: < 75% of the mean minus 2 standard deviations (SD) CV value for age
3. Prolonged distal latency (DL) in two or more nerves: > 130% of the mean + 2 SD DL value for age
4. Absent F wave or prolonged F wave minimal latency (ML) in two or more nerves: > 130% of the mean + 2SD F wave ML for age
5. Recommendations: F wave study should include a minimum of 10 trials

**A-2 Supportive**
1. When conduction block is absent, the following abnormal electrophysiological parameters are indicative of non-uniform slowing and thus of an acquired neuropathy:
2. Abnormal median sensory nerve action potential (SNAP) while the sural nerve SNAP is normal
3. Abnormal minimal latency index (TLI)[3]
4. Difference of > 10 m/s in motor CVs between nerves of upper or lower limbs (either different nerves from same limb for example left median versus left ulnar or the same nerve from different sides (for example left versus right ulnar nerves)

**B - Cerebrospinal fluid (CSF studies)**
1. CSF protein > 35 mg/dl
2. Cell count < 10 cells/mm³

**C - Nerve biopsy with predominant features of demyelination**

**EXCLUSION CRITERIA**
1. Clinical features or history of a hereditary neuropathy, other diseases or exposure to drugs or toxins that are known to cause peripheral neuropathy
2. Laboratory findings (include nerve biopsy or DNA studies) that show evidence for different etiology other than CIDP
3. Electrodiagnostic features of abnormal neuromuscular transmission, myopathy or anterior horn cell disease

**DIAGNOSTIC CRITERIA (MUST HAVE NO EXCLUSION CRITERIA)**
1. Confirmed CIDP
   i. Mandatory clinical features
   ii. Electrodiagnostic and CSF features
2. Possible CIDP
   i. Mandatory clinical features
   iii. One of the 3 laboratory findings

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Case Report

Atypical Progression of Thyrotoxic Manifestations while Awaiting Laboratory Confirmation

Venkatesan Nagarajan, Asmahan Al-Shubaili
Department of Neurology, Ibn Sina Hospital, Kuwait

ABSTRACT

An ill-nourished man, whose hyperthyroid state was unmasked by respiratory infection, had more than one attack of thyroid storm and rapidly went through several unusual complications which included thyrotoxic periodic paralysis, upper and lower motor neuron manifestations, neuropsychiatric and metabolic disturbances. Diagnosis of hyperthyroidism was established after 14 days with the arrival of thyroid function test which was drawn on admission. Though his symptoms improved after initiating treatment, he continued to suffer from thyroid associated ophthalmopathy and myasthenia gravis. Although these manifestations of hyperthyroidism are well known, their occurrence in a single patient is unusual. This report highlights the need for the physicians to be alert regarding these rare manifestations of thyrotoxicosis in their patients and initiate treatment as it is difficult to obtain rapid laboratory confirmation in emergency department.

KEY WORDS: Basedow’s paraplegia, periodic paralysis, hyperthyroidism, thyroid storm, thyrotoxicosis

INTRODUCTION

Thyroid hormone plays an important role in the development and metabolism of almost every tissue[1]. So either high or low levels of thyroid hormone are expected to produce diffuse systemic manifestations, but it is usually restricted to one or two organs. We report a case which did not show classical clinical signs of thyrotoxicosis at presentation. He was referred for respiratory infection but slipped into thyroid storm and exhibited involvement of multiple organs. Even after the diagnosis was established, he continued to display uncommon complications of thyrotoxicosis. Occurrence of all these rare manifestation in a single patient is unusual.

CASE HISTORY

A 33-year-old thin Southeast Asian man, looking ill and tired, was brought into the emergency department with history of breathing difficulty, chest tightness and generalized fatigue for several hours. His blood pressure was 130/90 mmHg, pulse 130 per minute, regular and temperature was 37.2 °C. Cardiovascular and abdominal examinations were normal. Chest examination revealed a pleural rub and few crackles over the right lower chest. Few non-tender cervical lymph nodes were palpable.

He was admitted with the diagnosis of pneumonia and started on dextrose infusion intravenously as a routine measure while being evaluated. Within a short time he became irritable, markedly breathless and cyanosed. The jugular veins became distended. His blood pressure increased to 190/110 mmHg and the pulse to 170/minute. He had a right ventricular gallop. He steadily deteriorated; oxygen saturation dropped and he was subsequently intubated. Decompensated acute congestive cardiac failure was considered and his condition stabilized with ventilation, intravenous frusemide and antibiotics. His ECG showed sinus tachycardia; cardiac enzymes and echocardiogram were normal. On suspicion thyroid function tests (TFT) were sent. He was extubated on the next day and through out the day he was fully oriented.

Thirty hours after admission, he suddenly became restless. He later became agitated, confused, was sweating profusely and had shallow breathing. He brought out frothy secretions after incessant coughing. Examination revealed bilateral crackles and wheezes and generalized muscle weakness.
He was reintubated and ventilated without muscle relaxant. Within two hours his sweating and basal crackles disappeared. Chest CT showed increased density of both lower lobes with alveolar infiltration, peribronchial thickening and sub-pleural cystic changes.

On the third day the patient was awake on ventilator, but was confused and minimally responsive to commands. The pupils were sluggishly reactive to light and oculocephalic reflex and orbicularis oculi muscles were weak. There was generalized hypotonia and weakness of all four limbs. The tendon reflexes were absent with a mute plantar response. There was no neck stiffness. Guillain-Barre syndrome (GBS) (acute polyradiculoneuropathy) was suspected and he was started on IV immunoglobulin 25 gram daily for five days. Thorough evaluations were carried out. HIV, hepatitis, connective tissue and toxic drugs screening were negative. Brain CT, nerve conduction studies (NCS) and the cerebrospinal fluid (CSF) examinations were normal. None of the investigations substantiated the diagnosis of GBS. Routine investigations showed hypercalcemia, hypomagnesemia, hypokalemia, and hypoalbuminemia. Persistent hypokalemia (3 mmol – normal range is 3.8-5.2 mmol) in spite of replacement, suggested the possibility of hypokalemic periodic paralysis. He was continued on symptomatic and replacement therapies. His ventilatory support was withdrawn on the eighth day. Even after extubation he remained sleepy and confused most of the time. He had occasional high temperature.

On the twelfth hospital day, neurological examination showed that the patient was conscious but drowsy, confused and confabulating. Visual acuity was reduced to 20/200 in both eyes and pupils were dilated with a sluggish reaction. All extraocular eye movements were restricted. Hanging jaw with wasting of both masseters and temporalis muscles was noted. The jaw jerk was absent. Weakness of eye closure with positive Bell’s phenomenon and weakness of palatal muscles with absent gag reflex were noted. He had difficulty in raising his head from the pillow and had slow tongue movements. The motor system examination showed spastic weakness of the muscles of all four limbs with predominant involvement of upper limbs and distal muscles. Now all the tendon reflexes were highly exaggerated with extensor plantar responses. Cerebellar and sensory system, first and eighth cranial nerves examination were limited because of his confusion. The patient could not sit or stand without support. He was thought to have encephalomyeloradiculoneuropathy of uncertain origin because of confusion, upper motor neuron and lower motor neuron signs. Infective causes were ruled out by normal CSF study. Among non-infective causes Bickerstaff’s brainstem encephalitis and combined central (acute disseminated encephalomyelitis) and peripheral demyelination (GBS) were actively pursued.

His EEG was moderately abnormal with slow irregular basic activity and intermittent theta activity in posterior leads. The neurophysiological tests - NCS, EMG, facial conduction, blink reflex, repetitive nerve stimulation and brainstem auditory evoked potential were normal. The patient was not co-operative for brain MRI. As none of the investigations pinpointed any diagnosis, Wernicke’s encephalopathy was put forward in view of cardiovascular as well as neurological involvement and in fact patient received one dose of parenteral thiamine without improvement before the TFT which took two weeks to arrive, sealed the diagnosis.

**Diagnosis**

Thyroid function test result was TSH < 0.005 mIU/l (0.270-4.20); FT4 > 100 pmol/l (12.00-22.00) and a firm diagnosis of hyperthyroidism or thyrotoxicosis was reached on the fourteenth day, which could explain all the clinical features. Other thyroid related test results were thyroxine 324 nmol/l (49.0-141.9), thyrioxin uptake 44.4% (30.0-40.0), anti-thyroid peroxidase antibody 353 U/ml (< 60 for males and < 100 for females) and thyroglobulin antibodies 66 U/ml (< 60 for males and < 100 for females) which points towards Graves’ disease as the possible cause for his thyrotoxicosis. Though there was no thyromegaly clinically, the ultrasonography of thyroid gland showed mild enlargement of both lobes with coarse echo pattern.

**Treatment**

Immediately after diagnosis, the patient was started on carbimazole 15 mg and propranolol 40 mg thrice daily orally. On the eighteenth day, the patient developed corneal ulcer in the right eye and pseudomonas was grown from it. First his confusion cleared and he demonstrated some aggressive behavior and suspicious tendency. With redness in his eyes due to corneal ulcer, he now exhibited the typical staring look. Next his limb weakness improved slowly and he started walking. Later his cranial muscle weakness became better and lastly his extra ocular muscles started moving almost after a month of treatment. As there was a concern about his compliance with treatment, radioactive Iodine was given for two weeks after stopping carbimazole. B-blocker propranolol was continued for few weeks after radio-iodine treatment. Three months later, when his recovery was almost complete, his eyelids started dropping and he complained of difficulty in getting up from sitting posture. He could not give a definite history of diurnal variation but mild
fatigability was noted in his ptosis. The tensilon test at bed side and repetitive nerve stimulation confirmed the diagnosis myasthenia gravis (MG). There was no thymic enlargement in the CT mediastinum. He was started on pyridostigmine and oral prednisolone. Once again all his symptoms started improving except moderate restriction of eye movements. Presently he is doing well with additional medications.

**DISCUSSION**

Hyperthyroidism was first described by Robert J Graves in 1835[1]. Though most of the cases can be diagnosed clinically by its familiar presentation, there is a wide variation of clinical signs and symptoms in unusual presentation. Even among general population with a prevalence of 1-2%, roughly 0.5% of thyrotoxicosis remains undiagnosed[2]. Thyrotoxicosis is more common in women (2%) than in men (0.2%)[1]. Our patient did not exhibit any of the classical signs of thyrotoxicosis - thryomegaly, tremors and staring look. Thyroid enlargement is usually diffuse and symmetrical in thyrotoxicosis but occasionally it may not be palpable[2]. Though tremor is universal in thyrotoxicosis, it was not exhibited by our patient throughout the illness. The staring look was evident only in later part of his illness after he developed corneal ulcer.

Retrospective analysis suggests that respiratory infection unmasked his underlying thyrotoxicosis. Without being recognized he twice went into thyroid storm requiring intubation, diagnosed as heart failure and recovered only with symptomatic treatment. Thyroid storm is a medical emergency with an incidence of 1-2% and carries a mortality of 10-75%[3]. Possibly his first storm was precipitated by palpating the gland while looking for cervical adenitis and the second one by the drugs given.

Flaccid paralysis after the storm was thought to be due to GBS because of the absent jerks, facial weakness and respiratory failure. In thyrotoxicosis also there may be flaccid paraplegia with absent jerks and without bladder involvement, called “Basedow’s paraplegia”. The incidence of this polyneuropathy is around 19%[4]. Both possibilities were ruled out by normal nerve conduction studies. Next hypokalemic periodic palsy was considered. If such paralyses occur in thyrotoxicosis, it is called Thyrotoxic periodic paralysis. Its incidence is 10% and is most common in Asian males (M:F = 6:1) over 20 years of age. Attacks may be localized or generalized and each attack can last for minutes to days. Type of paralyses also differs during each attack. Except heart and sphincters, all other muscles can be affected[5,6]. It differs from familial hypokalemic periodic paralysis by the absence of family history, older age of onset (> 20 years), worsening or precipitation of symptoms by acetazolamide and prevention of attacks by B-blockers[3]. The flaccid paralysis responded to the replacement of potassium, respiratory support and B-blockers which are incidentally the treatment for thyrotoxic periodic paralysis. Another possibility is thyroid myopathy which can precede the thyrotoxicosis symptoms. It is more common in female in fifth decade and is characterized mainly by proximal weakness. Wasting can occur especially in elderly and weakness may involve bulbar, esophageal and respiratory muscles. Serum creatinine kinase is not raised as a rule but electromyography is usually abnormal in 80% cases[6].

Hashimoto’s encephalopathy is usually associated with hypo rather than hyperthyroidism. This subacute encephalopathy has varied presentation and commonly affects females. Two less distinct patterns of presentation are recognized. The vasculitic presentation is like acute stroke associated with focal neurologic signs, seizures and multifocal myoclonus. Another pattern is diffuse slowly progressive impairment of mental function with confusion, somnolence, and psychosis[7]. Mostly there will be raised CSF protein with occasional pleocytosis. In our patient the neuropsychiatric manifestation occurred abruptly along with thyroid storm and did not have myoclonus, seizures or abnormal CSF. Reversible pyramidal signs have been reported in thyrotoxicosis[8]. This patient also exhibited all the metabolic complications of thyrotoxicosis. They are hypercalcemia, hypomagnesemia, hypokalemia and hypoalbuminemia[9].

Thyroid associated ophthalmopathy (TAO) is an auto-immune disorder, with an incidence of 5% (but sub-clinical in 90%). It may precede (20%), concur (40%) or follow (40%) treatment for thyrotoxicosis and is independent of thyroid status[10]. There are four subtypes depending upon the site of immune attack[11]. Ocular myopathy type results from sensitized T-lymphocytes targeting extra ocular muscles. Congestive ophthalmopathy is due to antibodies cross reacting with orbital connective tissue and fat to produce typical symptoms of TAO – watering, congestion and proptosis. Combination of both is called mixed type and it is the commonest presentation of TAO. The fourth type, chronic eyelid disease is responsible for lid lag and corneal ulcer[7]. In our patient, it started as acute ocular myopathy with absent extraocular movements which was mistaken for cranial nerve palsy or brainstem involvement. Subsequently he evolved into mixed and chronic eye lid disease type with the development of corneal ulcer which was conveniently labeled as exposure keratitis due to bilateral facial palsy. The staring look was evident later after initiating on anti-thyroid regimen.
Always suspect MG when a thyrotoxicosis patient with lid retraction develops ptosis\textsuperscript{[12]}. The history of diurnal variation and clinically demonstrable fatigability will clinch the diagnosis. Apart from tensilon test and repetitive nerve stimulation, acetylcholine receptor antibody and single fiber electromyography are the other tests to confirm MG. This could not be done due to administrative reasons. Both MG and Graves’ disease are autoimmune disorder against surface receptors with high prevalence and correlation\textsuperscript{[12]}. Usually their response to treatment with pyridostigmine will be dramatic.

CONCLUSION

This report highlights the need for physicians to be alert regarding these rare manifestations of thyrotoxicosis in their patients and initiate treatment as it is difficult to obtain rapid laboratory confirmation in the emergency department.

REFERENCES

Comparative Hydrocarbon Utilization by Hydrophobic and Hydrophilic Variants of Pseudomonas Aeruginosa

Obuekwe CO, Al-Jadi ZK, Al-Saleh ES
Microbiology Division, Department of Biological Sciences, Faculty of Science, Kuwait University, Safat, Kuwait City, Kuwait. E-mail: okey@kuc01.kuniv.edu.kw

J Appl Microbiol 2008; 105:1876-1887

Aims: To investigate hydrocarbon degradation by hydrophobic, hydrophilic and parental strains of Pseudomonas aeruginosa.

Methods and results: Partitioning of hydrocarbon-degrading P. aeruginosa strain in a solvent/aqueous system yielded hydrophobic and hydrophilic fractions. Exhaustive partitioning of aqueous-phase cells yielded the hydrophilic variants (L), while sequential fractionation of the hydrophobic phase cells yielded successive fractions exhibiting increasing cell-surface hydrophobicity (CSH). In hydrocarbon adherence assays (bacterial attachment to hydrocarbon), L had a value of 20%, which increased from 61.7% in first hydrophobic fraction (H(1)) to 72.2% in the third (H(3)). Crude oil degradation by L was 70%, but increased from 82% in H(1) to 93% in H(3). L variant produced most exopolysaccharides and reduced surface tension from about 73 to 49 mN m(-1). Rhamnolipid production was highest in L, but was not detected in all crude oil cultures.

Conclusions: Hydrophobic subpopulations of hydrocarbon-degrading P. aeruginosa exhibited greater hydrocarbon-utilizing ability than hydrophilic ones, or the parental strain.

Significance and impact of the study: Results demonstrate that a population of P. aeruginosa consists of cells with different CSH which affect hydrocarbon utilization. This potentially provides the population with the capacity to utilize different hydrophobic substrates found in petroleum. Judicious selection of such hydrophobic subpopulations can enhance hydrocarbon pollution bioremediation.

Survival of Male Breast Cancer Patients: Population-Based Cohort Study

Thalib L, Hall P
Department of Community Medicine and Behavioral Sciences (Biostatistics), Faculty of Medicine, Kuwait University, PO BOX 24923, Safat 13110 Kuwait

Cancer Sci 2008 Dec 11 [Epub ahead of print]

Little information is available on the survival of male breast cancer patients because the disease is extremely rare in men. Moreover, previous reports on the prognosis of male breast cancer have been conflicting. We took advantage of a number of large, nationwide registries in Sweden to evaluate the prognostic value of sex in breast cancer patients. A population-based cohort of 269 male and 30 011 female breast cancer patients born after 1935 and diagnosed with primary breast cancer between 1970 and 1997 was generated by linking a number of Swedish registries, including the Swedish Cancer Registry, the Cause of Death Registry, the Swedish Generation Registry, and the Registry of Population and Population Changes.
We used this cohort to quantify the association between the sex of the patient and breast cancer-specific mortality, using the Cox proportional hazards. The sex of the patient did not significantly influence the prognosis of breast cancer. Adjusting for age at diagnosis and calendar period did not alter the results. Nor did the results change when the analyses were repeated for all causes of mortality. Our study, one of the largest to date, failed to find evidence to support the proposed association between the sex of breast cancer patients and survival. Given the previous reports, which advocated that male breast cancer patients have poorer survival and need aggressive treatment strategies, our findings are reassuring and clinically very important.

Fine needle Aspiration Cytology of Breast Masses in Children and Adolescents: Experience with 1404 Aspirates

Kapila K, Pathan SK, Al-Mosawy FA, George SS, Haji BE, Al-Ayadhy B
Department of Pathology, Faculty of Medicine, Kuwait University, and Cytology Laboratory, Mubarak Al-Kabeer Hospital, Safat, Kuwait. E-Mail: kkapila@yahoo.com

Acta Cytol 2008; 52:681-686

Objective: To study the distribution and efficacy of fine needle aspiration cytology (FNAC) in the diagnosis of breast lesions in pediatric and adolescent patients.

Study design: From January 1993 to December 2006, the cytology reports of 1404 breast aspirates (178 males and 1226 females) performed on children and adolescents (ranging from 1 to 21 years) were reviewed. Of these 41, 179, 506 and 678 aspirates belonged to the age group 1-<12, 12-<16, 16-<19 and 19-21 years, respectively.

Results: The morphologic spectrum seen in females was inflammatory lesions (4%), benign ductal cells (20%), ductal hyperplasia (0.6%), papillary lesions (0.7%), benign neoplasms (69%), suspicious cytology (0.3%) and cancer (0.3%). Of the benign neoplasms, 98% (831 of 851) were fibroadenomas, with 12 cases of phyllodes (benign), 5 cases of lipoma and 3 cases of adenoma. There were 3 cases of malignancy (2 adenocarcinoma and 1 non-Hodgkin's lymphoma). Only 3% of the male breast aspirates provided a diagnostic challenge, while 89% of them showed benign ductal cells.

Conclusion: FNAC of children and adolescent breast masses is helpful and can reduce the need for open surgery to prevent later deformity. The aspirates are mostly benign and can be managed conservatively.

Prevalence of Obesity among Adolescents (10 to 14 Years) in Kuwait

El-Bayoumy I, Shady I, Lotfy H
Public Health Department, Tanta Faculty of Medicine, Shaab, Kuwait. faraibr@hotmail.com

Asia Pac J Public Health 2009; 21:153-159

The purpose of this cross-sectional study was to find out the prevalence of obesity and overweight among intermediate school adolescents aged 10 to 14 years. The study comprised a multistage stratified random sample that included 5402 children (2657 males and 2745 females). They represent 12.7% of the total number of children between 10 and 14 years during the educational year 2005-2006. The weights and heights of adolescents were measured, from which the body mass index (BMI) was calculated, which is the weight in kilograms divided by the height in meters squared (kg/m²). BMI values higher than 95 percentile were accepted as being obese and those in between 85 and 94 percentile were accepted as overweight. Dietary intake was assessed by the investigators using food exchange lists designed by American Diabetic Association and physical fitness was measured by modified Harvard step test. Data
regarding monthly income of the chosen sample were collected from parents of those children. The overall prevalence of overweight and obesity in adolescent Kuwaiti children aged 10 to 14 years was 30.7% and 14.6%, respectively. The overall prevalence of overweight and obesity among males was 29.3% and 14.9%, respectively (P < .001) and the prevalence of overweight and obesity among females was 32.1% and 14.2%, respectively (P < .001). High daily caloric intake by the obese and overweight children and physical inactivity was reported among the majority of them. Health education programs should be conducted to control this syndrome in order to prevent future risk of obesity-related disease, and physical activity programs should be incorporated in the schools. Any management plan for overweight and obese children should include 3 major components: diets, exercise, and family-based behavior and they should not be placed on restrictive diets because adequate calories are needed for proper growth.

Antioxidant Therapy is Associated with a Reduction in the Serum Levels of Mediators of Renal Injury following Lithotripsy for Renal Calculi

Kehinde EO, Al-Awadi KA, Al-Hunayan A, Mojiminiyi OA, Memon A, Abdul-Halim H, Fatinkun T. Department of Surgery (Division of Urology), Faculty of Medicine, Kuwait University, Safat, Kuwait. E-Mail: ekehinde@hsc.edu.kw

J Endourol 2008; 22:2537-2545

Objective: To investigate the effects of antioxidant therapy on the levels of mediators of shock wave induced renal injury in patients with renal calculi treated with extracorporeal shock wave lithotripsy (ESWL).

Patients and methods: One hundred and twenty patients with renal calculi were divided into three treatment groups: Group A patients (n = 39) served as a control group; Group B patients (n = 41) were given 2 capsules of Nature Made(R) antioxidants 2 hours before, and 2 and 8 hours after ESWL and Group C patients (n = 40) were given 2 capsules of the antioxidants at 2 and 8 hours after ESWL. Blood and urine samples were obtained from all patients just before the start of treatment with ESWL, and at 2 and 24 hours and on day 7 and 28 after ESWL. Levels of mediators of renal injury such as serum alkaline phosphatase (ALP), C-reactive protein (CRP) and lactate dehydrogenase (LDH) were measured. Urinary levels of albumin and ALP were also determined as measures of renal tubular injury.

Results: Patients given antioxidants had significantly reduced mean serum concentration of ALP (p < 0.001) at 24 hours, lower serum ALP and LDH on day 7 and 28, and lowest CRP on day 28 after ESWL. They also had higher urine albumin (p < 0.001) and ALP (p < 0.001) levels (from 24 hours to day 28) compared with patients who were not given antioxidants.

Conclusion: These findings suggest that oral antioxidant therapy prior to lithotripsy may reduce the severity of long term renal injury caused by the shock waves.

Medical Laboratory Sciences Graduates: Are They Satisfied at Work?

Al-Enezi N, Shah MA, Chowdhury RI, Ahmad A Ministry of Health, Sulaibekhat, Kuwait. E-Mail: naser@brikwood.com.kw

Educ Health (Abingdon) 2008; 21:100

Objective: In this study, the overall job satisfaction of medical laboratory scientist graduates of one Kuwaiti University was examined in relation to the environment and organizational features of their places of employment.
**Materials and Methods:** A questionnaire was distributed to 105 graduates of the Medical Laboratory Sciences (MLS) Department, Faculty of Allied Health Sciences, Kuwait University from the years 1982 to 2001 who are currently working in Ministry of Health hospitals. Of those, 85 questionnaires were returned and this was a response rate of 80 percent.

**Results:** Fifty-six percent of respondents were satisfied overall with their jobs, but 44% were not satisfied. Overall job satisfaction was found to be associated with having the opportunity of applying their academic knowledge and laboratory skills to their work when job conditions were conducive to the work and there was collegiality in the laboratory. Reporting to only one supervisor also showed a positive relationship with overall job satisfaction. In contrast, perceptions of unhealthy working conditions, where employees tended to be a hindrance to another employee, were associated with lower overall job satisfaction. Forty-nine percent of all respondents reported that they were not satisfied with organizational practice, 44% were not satisfied with the work environment, and 39% were not satisfied with their autonomy and freedom to work.

**Conclusion:** A high percentage of laboratory technologists were not satisfied overall with their jobs or with specific aspects of their jobs. Particularly important in this respect were whether technologists felt that their work appropriately used their knowledge, feelings of technical competency, work related rules/procedures, and presence of unhealthy competition. These issues of health worker dissatisfaction need to be addressed by the health authority managers responsible for these services and by academics who train MLS workers.

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**Diarrhoeagenic Escherichia Coli are Not a Significant Cause of Diarrhoea in Hospitalised Children in Kuwait**

Albert MJ, Rotimi VO, Dhar R, Silpikurian S, Pacsa AS, Molla AM, Szucs G

**BMC Microbiol 2009; 9:62**

**Background:** The importance of diarrhoeagenic Escherichia coli (DEC) infections in the Arabian Gulf including Kuwait is not known. The prevalence of DEC enterotoxigenic [ETEC], enteropathogenic [EPEC], enteroinvasive [EIEC], enterohemorrhagic [EHEC] and enteroaggregative [EAEC]) was studied in 537 children [less than or equal to] 5 years old hospitalised with acute diarrhoea and 113 matched controls from two hospitals during 2005-07 by PCR assays using E. coli colony pools.

**Results:** The prevalence of DEC varied from 0.75% for EHEC to 8.4% for EPEC (mostly atypical variety) in diarrhoeal children with no significant differences compared to that in control children (P values 0.15 to 1.00). Twenty-seven EPEC isolates studied mostly belonged to non-traditional serotypes and possessed beta and theta intimin subtypes. A total of 54 DEC isolates from diarrhoeal children and 4 from controls studied for antimicrobial susceptibility showed resistance for older antimicrobials, ampicillin (0 to 100%), tetracycline (33 to 100%) and trimethoprim (22.2 to 100%); 43.1% of the isolates were multidrug-resistant (resistant to 3 or more agents). Six (10.4%) DEC isolates produced extended spectrum beta-lactamasases and possessed genetic elements (blaCTX-M, blaTEM and ISEcp1) associated with them.

**Conclusion:** We speculate that the lack of significant association of DEC with diarrhoea in children in Kuwait compared to countries surrounding the Arabian Gulf Region may be attributable to high environmental and food hygiene due to high disposable income in Kuwait.
Forthcoming Conferences and Meetings

Compiled and edited by
Babichan K Chandy

Kuwait Medical Journal 2009; 41 (2): 170-179

Mammograms to MRI: Breast Imaging and Interventions 2009
Jun 15 - 18, 2009
Kiawah Island Resort, SC, United States
Contact: Office of Continuing Medical Education, Duke University School of Medicine, 3100 Tower Boulevard, Suite 1300, Durham, NC 27707
Phone: 919-401-1200; Fax: 919-401-1213
E-Mail: cme@mc.duke.edu

MILAN Breast Cancer Conference
Jun 17 - 19, 2009
Milan, Italy
Contact: Cristina Rai
Phone: 39-0-24-804-951; Fax: 39-0-243-911-650
E-Mail: congress@cq-travel.com

20th Congress of the European Society of Paediatric and Neonatal Intensive Care ESPNIC
Jun 17 - 20, 2009
Verona, Italy
Contact: Liraz Bregman
Phone: 41-229-080-488; Fax: 41-227-322-850
E-Mail: espnic@kenes.com

The 1st World Congress on Controversies in Psychiatry
Jun 18 - 21, 2009
Berlin, Germany
Contact: Organizing Secretariat
Phone: 97-235-666-166
E-Mail: copsy@comtecmed.com

5th World Congress of Paediatric Cardiology and Cardiac Surgery
Jun 21 - 26, 2009
Cairns, QLD, Australia
Contact: ICMS Pty Ltd
Phone: 61-396-820-244; Fax: 61-396-820-288
E-Mail: pccs2009@icms.com.au

Autism, ADHD, and other Pediatric Behavior Disorders
Jun 21 - June 28, 2009
Rome, Italy
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: sandra@continuingeducation.net

American College of Surgeons Oncology Group 2009 Annual Symposium
Jun 18 - 20, 2009
Durham, NC, United States
Contact: Office of Continuing Medical Education, Duke University School of Medicine, 3100 Tower Boulevard, Suite 1300, Durham, NC 27707
Phone: 919-401-1200; Fax: 919-401-1213
E-Mail: cme@mc.duke.edu

The 12th Annual Duke Cardiotoracic Update and TEE Review
Jun 18 - 21, 2009
Hilton Head, SC, United States
Contact: Office of Continuing Medical Education, Duke University School of Medicine, 3100 Tower Boulevard, Suite 1300, Durham, NC 27707
Phone: 919-401-1200; Fax: 919-401-1213
E-Mail: cme@mc.duke.edu

The 1st World Congress on Controversies in Psychiatry
Jun 18 - 21, 2009
Berlin, Germany
Contact: Organizing Secretariat
Phone: 97-235-666-166
E-Mail: copsy@comtecmed.com

BIT’s 2nd World Cancer Congress
Jun 22 - 25, 2009
Beijing, China
Contact: Annie Sun
Phone: 0086-411-84-799-479; Fax: 0086-411-84-799-629
E-Mail: annie@cancercon.com
British Association of Urological Surgeons 2009
Annual Meeting
Jun 22 - 25, 2009
Glasgow, Scotland, United Kingdom
Contact: Meeting Organiser
Phone: 44-0-2-078-696-950; Fax: 44-0-2-074-045-048
E-Mail: admin@baus.org.uk

Gastroenterology for the PCP
Jun 22 - Jul 04, 2009
Harwich, England, United Kingdom
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: sandra@continuingeducation.net

Lung Health and the Strengthening of Health Systems in Africa
Jun 24 - 26, 2009
Ouagadougou, Burkina Faso
Contact: Conference Secretariat
Phone: 22-650-304-346; Fax: 22-650-317-979
E-Mail: ocarolineella@yahoo.fr

2nd World Psoriasis and Psoriatic Arthritis Conference 2009
Jun 24 - 28, 2009
Stockholm, Sweden
Contact: Veronika Lindberg
Phone: 46-855-610-910; Fax: 46-855-610-919
E-Mail: I fpaf@psos.se

Cardiology Essentials and Case Studies
Jun 25 - Jul 08, 2009
Amsterdam, Netherlands
Contact: Eileen Tener, ACC
Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

12th Annual Congress of the German Society for Wound Healing and Wound Care
Jun 25 - 27, 2009
Kassel, Germany
Contact: Jana Rausch
Phone: 49-0-3-641-353-313; Fax: 49-0-36-413-533-272
E-Mail: jana.rausch@conventus.de

American Academy of Dermatology and European Academy of Dermatology & Venereology State of the Art in Dermatology
Jun 25 - 28, 2009
Munich, Germany
Contact: Meeting Organiser
Phone: 847-240-1485; Fax: 847-330-1135
E-Mail: mstein@aad.org

UCSD Conference on Limb Salvage and Functional Reconstruction: Orthopaedic, Vascular and Wound Care Team Approval
Jun 26 - 28, 2009
Del Mar, CA, United States
Contact: Meeting Organiser
Phone: 858-534-3940 / 888-229-OCME (6263)
E-Mail: ocme@ucsd.edu

1st Meeting of the European Academy of Otorhinolaryngology and Head and Neck Surgery (EAORL-HNS)
Jun 27 - 30, 2009
Mannheim, Germany
Contact: Frau Ganthaler
Phone: 43-0-158-804-224; Fax: 43-0-158-804-185
E-Mail: ganthaler@mondial-congress.com

European Society of Therapeutic Radiology and Oncology (ESTRO) 2009 Course
Jun 27 - Jul 02, 2009
Bali, Indonesia
Contact: Meeting Organiser
Phone: 62-21-31-931-172; Fax: 62-21-31-931-172 / 62-21-55-960-179; E-Mail: estrobali@pharma-pro.com
European Society for Human Reproduction and Embryology: 25th Annual Meeting
Jun 28 - Jul 01, 2009
Amsterdam, Netherlands
Contact: ESHRE Central Office
Phone: 32-22-690-969; Fax: 32-22-695-600
E-Mail: info@eshre.com

9th World Congress of Biological Psychiatry
Jun 28 - Jul 02, 2009
Paris, France
Contact: Ms Gesche Ohle
E-Mail: GOhle@cpo-hanser.de

Pediatric Update
Jun 29 - Jul 02, 2009
Hilton Head Island, SC, United States
Contact: Catherine Burrison
Phone: 1-800-335-2582
E-Mail: cburrison@seapines.com

Orthopaedics and Sports Medicine for Primary Care and Family Practitioners
Jul 02 - 12, 2009
Copenhagen, Denmark
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: sandra@continuingeducation.net

4th Europediatrics 2009
Jul 03 - 06, 2009
Moscow, Russian Federation
Contact: Meeting Organiser
Phone: 302-106-889-100; Fax: 302-106-844-777
E-Mail: europediatrics2009@acnc.gr

World Glaucoma Congress
Jul 08 - 11, 2009
Boston, MA, United States
Contact: Congress Secretariat
Phone: 31-206-793-411; Fax: 31-206-737-306
E-Mail: info@worldglaucoma.org

North American Clinical Dermatologic Society 50th Annual Meeting
Jul 08 - 18, 2009
Boston, MA, United States
Contact: Meeting Organiser
Phone: 858-558-0677; Fax: 858-558-3077
E-Mail: jakoperski@yahoo.com

10th Annual Update in Gastroenterology - 2009
Jul 08 - 20, 2009
Amsterdam, Netherlands
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: sandra@continuingeducation.net

Society for Pediatric Dermatology 35th Annual Meeting
Jul 09 - 12, 2009
Philadelphia, PA, United States
Contact: Meeting Organiser
Phone: 317-202-0224; Fax: 317-205-9481
E-Mail: spd@hp-assoc.com

Modern Endocrinology: An Update & Refresher for the Primary Care Physician
Jul 10 - 17, 2009
Seattle, WA, United States
Contact: Eileen Tener, ACC
Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

North American Society for Dialysis and Transplantation: 28th Annual Meeting
Jul 12 - 16, 2009
Maui, HI, United States
Contact: Meeting Organiser
Phone: 1-281-997-1944
E-Mail: lbrazil@nasdat.org

Seminar on Legal-Medical Issues
Jul 15 - 28, 2009
Harwich, England, United Kingdom
Contact: Eileen Tener, ACC
Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

XVII World Congress of Aesthetic Medicine
Jul 17 - 19, 2009
Vancouver, BC, Canada
Contact: Natalie Lamppu
Phone: 604-685-0450; Fax: 604-685-0451
E-Mail: nlamppu@caam.ca

Anti Aging and Aesthetic Medicine Alaska Cruise
Jul 19 - 26, 2009
Vancouver, AK, United States
Contact: Dr. Martin Gerretsen
Phone: 1-888-647-7327; Fax: 1-888-547-7337
E-Mail: martin@seacourses.com

5th IAS Conference on HIV Pathogenesis, Treatment and Prevention (IAS 2009)
Jul 19 - 22, 2009
Cape Town, South Africa
Contact: Conference Secretariat
E-Mail: registration@ias2009.org

Washington State Dermatology Association Pacific Northwest Dermatological Society 76th Annual Meeting
Jul 23 - 26, 2009
Seaside, OR, United States
Contact: Meeting Organiser
Phone: 206-956-3038; Fax: 206-441-5863
E-Mail: smc@wsma.org
Urology Update for the Non-Urologist  
July 25 - Aug 01, 2009  
Miami, FL, United States  
Contact: Eileen Tener, ACC  
Phone: 813-333-6878  
E-Mail: ETener@CruisersParadise.com

Pediatrics Update 2009 - with a Focus on Gastrointestinal Diseases  
Aug 08 - 15, 2009  
Seattle, WA, United States  
Contact: Continuing Education, Inc.  
Phone: 1-800-422-0711; Fax: 727-522-8304  
E-Mail: Sandra@continuingeducation.net

Sports Medicine and Orthopaedics with Hands on Splinting/casting and Joint Injection Models  
Aug 08 - 18, 2009  
Southampton, England, United Kingdom  
Contact: Continuing Education, Inc.  
Phone: 1-800-422-0711; Fax: 727-522-8304  
E-Mail: Sandra@continuingeducation.net

3rd Annual LAVA (Latest Advances in Interventional Techniques)  
Jul 27 - 30, 2009  
Maui, HI, United States  
Contact: Stanford Radiology CME  
Phone: 650-473-5052 888-556-2230; Fax: 650-473-5062  
E-Mail: radiologycme@med.stanford.edu

Advanced Topics in Multidetector CT Scanning  
Jul 31 - Aug 02, 2009  
Seattle, WA, United States  
Contact: Johns Hopkins University School of Medicine, Thomas B. Turner Building, 720 Rutland Avenue, Room 20, Baltimore, Maryland 21205-2195  
Phone: 410-502-9634  
E-Mail: cmenet@jhmi.edu

13th World Conference on Lung Cancer  
Jul 31 - Aug 04, 2009  
San Francisco, CA, United States  
Contact: Robertson  
Phone: 1-604-681-2153; Fax: 1-604-681-1049  
E-Mail: wclc2009@meet-ics.com

7th Annual Comprehensive Pain Board Review Symposium  
Aug 03 - 07, 2009  
Madison, WI, United States  
Contact: CME Office Fax: 608-262-8421 / 608-240-6040  
E-Mail: ocpd@mailplus.wisc.edu

14th Congress of Indonesian Society of Obstetrician and Gynecologists  
Aug 03 - 09, 2009  
Surabaya, Indonesia  
Contact: Brahmana Askandar, MD  
Phone: 62-818-316-500  
E-Mail: Brahmana@kogi2009.com

World Congress on Thyroid Cancer  
Aug 06- 10, 2009  
Toronto, ON, Canada  
Contact: Meeting Organiser  
Phone: 416-978-2719 / 1-888-512-8173; Fax: 416-946-7028  
E-Mail: help-ENT0909@cmetoronto.ca

Neurology Conference Cruise  
Aug 07 - 14, 2009  
Vancouver, BC, Canada  
Contact: Continuing Education, Inc.  
Phone: 1-800-422-0711; Fax: 727-522-8304  
E-Mail: Sandra@continuingeducation.net

23rd Annual Echocardiographic Symposium  
Aug 09 - 13, 2009  
Vail, CO, United States  
Contact: Mayo Clinic Medical Education Office  
Phone: 904-953-7114 / 507-284-2509 / 480-301-4580;  
Fax: 904-953-2954 / 507-284-0532 / 480-301-8323  
E-Mail: -jax@mayo.edu / cme@mayo.edu / mcs.cme@mayo.edu

SkinCare Physicians Controversies and Conversations in Laser and Cosmetic Surgery  
Aug 14 - 16, 2009  
Southampton, Bermuda  
Contact: Meeting Organiser  
Phone: 617-731-1600  
E-Mail: controversies@skincarephysicians.net

The Bali Ophthalmology Retreat  
Aug 14 - 16, 2009  
Bali, Indonesia  
Contact: Bali Ophthalmology Retreat 2009 Secretariat  
Phone: 62-215-646-688; Fax: 62-215-642-072  
E-Mail: Esti.Hastarini@klinikmatanusantara.com
American Dermatological Association 2009 Annual Meeting
Aug 19 - 23, 2009
Park City, UT, United States
Contact: Meeting Organiser
Phone: 954-452-1113; Fax: 305-945-7063
E-Mail: ameriderm1930@aol.com

European Plastic Surgery Research Council
Aug 20 - 23, 2009
Hamburg, Germany
Contact: Lars Steinstraesser
E-Mail: info@epsrc.eu

Psychiatry at Sea Cruise (to Alaska)
Aug 22 - 29, 2009
Vancouver, AK, United States
Contact: Dr Martin Gerretsen
Phone: 1-888-647-7327; Fax: 1-888-547-7337
E-Mail: cruises@seacourses.com

7th Baltic Bone and Cartilage Conference
Aug 23 - 26, 2009
Nyborg, Denmark
Contact: Nanett Mosumgaard
Phone: 45-65-414-838 Fax: 45-65-919-653
E-Mail: nanett.mosumgaard@ouh.regionsyddanmark.dk

Trauma Conference
Aug 23 - 28, 2009
Thredbo, NSW, Australia
Contact: Bron Chesterman
Phone: 0-882-676-660; Fax: 0-882-676-668
E-Mail: bron@learningandleisure.com.au

20th Annual Anesthesiology Update
Aug 28 - 30, 2009
Napa Valley, CA, United States
Contact: Continuing Medical Education Office, 3560 Business Drive, Suite 130, Sacramento, CA 95820
Phone: 916-734-5390 / 866-263-4338 / 866-CME-4EDU
E-Mail: cmereg@ucdavis.edu

Clinical Endocrinology for Primary Care Physicians
Aug 28 - 30, 2009
Monterey, CA, United States
Contact: Linda Main
Phone: 303-798-9682
E-Mail: linda@mer.org

International Society for Hemodialysis & Hong Kong Society of Nephrology: 2nd Congress of International Society for Hemodialysis 2009
Aug 28 - 30, 2009
Hong Kong, Hong Kong
Contact: ISHD 2009 Congress Secretariat
Phone: 85-225-599-973; Fax: 85-225-479-528
E-Mail: enquiry@ishd2009.org

Cardiology, Infectious Diseases, and Respiratory Medicine
Cruise
Aug 31 - Sep 13, 2009
Athens, Greece
Contact: Dr. Martin Gerretsen
Phone: 1-888-647-7327 Fax: 1-888-547-7337
E-Mail: cruises@seacourses.com

EBA 2009 - European Burns Association Congress
Sept 02 - 05, 2009
Lausanne, Switzerland
Contact: Gerald Howard
Phone: 41-0-223-399-635; Fax: 41-0-223-399-601
E-Mail: gerald.howard@mci-group.com

European Society for Paediatric Nephrology: 42nd Annual Scientific Meeting ESPN 2009. A Joint Meeting with the Renal Association
Sep 02 - 05, 2009
Birmingham, England
United Kingdom
Contact: ESPN Conference Office
Phone: 44-8-704-584-138; Fax: 44-8-704-429-940
E-Mail: ESPN2009@mci-group.com

6th European Congress on Tropical Medicine and International Health
Sep 06 - 10, 2009
Verona, Italy
Contact: Stephane Talboom
Phone: 41-22-74-156-60; Fax: 41-22-74-156-64
E-Mail: info@tropverona.org

Infectious Disease Conference Cruise
Sep 06 - 13, 2009
Rome, Italy
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: Sandra@continuingeducation.net

Pharmacokinetics for the Pharmaceutical and Biomedical Scientist
Sep 06 - 15, 2009
Msida, Malta
Contact: NA
Phone: +356 32902845; Fax: +356 320281
E-Mail: janmif@um.edu.mt

European Surgical Institute: Minimally Invasive Techniques in Gynaecology
Sep 07 - 10, 2009
Norderstedt, Germany
Contact: European Surgical Institute
Phone: 49-0-4-052-973-200 Fax: 49-0-4-052-973-209
E-Mail: info@esi-online.de
Australasian HIV/AIDS Conference 2009 - 21st
Australasian Society for HIV Medicine Conference
Sep 09 - 12, 2009
Brisbane, QLD, Australia
Contact: Australasian Society for HIV Medicine
Phone: 61-282-040-770; Fax: 61-292-124-670
E-Mail: conferenceinfo@ashm.org.au

ESRA - 28th Annual ESRA Congress - European Society of Regional Anaesthesia and Pain Therapy
Sep 09 - 12, 2009
Salzburg, Austria
Contact: Liraz Bregman
Phone: 41-229-080-488; Fax: 41-227-322-850
E-Mail: esra2009@kenes.com

European Society for Dermatological Research 39th Annual ESDR Meeting
Sep 09 - 12, 2009
Budapest, Hungary
Contact: Meeting Organiser
Phone: 41-223-214-890; Fax: 41-223-214-892
E-Mail: office@esdr.org

Practical Surgical Pathology Symposium
Sep 10 - 12, 2009
Rochester, MN, United States
Contact: Mayo Clinic Medical Education Office
Phone: 904-953-7114 / 507-284-2509 / 480-301-4580;
Fax: 904-953-2954 / 507-284-0532 / 480-301-8323
E-Mail: cme-jax@mayo.edu / cme@mayo.edu / mcs.cme@mayo.edu

Cardiothoracic Imaging Update
Sep 11 - 13, 2009
Quebec City, QC, Canada
Contact: Brenda Lewicki
Phone: 613-798-5555 ext 16-894
E-Mail: info@ottawaradcme.com

California Society of Dermatology & Dermatologic Surgery 2009 CalDerm Annual Meeting Sep 11 - 13, 2009
San Diego, CA, United States
Contact: Meeting Organiser
Phone: 916-498-1712; Fax: 916-244-0330
E-Mail: membership@calderm.org

13th Congress of the European Federation of Neurological Societies - EFNS 2009
Sep 12 - 15, 2009
Florence, Italy
Contact: Liraz Bregman
Phone: 41-229-080-488; Fax: 41-229-080-850
E-Mail: efns09@kenes.com

4th All African Congress of Anaesthesiologists
Sep 12-17, 2009
Nairobi, Kenya
Contact: Dr. Jane Kabutu
Phone: 254-0-202-738-327; Fax: 254-202-738-327
E-Mail: jkabutu@aaackenya09.org

2nd European Congress of Immunology
Sept 13 - 16, 2009
Berlin, Germany
Contact: Congress Secretariat
E-Mail: eci2009registration@kit-group.org

38th Annual Meeting of the American College of Clinical Pharmacology
Sept 13 - 15, 2009
San Antonio, TX, United States
Contact: American College of Clinical Pharmacology,
3 Ellinwood Court, New Hartford, NY 13413-1105
Phone: 315-768-6117 Fax: 315-768-6119
E-Mail: linda@accp1.org, tami@accp1.org

19th World Congress on Ultrasound in Obstetrics and Gynecology
Sep 13 - 17, 2009
Hamburg, Germany
Contact: Congress Secretariat
Phone: 44-0-2-074-719-955; Fax: 44-0-2-074-719-959
E-Mail: congress@isuog.org

The 8th Congress of the Baltic Association of Dermatovenereologists
Sept 17 - 19, 2009
Vilnius, Lithuania
Contact: Vita Gircyte
Phone: 37-0-52-051-350; Fax: 37-0-52-051-340
E-Mail: info@aimbaltic.lt

NICE Spine Course 2009
Sep 17 - 19, 2009
Nice, France
Contact: Christina Loicht
Phone: 0-492-073-576; Fax: 0-492-073-586
E-Mail: christina@impact-events.net

European Course on Laryngology and Phonosurgery
Sept 17 - 19, 2009
Gießen, Germany
Contact: HNO-Klinik Gießen, Klinikstrasse 29, 35390 Gießen
Phone: 419-943-701; Fax 496-419-943-709
E-Mail: christoph.aren@hno.med.uni-giessen.de

The 12th International Congress of Pediatric Hepatology, Gastroenterology and Nutrition
Sep 22 - 25, 2009
Sharm El-Sheikh, Egypt
Contact: Mortada El-Shabrawi
Phone: 20-123-133-705; Fax: 20-237-619-012
E-Mail: mortada_elshabrawi@yahoo.com
Forthcoming Conferences and Meetings

March 2009

Royan International Twin Congress. 10th Congress on Reproductive Biomedicine
5th Congress on Stem Cell Biology and Technology
Sep 23 – 25, 2009
Tehran, Iran
Contact: Congress Secretariat
Phone: 98 21 22305236; Fax: 98 21 22306481
E-Mail: congress@royaninstitute.org

2009 - Primary Care Sports Medicine
Sep 23 - 25, 2009
Burlington, VT, United States
Contact: Continuing Medical Education Office
Phone: 802-656-2292
E-Mail: uvmcme@uvm.edu

World Psychiatric Association Regional Meeting
Sep 24 - 26, 2009
Abuja, Nigeria
Contact: Dr. Oye Gureje
E-Mail: ogureje@comui.edu.ng

The 7th Pediatric Intensive Review Course
Sep 26 - Oct 01, 2009
Riyadh, Saudi Arabia
Contact: Dr. Abdullah Al-Dowaish
Phone: 966-1-442-7763; Fax: 966-1-442-7784
E-Mail: mdatu@kfshrc.edu.sa

The 7th Greek Legal and Medical Conference
Sep 26 - Oct 02, 2009
Kommeno, Bay, Greece
Contact: Miss Eugenia Mitrakas
Phone: 61-396-902-033; Fax: 61-396-962-937
E-Mail: eugenia@greekconference.com.au

35th European Congress of Cytology
Sep 27 - 30, 2009
Lisbon, Portugal
Contact: Congress Secretariat c/o Forum d’Ideias
Phone: 351-212-189-393; Fax: 351-212-189-392
E-Mail: cytologylisboa2009@forumdideias.com

1st PANARAB Conference on Thoracic, Cardiac and Vascular Surgery
Sep 29 - Oct 01, 2009
Amman, Jordan
Contact: Abdullah Al-Qudah, MD
Phone: 962-795-590-788; Fax: 96-264-652-723
E-Mail: al_qudah_as@hotmail.com

2009 Meeting of the International Continence Society
Sep 30 - Oct 04, 2009
San Francisco, CA, United States
Contact: Meeting Organiser
E-Mail: arstone@ucdavis.edu

The American Society of Dermatopathology ASDP
46th Annual Meeting
Oct 01-04, 2009
Chicago, IL, United States
Contact: Meeting Organiser
Phone: 847-400-5820; Fax: 847-480-9282
E-Mail: ksantos@asdp.org

4th Annual AAOS Orthopaedic Practice Management Course
Oct 02-04, 2009
Rosemont, IL, United States
Contact: Customer Service
Phone: 1-800-626-6726/1-847-823-7186; Fax: 847-823-8125
E-Mail: custserv@aaos.org

Michigan Ear Institute Temporal Bone Surgical Dissection Course
Oct 05-09, 2009
Farmington Hills, MI, United States
Contact: Organiser
Phone: 248-865-4444; Fax: 248-865-6161

Endourological Society: 27th World Congress on Endourology and SWL (WCE 2009)
Oct 06 - 10, 2009
Munich, Germany
Contact: Meeting Organiser
Phone: 49-210-296-920; Fax: 492-102-969-230
E-Mail: beate.ruloff@ruloff.de

18th Congress of the European Academy of Dermatology and Venerology (EADV)
Oct 07 - 11, 2009
Berlin, Germany
Contact: EADV Office
Phone: 322-650-0090; Fax: 322-650-0098
E-Mail: office@eadv.org

Pediatric and Adolescent Medicine Update for the Primary Care Physician
Oct 09 - 21, 2009
Southampton, England, United Kingdom
Contact: Eileen Tener, ACC
Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

The 50th Annual Meeting of the European Society for Pediatric Research - ESPR 2009
Oct 09 - 12, 2009
Hamburg, Germany
Contact: Liraz Bregman
Phone: 41-229-080-488; Fax: 41-227-322-850
E-Mail: espr09@kenes.com
Allergy and Immunology Conference Cruise  
**Oct 10 - 17, 2009**  
Honolulu, HI, **United States**  
Contact: Continuing Education, Inc.  
Phone: 1-800-422-0711; Fax: 727-522-8304  
E-Mail: Sandra@continuingeducation.net

8th International Congress on **Coronary Artery Disease** - ICCAD 2009  
**Oct 11 - 14, 2009**  
Prague, **Czech Republic**  
Contact: Liraz Bregman  
Phone: 41-229-080-488; Fax: 41-227-322-850  
E-Mail: coronary@kenes.com

16th International Meeting of the European Society of **Gynaecological Oncology**: ESGO 2009  
**Oct 11-15, 2009**  
Belgrade, **Serbia**  
Contact: Natalie Shabi  
Phone: 41-229-080-488; Fax: 41-227-322-850  
E-Mail: wccs2009@kenes.com

37th Annual Meeting of the International Society for **Pediatric Neurosurgery**  
**Oct 11-15, 2009**  
Los Angeles, CA, **United States**  
Contact: Gordon Mccomb  
E-Mail: gmcomb@chla.usc.edu

The 4th International Congress on **Pulmonary Diseases, Intensive Care and Tuberculosis**  
**Oct 12 - 15, 2009**  
Tehran, **Islamic Republic of Iran**  
Contact: Maliheh Bitaraf  
Phone: 982-120-109-507; Fax: 982-120-109-484  
E-Mail: fic@nritld.ac.ir

Child Neurology Society (CNS) 38th Annual Meeting,  
**Oct 14 - 17, 2009**  
Louisville, KY, **United States**  
Contact: CNS National Office, 1000 West County Road E, Suite 290 Saint Paul, MN 55126  
Phone: 651-486-9447; Fax: 651-486-9436  
E-Mail: nationaloffice@childneurologysociety.org

Twenty-First Century Obstetrics and Gynecology (In Conjunction with the ACOG District I and III Annual Meeting)  
**Oct 16 - 18, 2009**  
Orlando, FL, **United States**  
Contact: American College of Obstetricians and Gynecologists, 409 12th St., S.W., PO Box 9692  
Phone: 202-638-5577  
E-Mail: coding@acog.org / meetings@acog.org

Lymphoma and Myeloma 2009: An International Congress on **Hematologic Malignancies**  
**Oct 22 - 24, 2009**  
New York, NY, **United States**  
Contact: Customer Service  
Phone: 713-792-2223; Fax: 713-794-1724  
E-Mail: meetings@imedex.com

8th Annual Symposium on Advances in **Breast MRI**  
**Oct 22 - 24, 2009**  
Las Vegas, NV, **United States**  
Contact: Stanford Radiology Continuing Medical Education Program 480 California Avenue, Suite 301, Palo Alto, CA 94306 USA  
Phone: 1-888-556-2230 / 1 650 473-5052; Fax: 1 650 473-5062  
E-Mail: radiologyme@med.stanford.edu

AAOS **Knee Arthroplasty**: Uni, Total & Revision Insights, New Techniques - What You Need to Know  
**Oct 22 - 24, 2009**  
Rosemont, IL, **United States**  
Contact: Customer Service  
Phone: 1-800-626-6726 / 1-847-823-7186; Fax: 847-823-8125  
E-Mail: custserv@aaos.org
3rd Annual Urology Today
Oct 22 - 25, 2009
City: Asheville, NC, United States
Contact: Office of Continuing Education, Medical Center Blvd, Winston-Salem, NC 27157
Phone: 336-713-7755; Fax: 336-713-7702
E-Mail: rnhoc@wfubmc.edu

ACG 2009: American College of Gastroenterology
Annual Scientific Meeting and Postgraduate Course
Oct 23 - 28, 2009
San Diego, CA, United States
Contact: ACG Office
Phone: 301-263-9000

2009 American Academy of Ophthalmology Joint meeting with the Pan-American Association of Ophthalmology (PAAO)
Oct 24 - 27, 2009
San Francisco, CA, United States
Contact: American Academy of Ophthalmology
Phone: 415-447-0320
E-Mail: meetings@aao.org

The 3rd World Congress on Controversies in Neurology
Oct 24 - 26, 2009
Prague, Czech Republic
Contact: Organizing Secretariat
Phone: 00-97-235-666-166
E-Mail: cony@comtecmed.com

8th International Congress on Endocrinology and Metabolism
Oct 27 - 30, 2009
Tehran, Iran
Contact: Dr. H. Delshad
Phone: 98-2-122-418-931; Fax: 98-2-122-418-931
E-Mail: info@endocrine.ac.ir

20th Annual Coronary Interventions
October 28, 2009 - October 30, 2009
San Diego, CA, United States
Contact: Gretchen Ploen
Phone: 858-652-5400; Fax: 858-652-5565
E-Mail: Med.edu@scrippshealth.org

JOPEOI European Indian Ocean Perinatology Congress / Journées Obstétrico-Pédiatriques Europe Océan Indien
Oct 29 - 31, 2009
Antananarivo, Madagascar
Contact: Dr. K. DE BARGOU
Phone: 33-607-686-118; Fax: 33-143-839-985
E-Mail: kambarg@orange.fr

Michigan Ear Institute Temporal Bone Surgical Dissection Course
Nov 02 - 06, 2009
Farmington Hills, MI, United States
Contact: Meeting Organiser
Phone: 248-865-4444; Fax: 248-865-6161

American College of Phlebology 23rd Annual Congress
Nov 05 - 08, 2009
Palm Desert, CA, United States
Contact: Meeting Organiser
Phone: 246-6800; Fax: 510-346-6808
E-Mail: ddeponzi@acpmail.org

AAOS Spinal Surgery
Nov 05 - 07, 2009
Rosemont, IL, United States
Contact: Customer Service
Phone: 1-800-626-6726 / 1-847-823-7186; Fax: 847-823-8125
E-Mail: custserv@aaos.org

International Society for Dermatologic Surgery 30th Annual Meeting of the ISDS
Nov 05 - 08, 2009
Vienna, Austria
Contact: Meeting Organiser
Phone: 49-61-519-518-892; Fax: 49-61-519-518-893
E-Mail: info@isdsworld.com

5th International Congress on Myeloproliferative Disorders and Myelodysplastic Syndromes
Nov 05 - 07, 2009
New York, NY, United States
Contact: Customer Service
Phone: 770-751-7332; Fax: 770-751-7334
E-Mail: meetings@imedex.com

17th Annual Trauma/Surgical Critical Care Symposium
Nov 06 - 09, 2009
Indianapolis, IN, United States
Contact: Indiana University School of Medicine, Division of Continuing Medical Education, Attn: REGISTRAR, 714 N. Senate Ave, EF 200, Indianapolis, IN 46202
Phone: 317-274-8353 / 888-615-8013; Fax: 317-274-4638
E-Mail: marmin@iupui.edu

American College of Allergy, Asthma and Immunology Annual Meeting 2009
Nov 06 - 11, 2009
Miami Beach, FL, United States
Contact: Meeting Organiser
Phone: 847-427-1294; Fax: 847-427-1200
E-Mail: mail@acaai.org / meetings@acaai.org
3rd Asia pacific Congress on Controversies in Obstetrics, Gynecology and infertility
Nov 12- 15, 2009
Bangkok, Thailand
Contact: Congress Secretariat
Phone: 97-235-666-166
E-Mail: cogi@comtecmed.com

The Arthroscopy Association of North America 2009 Fall Course
Nov 12 - 15, 2009
Palm Desert, CA, United States
Contact: Arthroscopy Association of North America
Phone: 847-292-2262; Fax: 847-292-2268
E-Mail: info@aana.org

Emergency Medicine Update
Nov 15 - 22, 2009
Miami, FL, United States
Contact: Eileen Tener, ACC
Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

The 3rd Iranian Asthma Meeting
Nov 17 - 19, 2009
Tehran, Iran
Contact: Iranian Society of Asthma & Allergy
Phone: 982-166-938-545; Fax: 982-166-428-995
E-Mail: isaacng@tums.ac.ir

11th World Congress on Pediatric Dermatology (WCPD 2009)
Nov 17 - 20, 2009
Bangkok, Thailand
Contact: Liraz Bregman
Phone: 00-41-229-080-488; Fax: 00-41-227-322-850
E-Mail: wcpd@kenes.com

Allergy, Asthma and Clinical Immunology Symposium
Nov 17 - 18, 2009
Riyadh, Saudi Arabia
Contact: Ms Ghalia Al Otaibi
Phone: 96-614-647-272 ext. 31-912; Fax: 96-614-424-153
E-Mail: gotaibi@kfshrc.edu.sa

40th Union World Conference on Lung Health
Dec 03 - 07, 2009
Cancun, Quintana Roo, Mexico
Contact: Conference Unit
Phone: 33-143-299-087; Fax: 33-153-108-554
E-Mail: cancun2009@theunion.org Q. Quintana Roo

Pain Management Conference Cruise
Dec 05 - 12, 2009
Honolulu, HI, United States
Contact: Continuing Education, Inc.
Phone: 1-800-422-0711; Fax: 727-522-8304
E-Mail: Sandra@continuingeducation.net

XXI World Allergy Congress
Dec 06 - 10, 2009
Buenos Aires, Argentina
Contact: Mariu Denovi
Phone: 541-147-779-449; Fax: 541-147-711-536
E-Mail: info@worldallergy2009.com

2009 Annual Meeting of the American College of Neuropsychopharmacology
Dec 06 - 10, 2009
Hollywood, FL, United States
Contact: American College of Neuropsychopharmacology,
545 Mainstream Drive Suite 110, Nashville TN 37228
Phone: 615-324-2360; Fax: 615-324-2361
E-Mail: acnp@acnp.org

26th Annual Advances in Heart Disease
December 11 - 13, 2009
San Francisco, CA, United States
Contact: UCSF Office of Continuing Medical Education,
3333 California Street, Room 450, San Francisco, CA 94118
Phone: 415-476-4251 / 415-476-5808; Fax: 415-476-0318 / 415-502-1795
E-Mail: info@ocme.ucsf.edu

Rheumatology for the Primary Care Physician
Dec 12 - 20, 2009
Fort Lauderdale, FL, United States
Contact: Eileen Tener, ACC Phone: 813-333-6878
E-Mail: ETener@CruisersParadise.com

The 5th International Conference on Ocular Infections
Feb 18 - 21, 2010
Palm Beach, FL, United States
Contact: Hila Dayan
Phone: 41-225-330-948
E-Mail: hdayan@paragon-conventions.com

Multidisciplinary Head and Neck Cancer Symposium
Feb 25 - 27, 2010
Chandler, AZ, United States
Contact: Meeting Organiser
Phone: 703-502-1550; Fax: 703-502-7852
WHO-Facts Sheet

1. Childhood Diarrhea
2. Addressing Mental Disorders in Children
3. More People Dying from TB are HIV-infected
4. Drug Resistance Could Set Back Malaria Successes
5. Climate Change Global Risks, Challenges and Decisions

Compiled and edited by
Babichan K Chandy


1. CHILDHOOD DIARRHEA

An Inconvenience for Some, A Death Threat for Many; A Major Priority for Research

The World Health Organization (WHO), in consultation with global experts, has identified priorities for research on diarrhea - the cause of almost 20% of child deaths globally. The list of research questions focus on how to make the best use of interventions that are available today, in order to make the most difference, and ultimately save as many children's lives as possible.

Each year, nearly two million children die from diarrhea. If childhood diarrhea is not addressed urgently, the world will fail to achieve the fourth Millennium Development Goal (MDG4) target of reducing child deaths by two-thirds by 2015.

Despite the persistently high burden of disease, research into childhood diarrhea has been steadily decreasing since the 1980s. Funds available for research into diarrhea are much lower than those devoted to other diseases that cause comparatively few deaths.

While a lot is already known about effective treatments for diarrhea, we still lack critical knowledge on how to make sure the children who need it most get access to that treatment. WHO has led a process to identify which types of research are most needed and would have greatest impact on mortality. The resulting top 20% of research questions are mainly targeted at better understanding the barriers to implementation, effectiveness and optimizing the use of available interventions and programmes such as Oral Rehydration Salts (ORS) and zinc, exclusive breastfeeding and the integrated management of childhood illness. The life-saving treatment for diarrhea is simple: (ORS) and zinc tablets. ORS is essentially a pinch of salt and a handful of sugar mixed with clean water. The cost of treating a child with ORS and zinc is approximately US$0.30 (€0.25, £0.20).

Children in poor countries get diarrhea on average four times per year - each of these episodes can be life-threatening. ORS and zinc bring the risk of death down to almost zero. More than 50 million children's lives have been saved by ORS since its creation 25 years ago, which has meant a large chunk of the adult population in developing countries is alive today as a result of this cheap, easily prepared solution. The great challenge we now face is how to reach all children who are still suffering and dying from diarrhea.

For more information contact: Olivia Lawe-Davies, Communications Officer, Child and Adolescent Health and Development, WHO, Geneva. Telephone: +41 22 791 1209; Mobile: +41 794 755 545; Email: lawedavieso@who.int

2. ADDRESSING MENTAL DISORDERS IN CHILDREN

On the occasion of World Autism Day on 2nd April, the World Health Organization (WHO) reaffirmed its commitment to provide technical assistance to member states to deliver integrated health services to people with autism and other mental and developmental disorders of childhood.

Dr Ala Alwan, WHO Assistant Director-General for Noncommunicable Diseases and Mental Health said “It is a deep concern that the global burden of disease attributed to mental disorders continues...”

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/
to grow, particularly in developing countries. It is essential to prioritize, implement and fund projects on autism spectrum disorders and other mental disorders in children in developing countries.”

Currently, the vast majority of children with mental health needs in developing countries do not receive any treatment or care. The immediate challenge in these countries is generating sufficient resources for primary health care to ensure early identification and treatment of mental disorders among children. These disorders are included as priority conditions in WHO’s Mental Health Gap Action Programme 2008-2013, launched in 2008.

“A prioritized agenda for autism and other mental disorders in children should generate and strengthen the evidence base for cost-effective prevention and control strategies. Scaling up of services is the real need. This will also improve educational attainments and will contribute to a better informed and healthier generation of children.” said Dr Benedetto Saracenos, Director, Mental Health and Substance Abuse at WHO.

World autism awareness day:

On 18 December 2007, the United Nations General Assembly adopted resolution 62/139 which declared April 2, as World Autism Awareness Day.

Autism spectrum disorders are characterized by varying degrees of impairment in communication skills and social interactions and in restricted, repetitive patterns of behaviour. The condition causes disabilities that can be lifelong. Emerging evidence indicates that early intervention results in improved outcomes.

Autism spectrum disorders and other mental disorders among children bring significant economic hardships to families, given the lack of health resources often found in developing countries. The stigmatization and discrimination associated with these illnesses also remain substantial obstacles to diagnosis and treatment. The absence of autism spectrum disorders and other mental disorders among children from lists of the leading causes of death has contributed to their long-term neglect by both public policy-makers in developing countries, as well as donors.

UN Convention on the Rights of the Child 1990

States parties
• recognize that a mentally or physically disabled child should enjoy a full and decent life, in conditions which ensure dignity, promote self-reliance, and facilitate the child’s active participation in the community (Article 23.1);
• agree that the education of the child shall be directed to: a. the development of the child’s personality, talents and mental and physical abilities to their fullest potential (Article 29 1.a);
• shall take all appropriate measures to promote physical and psychological recovery and social reintegration of a child victim...re-integration shall take place in an environment which fosters the health, peer-respect and dignity of the child (Article 39)

Some bare facts about mental disorders:
• Mental, neurological and behavioural disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation, poor quality of life and increased mortality. These disorders are the cause of staggering economic and social costs.
• Hundreds of millions of people worldwide are affected by mental, behavioural, neurological and substance use disorders. For example, estimates made by WHO in 2002 showed that 154 million people globally suffer from depression and 25 million people from schizophrenia; 91 million people are affected by alcohol use disorders and 15 million by drug use disorders. A recently published WHO report shows that 50 million people suffer from epilepsy and 24 million from Alzheimer and other dementias.
• In addition to the above figures, many other disorders affect the nervous system or produce neurological sequelae. Projections based on a WHO study show that worldwide in 2005, 326 million people suffer from migraine; 61 million from cerebrovascular diseases; 18 million from neuroinfections or neurological sequelae of infections. Number of people with neurological sequelae of nutritional disorders and neuropathies (352 million) and neurological sequelae secondary to injuries (170 million) also add substantially to the above burden.
• About 877,000 people die by suicide every year.
• One in four patients visiting a health service has a psychological disorder but most of these disorders are neither diagnosed nor treated.
• Mental illnesses affect and are affected by chronic conditions such as cancer, heart and cardiovascular diseases, diabetes and HIV/AIDS. Untreated, they bring about unhealthy behaviour, non-compliance with prescribed medical regimens, diminished immune functioning, and poor prognosis.
• Cost-effective treatments exist for most disorders and, if correctly applied, could enable most of those affected to become functioning members of society.
• Barriers to effective treatment of mental illness include lack of recognition of the seriousness of mental illness and lack of understanding about the benefits of services. Policy makers, insurance
companies, health and labour policies, and the public at large – all discriminate between physical and mental problems.

• Most middle and low-income countries devote less than 1% of their health expenditure to mental health. Consequently mental health policies, legislation, community care facilities, and treatments for people with mental illness are not given the priority they deserve.

For more information please contact: Dr Shekhar Saxena, Programme Manager, Mental Health and Substance Abuse, WHO. Tel:+41.22.791.3625; Email: saxenas@who.int

3. MORE PEOPLE DYING FROM TB ARE HIV-INFECTED

World TB Day and the launch of the WHO Global TB Control Report

The total number of new tuberculosis (TB) cases remained stable in 2007, and the percentage of the world’s population becoming ill with TB has continued the slow decline that was first observed in 2004, according to a new report released by the World Health Organization (WHO) in March, 2009.

However, Global TB Control Report also revealed that one out of four TB deaths is HIV related, twice as many as previously recognized. In 2007, there were an estimated 1.37 million new cases of tuberculosis among HIV-infected people and 456,000 deaths. This figure reflects an improvement in the quality of the country data, which are now more representative and available from more countries than in previous years.

“We have to stop people living with HIV from dying of tuberculosis,” said Mr Michel Sidibe, Executive Director of UNAIDS. “Universal access to HIV prevention, treatment, care and support must include TB prevention, diagnosis and treatment. When HIV and TB services are combined, they save lives.”

TB/HIV co-infection and drug-resistant forms of tuberculosis present the greatest challenges, the report says. In 2007, an estimated 500,000 people had multidrug-resistant TB (MDR-TB), but less than 1% of them were receiving treatments that was known to be based on WHO’s recommended standards.

Given the current financial crisis, the report documents concerns over an increasing shortage in funding. Ninety-four countries in which 93% of the world’s TB cases occur provide complete financial data for the report. To meet the 2009 milestones in the Stop TB Partnership’s Global Plan to Stop TB, the funding shortfall for these 94 countries has risen to about US$ 1.5 billion. Full funding of the Global Plan will achieve its aim of halving TB prevalence and deaths compared with 1990 levels by 2015.

For more information contact: Glenn Thomas, WHO Stop TB Department. Mobile: +41 795090677; E-mail: thomassg@who.int

4. DRUG RESISTANCE COULD SET BACK MALARIA SUCEESSES

The World Health Organization (WHO) announced in February, 2009 that the emergence of artemisinin resistant parasites at the Thai-Cambodia border could seriously undermine global malaria control efforts achieved.

Surveillance systems and research studies supported by WHO to monitor antimalarial drug efficacy in countries are providing new evidence that parasites resistant to artemisinin have emerged along the border between Cambodia and Thailand where workers walk for miles every day to clear forests. The risk that they may be infected with a drug-resistant form of malaria could set back recent successes to control the disease.

Huge strides have been made in the last ten years to reduce the burden of malaria, one of the world’s major killer diseases. Strong malaria control programs have helped lower infection rates in several countries. The recent shift from failing drugs to the highly effective artemisinin-based combination therapies (ACTs) has been a breakthrough. Appropriate treatment with ACTs succeeds in more than 90% of cases. However, malaria drug resistance now emerging along the Thai-Cambodia border threatens these gains.
With a US$ 22.5 million grant from the Bill & Melinda Gates Foundation, WHO will endeavour to contain artemisinin resistant malaria parasites before they spread. Resistance along the Thai-Cambodia border started with chloroquine, followed by resistance to sulfadoxine-pyrimethamine and mefloquine, drugs used in malaria control several years ago. Malaria poses a risk to half of the world’s population and more than one million people die of the disease each year. The malaria map, or the area where it is prevalent, has been reduced considerably over the past fifty years, but the disease has defied elimination in areas of intense transmission.

Obstacles to malaria control include drug resistance in the parasite that causes the disease, as well as resistance of the vector mosquito to insecticides, environmental factors and counterfeit medicines. The likelihood of drug resistance is increased with the use of single drug therapy for malaria, especially monotherapies of artemisinin and its derivatives. Monotherapy fosters resistance because it is easier for the parasite to adapt and eventually overcome the obstacles presented by a single drug than a combination of drugs delivered together. This makes it crucial for monotherapies to be removed from the market. WHO treatment policy is to treat all cases of uncomplicated falciparum malaria with artemisinin combination therapy (ACTs).

The grant will be used to meet the following key objectives:

- Eliminate artemisinin tolerant parasites by detecting all malaria cases in target areas and ensuring effective treatment
- Reduce exposure of the parasites to artemisinin to limit emergence of resistance
- Prevent transmission of artemisinin tolerant malaria parasites through mosquito control and personal protection
- Limit the spread of artemisinin tolerant malaria parasites by mobile populations
- Support the containment and elimination of artemisinin tolerant parasites through comprehensive behavior change, communication, community mobilization and advocacy
- Undertake basic and operational research to fill knowledge gaps and ensure that strategies applied are evidence-based
- Provide effective management, surveillance and coordination to enable a rapid and high quality implementation of the strategy.

For more information, please contact:
Ravini Thenabadu, Communications Officer, Global Malaria Programme, WHO, Geneva. Telephone: +41 22 791 2339, Mobile: +41 79 500 6549, Email: thenabadur@who.int

5. CLIMATE CHANGE GLOBAL RISKS, CHALLENGES AND DECISIONS

The health impact of climate change is a critical issue that policy makers should be aware of while setting priorities for action and investment to mitigate the impact of global climate change. This was the key message that WHO experts delivered at the Climate Change Global Risks, Challenges and Decisions conference in Copenhagen. Building on research, WHO has identified three key health arguments for stronger climate change measures:

1. Climate change has adverse consequences for health: as carbon goes up health goes down

WHO and the International Panel on Climate Change (IPCC) data identify risks to human health as a serious signal of the consequences of climatic disruption of this planet’s natural processes which we depend on for food, water, and physical safety. Health hazards from climate change are diverse, global and difficult to reverse over human time scales. They range from increased risks of extreme weather events, to effects on infectious disease dynamics and sea level rise leading to salinization of land and water sources.

Based on WHO estimates around 150,000 deaths now occur in low-income countries each year due to climate change from four climate-sensitive health outcomes – crop failure and malnutrition, diarrheal disease, malaria and flooding. Almost 85% of these excess deaths are in young children.

2. Reducing green house gases emissions can be beneficial to health: as carbon goes down health goes up

Feasible improvements in environmental conditions could reduce the global disease burden by more than 25%. A large part of the current burden is linked to energy consumption and transport systems. Changing these systems to reduce climate change would have the added benefit of addressing some major public health issues, including outdoor air pollution (800 000 annual global deaths); traffic accidents (1.2 million annual deaths); physical inactivity (1.9 million deaths); and indoor air pollution (1.5 million annual deaths).

3. The health impacts of climate change are felt unequally: effective response requires global action

Whether it’s the 70,000 excess deaths from the heat wave in Europe in 2003, or new malarial deaths in the central African highlands, the people at greatest risk for climate-related health disorders and premature deaths are the poor, the geographically vulnerable, the very young, women and the elderly. The populations considered to be at greatest risk are those living in...
small island developing states, mountainous regions, water-stressed areas, megacities and coastal areas in developing countries (particularly the large urban agglomerations in delta regions in Asia), and also poor people and those lacking access to health services.

Putting these three health arguments at the center of discussions at the forthcoming Conference of the Parties (COP-15) in Copenhagen later this year would ensure that in the new post-Kyoto agreement we will all share in the health and economic benefits that can accrue from countering climate change.

WHO will work to achieve four objectives: raising global awareness of these health arguments; making the health case for strong greenhouse gas reductions (mitigation) in all sectors (e.g., transport, housing, energy, agriculture) at national, regional and international levels; promoting and supporting the generation of scientific evidence; and strengthening health systems to cope with the health threat posed by climate change, including emergencies related to extreme weather events and sea-level rise.

WHO’s member States have highlighted the importance of action to protect health from climate change. Countries have asked WHO to step up support for national and international efforts assess and address the implications of climate change for health and health systems.

For more information contact: Nada OSSEIRAN; Advocacy & Communications Officer, Public Health & Environment Dept. World Health Organization, Tel: +4122 7914475; Email osseirann@who.int

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**Erratum**


**Surveillance of Healthcare-Associated Infections in Adult Patients with Leukemia in Kuwait Cancer Control Center**

Abeer Aly Omar¹, Haifaa Al-Mousa²

¹Infection Control Office, Kuwait Cancer Control Center (KCCC), Ministry of Health, Kuwait
²Infection Control Directorate, Ministry of Health, Kuwait

The above paper contained an error in Fig. 1 legends which is corrected as below. The Publisher regrets the error.

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**Fig. 1:** Susceptibilities of the isolated Gram-negative bacteria to ciprofloxacin. The only sensitive strain of *E. coli* was isolated from SST infection.