Umm Al-Qura University Medical Journal

A Biannual Peer-reviewd Research Journal

Massage from Editor-in-Chief

Includes
ORIGINAL SCIENTIFIC ARTICLE

Review
Rheumatoid Arthritis and Vitamin D

Brief Communication
Accreditation Is It a Fashion or Necessity?

Case Report
Congenital Epulis

Volume: 2 Number: 4 July 2011 ISSN 1658-4732

CONTACT

Post:
Editor
UQU Medical Journal
Faculty of Medicine
Umm al Qura University
PO Box: 7607
MAKKAH
Kingdom of Saudi Arabia

Website:
www.uqumedicalju.com

E-mail:
mj@uqu.edu.sa

UQU
Medical Journal

Volume:2 Number: 4 (July 2011)

Editorial Policy
The UQU Medical Journal publishes original material of interest to the healthcare practitioners and scientists in the broad field of medicine. Articles describing original clinical or laboratory investigations and case reports will be considered for publication. From time to time invited articles, editorials and review of selected topics will be published. Manuscripts, including illustrations and tables must be original and not under consideration by another publication.

The UQU Medical Journal has agreed to receive manuscripts in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals," as cited in N. Engl. J. Med., 1997, 336:309-15. In preparing manuscripts, authors should follow the "Uniform Requirements for Manuscript Submitted to Biomedical Journals" and specific author instructions by the International Committee of Medical Journal Editors. *The Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication, Updated October 2008, can be obtained from the website* http://www.icmje.org

UQU Med J

UQU Medical Journal

Vol. 2 No. 4

July 2011

The Umm al Qura University Medical Journal (UQU Medical Journal) published in print and electronic editions is the official publication of the Faculties of Medicine at the Umm al Qura University, Makkah, Kingdom of Saudi Arabia

Print Edition

ISSN 1658 - 4732

Electronic Edition

ISSN 1658 - 4740

The preferable mode of submission of manuscripts is online via the Journal's online submission and review system on the website: www.uqumedicalju.com

Printed and published by the Umm al Qura University Press, Makkah, Kingdom of Saudi Arabia

EDITORIAL TEAM OF THE UQU MEDICAL JOURNAL

Contents

Vol: 2 No: 4 July 2011

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 61-62 (2011)
07-012 © The University Press
UQU Medical Journal

Message from the editor-in-Chief

Professor; Tarik M Malatani

Peer review i.e. asking peers of the authors of scientific studies to review the studies critically before publication-is the process that is supposed to ensure the scientific quality of journals. It is a sacred process-and the phrase `peer reviewed journal' is supposed to guarantee quality. But clearly peer review is deficient. Despite being central to the scientific process it was itself largely unstudied until various pioneers-including Stephen Lock, former editor of the BMJ, and Drummond Rennie, deputy editor of JAMA-urged that it could and should be studied. Studies so far have shown that it is slow, expensive, ineffective, something of a lottery, prone to bias and abuse, and hopeless at spotting errors and fraud.

The benefits of peer review have been much harder to establish. As Rennie says, 'If it was a drug it would never get onto the market'. Nevertheless, no journal would dare to abandon peer review. Editors are convinced-even though they are finding it had to prove-that peer review is invaluable.

Yet the reputation of the Journal is dependent on the contributions of authors. But the standard of the journal, the level of excellence, is also a consequence of the efforts of expert reviewers who evaluate submitted manuscripts and determine their appropriateness for the Umm Al-qura University Medical Journal. In addition to expressing gratitude to our Editorial Board members, we are indebted to our ad hoc reviewers for the thoughtful comments that are integral to the eventual end product and without their timely and prompt help it would not have been possible for this dream to come true. They are:

Dr. Nawab Mohammad Khan (USA)
Dr. Fazal Karim Aasi (USA)
Dr. Khalid Zawawi (KSA)
Dr. Maher Abdulkareem (KSA)
Dr. Mohammed Mere'ani (KSA)
Dr. Nasser Elhawary (KSA)
Dr. Ghassan Nouman (KSA)
Dr. Syed Tabrez Ali (KSA)
Dr. Fhad Raees Ahmed (KSA)
Dr. Essam Jiffri (KSA)
Dr. Suzan Attar (KSA)
Dr. Osama Bawazir (KSA)
Dr. Alaa Sultan (KSA)

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 63-77 (2011)
07-012 © The University Press

UQU Medical Journal

Review

Rheumatoid Arthritis and Vitamin D: A review

Firas Sultan Azzeh.,* Amany Mokhtar **

(1) Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia (2) Department Of Community, Environmental, And Occupational Medicine, Faculty Of Medicine Ain Shams University , Cairo , Egypt*

Correspondence:
Dr. Firas Sultan Azzeh
Department of Clinical Nutrition, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia, P.O. Box: 7067
Fax: 0096625720000-4238
E-mail: fsazzeh@uqu.edu.sa

Received: September 30, 2011 *Accepted: October 20, 2011*

التهاب المفاصل الروماتيزمي وفيتامين د: مراجعة

د. فراس سلطان العزة [1]*., د. و أماني مختار[2]**

(1) قسم التغذية الإكلينيكية، كلية العلوم الطبية التطبيقية، جامعة أم القرى، مكة المكرمة، المملكة العربية السعودية*
(2) قسم طب المجتمع و البيئة وطب الصناعات، كلية الطب ،جامعة عين شمس ، القاهرة ، جمهورية مصر العربية

الملخص العربي

التهاب المفاصل الروماتيزمي هو مرض التهابي مـ زمن يجعـ ل الغشـ اء الزليلـ ي فـ ي المفاصـ ل ملتهبـ ا، ممـ ا يتسـ ببب فـ ي انتفـ اخ المفصل و يجعله صلبا وبه الم ويحد حركته مما يسيء حركة المفاصل ويتسبب في تشوهه وإعاقته.

يسمى فيتامين د "بفيتامين أشعة الشمس"لحيث يتحول في الجسم إلـ ى وهـ ون 25ثلـ -ائي هيدروكسـ ي فيتـ امين دلفعـ ل التـ أثير الضـ وئي للأشـ عة فـ وق البنفسـ جيوتيلـ ى فيتـ امين د دورا هامـ ا، جنب ا إلـ ى جنـ ب مـ ع عنصـ ري الكالسـ يوم والفسفور، في المحافظة على صحة العظام والأسنان.

حـ ت بعـ ض الدراسـ ات المسـ تقبلية انـ ه يوجـ د علاقـ ة عكسـ اوبلـ فيتـ يزامين د ومـ رض التهـ اب المفاصـ ل الروماتيزمي على الرغم من أن فيتامين د له دور مؤثر في تخفيض حدة أمراض المناعة الذاتيـ ة مثـ ل مـ رض الـ وع الأول لمرض السـ كري و مـ رض تصـ لب الأنسـ جة المتعـ دد، إلا أن دوره فـ ي تخفـ يض حـ دة التهـ اب المفاصـ ل الروماتيزمي لا يـ زال قضية جدل. تم كتابة هذه المراجعة العلمية لتوضيح العلاقة المحتملة بين مـ رض التهـ اب المفاصـ ل الروماتيزمي و فيتـ امين د.

ABSTRACT

Rheumatoid arthritis is a chronic inflammatory disease in which the synovial membrane of the joint becomes inflamed, resulting in a swelling, stiffness, pain, limited range of motion, joint deformity and disability.

Vitamin D is the "sunshine vitamin" which is converted in the body to a hormone 1, 25-dihydroxyvitamin D_3 by the photolytic action of ultraviolet light on the skin. Vitamin D plays an important role, along with the essential minerals calcium and phosphorus, in the maintenance of healthy bones and teeth.

An inverse association between vitamin D intake and rheumatoid arthritis was found in some prospective studies. Although vitamin D has been implicated in a decreased risk of autoimmune diseases such as type 1 diabetes and multiple sclerosis, its role in decreasing the risk of rheumatoid arthritis remains equivocal. This scientific review is written to illustrate the possible relationships between rheumatoid arthritis and vitamin D.

***Keywords*:** Rheumatoid arthritis**,** Vitamin D, Vitamin D Receptors (VDR)

INTRODUCTION

Arthritic conditions encompass more than 100 different diseases and conditions affecting the joints, the tissues surrounding the joints and the connective tissue. Arthritic conditions are among the most common diseases in the world and include osteoarthritis, rheumatoid arthritis (RA) and gout.[1] Arthritic conditions affect nearly one in sex North Americans and are the leading cause of disability among Americans 18 years of age and older,[2] and about 22% of U.S. adults have arthritis.[3]

Common misconceptions about arthritic conditions are they only affect older persons, that they are an inevitable consequence of aging, they are diagnosed in people of all ages, including children and teens.[4] Juvenile RA affects 70.000 to 100.000 children in US and is one of the most common chronic conditions of childhood.[1] There are several factors known to increase the risk of arthritic conditions, three of which are modifiable: overweight, joint injuries and infections. Non-modifiable risk factors include female sex, age and family history.[1]

RA is an autoimmune disease, in which a person's immune system attacks his or her own healthy tissues.[4] RA is the most common inflammatory arthritis across the world. Although the etiology of RA remains a mystery, a variety of studies suggest that a blend of environmental and genetic factors are responsible, and both affecting the prevalence of autoimmune disease.[5]

Vitamin D is the "sunshine vitamin" which is converted in the body to a hormone 1, 25-dihydroxyvitamin D_3 by the photolytic action of ultraviolet light on the skin. Vitamin D plays an important role, along with the essential minerals calcium and phosphorus, in the maintenance of healthy bones and teeth.[6] Moreover vitamin D sufficiency, especially during the childhood and adolescent years, is critically important not only for bone health, but also for the prevention of many serious chronic diseases, including cancer, cardiovascular heart disease, and autoimmune diseases. It has been suggested that vitamin D deficiency during

infancy and childhood may imprint an increased risk of these chronic diseases for the rest of one's life.[7]

An inverse association between vitamin D intake and RA was found in the prospective cohort study done by the Iowa Women's Health Study.[8] Vitamin D is a hormone essential for bone and mineral homeostasis and is also involved in the regulation of cells in the innate and adaptive immune system through the vitamin D receptor (VDR) as a suppressor of pro-inflammatory responses.[9] Although vitamin D has been implicated in a decreased risk of autoimmune diseases such as type 1 diabetes and multiple sclerosis, its role in decreasing the risk of RA remains equivocal.[10,11] The aim of this review is to summarize the currently available information for the effect of vitamin D on RA disease.

2. Rheumatoid Arthritis

2.1. Introduction

The name (RA) is based on the term "rheumatic fever", an illness which includes joint pain and is derived from the Greek word *rheumatos* ("flowing"). The suffix *-oid* ("resembling") gives the translation as *joint inflammation that resembles rheumatic fever*. The first recognized description of rheumatoid arthritis was made in 1800 by Dr. Augustin Jacob Landré-Beauvais (1772-1840) of Paris.[12]

Rheumatoid arthritis is a chronic inflammatory disease in which the synovial membrane of the joint becomes inflamed, resulting in a swelling, stiffness, pain, limited range of motion, joint deformity and disability.[13] RA affects approximately 0.8% of the population, is more common in older persons, and affects female three times more often than males.[14]

Although RA primarily affects the joints, it also can affect other tissues, resulting in anorexia, weight loss, fatigue, general itching and stiffness.[4] Extra-articular ("outside the joints") manifestations occur in about 15% of individuals with rheumatoid arthritis. It can be difficult to determine whether disease manifestations are directly caused by the rheumatoid process itself, or from side effects of the medications commonly used to treat it - for example, lung fibrosis from methotrexate, or osteoporosis from corticosteroids.[15]

2.2. Pathophysiology

Rheumatoid arthritis is an autoimmune disease, the cause for which is still unknown. It is a systemic (whole body) disorder principally affecting synovial joints. As shown in Figure.1, a normal joint (left) and a joint affected by rheumatoid arthritis (right) are compared. Early changes shown on the left side of the affected joint include inflammation and thickening of the synovial membrane. Late changes shown on the right side of the affected joint include development of pannus, erosion of articular cartilage and bone, and filling of the joint space by pannus.[4]

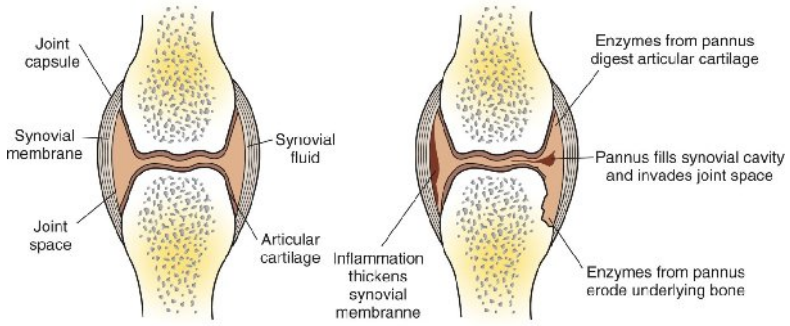

Figure 1. Pathophysiology of RA
(A normal joint at left and a joint affected by rheumatoid arthritis right)

As with most autoimmune diseases, it is important to distinguish between the causes that trigger the inflammatory process, and those that permit it to persist and progress. Chemical mediators (Cytokines) give rise to inflammation of joint synovium. Constitutional symptoms such as fever, malaise, loss of appetite and weight loss are also due to cytokines released in to the blood stream. Blood vessel inflammation (vasculitis) affecting many other organ systems can give rise to systemic complications.[16]

It has long been suspected that certain infections could be triggers for this disease. The "mistaken identity" theory suggests that an infection triggers an immune response, leaving behind antibodies that should be specific to that organism. The antibodies are not sufficiently specific, though, and set off an immune attack against part of the host. Because the normal host molecule "looks like" a molecule on the offending organism that triggered the initial immune reaction - this phenomenon is called molecular mimicry. Some infectious organisms suspected of triggering rheumatoid arthritis include *Mycoplasma*, *Erysipelothrix*, parvovirus B19 and rubella, but these associations have never been supported in epidemiological studies. Nor has convincing evidence been presented for other types of triggers such as food allergies. There is also no clear evidence that physical and emotional effects, stress and improper diet could be a trigger for the disease. The many negative findings suggest that either the trigger varies, or that it might in fact be a chance event.[17]

Epidemiological studies have confirmed a potential association between RA and two herpesvirus infections: Epstein-Barr virus (EBV) and Human Herpes Virus 6 (HHV-6).[18] Individuals with RA are more likely to exhibit an abnormal immune response to the Epstein-Barr virus.[19,20] The allele HLA-DRB1*0404 is associated with low frequencies of T cells specific for the EBV glycoprotein 110 and predisposes one to develop RA.[21]

The factors that allow the inflammation, once initiated, to become permanent and chronic, are much more clearly understood. The genetic association with HLA-DR4 is believed to play a major role in this, as well as the newly discovered associations with the gene PTPN22 and with two additional genes.[22] all involved in regulating immune responses. It has also become clear from recent studies that these genetic factors may interact with the most clearly defined environmental risk factor for rheumatoid arthritis, namely cigarette smoking.[23]

2.3. Diagnosis

When RA is being clinically suspected, immunological studies are required, such as rheumatoid factor (RF, a specific antibody).[24] A negative RF does not rule out RA; rather, the arthritis is called *seronegative*. During the first year of illness, RF is frequently negative. 80% of individuals eventually convert to seropositive status. RF is also seen in other illnesses, like Sjögren's syndrome, and in approximately 10% of the healthy population, therefore the test is not very specific.[24]

Because of this low specificity, a new serological test has been developed in recent years, which tests for the presence of so called anti-citrullinated protein antibodies (ACPA). [25] Like

RF, this test can detect approximately 80% of all RA cases, but is rarely positive if RA is not present, giving it a specificity of around 98%. In addition, ACP antibodies sometimes can be detected in early stages of the disease, or even before onset of clinical disease. Currently, the most common test for ACP antibodies is the anti-CCP (cyclic citrullinated peptide) test.[25] Also, several other blood tests are usually done to discriminate for other causes of arthritis, such as lupus erythematosus. The erythrocyte sedimentation rate (ESR), C-reactive protein, full blood count, renal function, liver enzymes and other immunological tests (e.g. antinuclear antibody/ANA).[26]

Disease activity can be assessed according to the Disease Activity Score including 28 joint counts (DAS28). Three components of DAS28 test are included: erythrocyte sedimentation rate, patient-assessed global score (0–100), and swollen and tender joint counts (both 0–28). High activity of the disease will be defined as a DAS28 > 5.1, moderate activity of disease will be defined as a 3.2 < DAS28 < 5.1, and low activity of disease will be defined as a DAS28 < 3.2.[5]

2.4. Classification criteria

The American College of Rheumatology (ACR) in 1987 (ACR; formerly, the American Rheumatism Association [ARA]) has defined the following criteria for the classification of rheumatoid arthritis:

- Morning stiffness of more than 1 hour most mornings for at least 6 weeks.
- Arthritis and soft-tissue swelling of >3 of 14 joints/joint groups, present for at least 6 weeks
- Arthritis of hand joints, present for at least 6 weeks
- Symmetric arthritis, present for at least 6 weeks
- Subcutaneous nodules in specific places
- Rheumatoid factor at a level above the 95th percentile
- Radiological changes suggestive of joint erosion

At least four criteria have to be met for classification as RA.[27]
A joint working group from the ACR and the European League Against Rheumatism (ACR/EULAR) developed a new approach to classifying RA. The work focused on identifying, among patients newly presenting with undifferentiated inflammatory synovitis, factors that best discriminated between those who were and those who were not at high risk for persistent and/or erosive disease. This new classification system redefines the current paradigm of RA by focusing on features at earlier stages of disease that are associated with persistent and/or erosive disease, rather than defining the disease by its late-stage features.[28]
In the new criteria set as shown in Table. 1, classification as “definite RA” is based on the confirmed presence of synovitis in at least 1 joint, absence of an alternative diagnosis that better explains the synovitis, and achievement of a total score of 6 or greater (of a possible 10) from the individual scores in 4 domains: number and site of involved joints (score range 0–5), serologic abnormality (score range 0–3), elevated acute-phase response (score range 0–1), and symptom duration (2 levels; range 0–1).[28]

Table 1. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis.[28]

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)	
2) with the synovitis not better explained by another disease	
Classification criteria for RA (score-base algorithm: add score categories (A-D); a score of ≥ 6/10 is needed for classification of a patient as having definite RA)	
A. Joint involvement	
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)	2
4-10 small joints (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	4
B. Serology (at least 1 test result is needed for classification)	5
Negative RF *and* Negative ACPA	
Low-positive RF *or* low-positive ACPA	0
High-positive RF *or* high-positive ACPA	2
C. Acute-phase reactants (at least 1 test result is needed for classification)	3
Normal CRP *and* normal ESR	
Abnormal CRP *or* abnormal ESR	0
D. Duration of symptoms	1
< 6 weeks	
≥ 6 weeks	0
	1

2.5. Treatment

There is no known cure for rheumatoid arthritis. However, many different types of treatment can be used to alleviate symptoms and/or to modify the disease process. The goal of treatment in this chronic disease must be for dual purposes: to alleviate the current symptoms, and to prevent the future destruction of the joints and resulting handicap if the disease is left unchecked. These two goals may not always coincide, while pain relievers may achieve the first goal, they do not have any impact on the long-term consequences. For these reasons, most authorities believe that most RA should be treated by at least one specific anti-rheumatic medication to which other medications and non-medical interventions can be added as needed.[29]

2.5.1. Pharmacological treatment

Pharmacological treatment of RA can be divided into disease-modifying antirheumatic drugs (DMARDs), anti-inflammatory agents and analgesics. Anti-inflammatories and analgesics improve pain and stiffness but do not prevent joint damage or slow the disease progression. Anti-inflammatory agents used in RA treatment include glucocorticoids and non-steroidal anti-inflammatory drug (NSAIDs), and analgesics include acetaminophen, opiates, diproqualone and lidocaine topical.[30]

DMARDs have been found to produce durable remissions and delay or halt disease progression. In particular they prevent bone and joint damage from occurring secondary to the uncontrolled inflammation. This is important as such damage is usually irreversible. [29]
There may be other reasons why starting DMARDs early is beneficial as well as prevention of structural joint damage. In the early stage of the disease, the joints are increasingly infiltrated by cells of the immune system that signal to one another and are thought to set up self-perpetuating chronic inflammation. Interrupting this process as early as possible with an effective DMARD (such as methotrexate) appears to improve the outcome of RA for years afterwards. Delaying therapy for as little as few months after the onset of symptoms can result in worse outcomes in the long term. There is therefore considerable interest in establishing the most effective therapy with early arthritis, when they are most responsive to therapy and have the most to gain.[31]

2.5.2. Dietary treatment

There is evidence that lower intakes of vegetables, fruits and dietary sources of vitamin C are associated with increased risk of developing RA.[32] However, a research by Canter and his colleague at 2007 which they concluded that there is presently no convincing evidence that selenium, vitamin A, or vitamin C are effective in the treatment of any type of arthritis.[33] Other researchers have been observed that there is a lower risk of developing RA in countries such as Italy and Greece, where oil-rich fish, olive oil, vegetables and fruits are consumed in greater amounts compared to other countries have a higher risk of developing RA.[32]

Omega-3 fatty acids (eicosapentaenoic acid; EPA, and docosahexaenoic acid; DHA) consumption from fish oil and other sources reduce the synthesis of chemicals known to stimulate joints inflammation and cartilage degradation with lesser need to take NSAIDs to relieve pain.[34,35] On the other hand, a diet low in arachidonic acid (omega-6 fatty acids) ameliorates clinical signs of inflammation in patients with RA.[36] Gamma-linolenic acid (GLA) is an omega-6 fatty acid found in oils of some plant seeds and cow milk. There is some preliminary evidence conducted by Little and Parsons in 2000 that GLA may be beneficial for RA. In the body, GLA can be converted into substances that reduce inflammation. [37]

Research on other supplements and herbs for RA symptoms is still in the early stages. Thunder god vine (*Tripterygium wilfordii*) has been used for centuries in traditional Chinese medicine. Extracts are prepared from the skinned root of the herb, as other parts of the plant are highly poisonous.[38] Findings from laboratory and animal studies suggest that thunder god vine may fight inflammation and suppress the immune system. In small clinical trials involving people with RA thunder god vine extracts appeared to provide some relief of symptoms.[39] Thunder god vine can cause severe side effects that can cause diarrhea, stomach upset, hair loss, headache, and skin rash. The herb can also affect the reproductive system, possibly causing menstrual changes in women and infertility in men. Long-term use of thunder god vine may decrease bone mineral density in women, potentially increasing the risk of osteoporosis.[38] In animal studies, extracts of turmeric (*Curcuma longa*) containing the chemical curcumin were found to protect joints from inflammation and damage.[40] Laboratory studies have identified anti-inflammatory compounds in ginger (*Zingiber officinale*) which is gingerol. It has been shown that gingerol is effective against cytokines synthesized and secreted at sites of inflammation. [41]

Citrus fruits, chocolate, alcohol, red meats, flour products, spices and fizzy drinks are often implicated in the aggravation of RA symptoms.[42] Previous investigations have found that a period of fasting followed by a regimented vegetarian diet can decrease RA disease activity. This led to investigations into whether protein and red meat intake play a role in increasing risk of developing RA.[43]

3. Vitamin D

Vitamin D deficiency has been a long-standing public health issue. This condition was first described in association with skeletal deformities by Glisson and his group during the mid-17th century in London, England. Despite numerous preventive strategies, vitamin D deficiency has remained a global health problem among children.[44]

The National Institution of Health (NIH) reported that serum concentration of 25-Hydroxyvitamin D3 [25 (OH) D_3] is the best indicator of the vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half life of 15 days. Different concentrations of serum 25 (OH) D_3 are associated with deficiency, adequacy for bone health, and optimal overall health Table. 2.[45] Several factors potentially affect vitamin D status; these include genetic factors, adiposity and factors affecting the cutaneous synthesis of vitamin D such as skin pigmentation, age, season, latitude, melanin concentration, clothing and use of sunscreens.[46]

Table 2. Serum 25-Hydroxyvitamin D [25(OH) D] concentration and health by NIH (2008) *

ng/ml**	nmol/L**	Health status
<11	<27.5	Associated with vitamin D deficiency and rickets in infant and young children.
<10-15	<25-37.5	Generally considered inadequate for bone and overall health in healthy individuals.
≥30	≥75	Proposed by some as desirable for overall health and disease prevention.
Consistently >200	Consistently >500	Considered potentially toxic, leading to hypercalcemia and hyperphosphatemia.

*Serum concentration of 25 (OH) D are reported in both nanograms per milliliter (ng/ml) and nanomoles per liter (nmol/L)**1 ng/ml = 2.5 nmol/L

It is clear that both genetic and environmental factors affect the prevalence of autoimmune diseases. Therefore, the fact that vitamin D has been implicated as a factor in several different

autoimmune diseases suggests that vitamin D might be one of the environmental factors that among others normally participates in the control of self-tolerance.[47] It has been found that polymorphisms in the Vitamin D Receptors (VDR) have been correlated with increased susceptibility of RA.[48,49]

4. Rheumatoid Arthritis and Vitamin D

The discovery of VDR in the cells of the immune system and the fact that activated dendritic cells produce vitamin D hormone suggested that vitamin D could have immunoregulatory properties.[50] VDR, a member of the nuclear hormone receptor super family, was identified in mononuclear cells, dendritic cells, antigen-presenting cells, and activated T-B lymphocytes.[47]

A physiological role for vitamin D in the immune system is supported by the presence of the VDR in primary lymphoid organs. The primary lymphoid organs (bone marrow and thymus) are the centers where the immune system develops and differentiates.[51,52] As a matter of fact, both genetic and environmental factors contribute to the etiology of autoimmnune diseases. T cells (lymphocytes that differentiate in the thymus) have been shown to play fundamental roles in autoimmune diseases. Quiescent CD4+ T cells express VDRs at low concentrations, which increases five-fold after their activation.[53] The effects of vitamin D on the acquired, antigen-specific immune response, are characterized by inhibition of T-lymphocyte proliferation.[54] particularly of the Th1 arm (Th 1 is a subset of the T helper cells that secretes cytokines).[55] Treatment of CD4 T cells with vitamin D inhibits Th1 cell proliferation and cytokine production.[56,57]

Addition of vitamin D was shown also to inhibit the expression of the Interleukin-6 (IL-6). IL-6 is an interleukin that acts as both a pro-inflammatory and anti-inflammatory cytokine.[59] Interestingly, in B cells vitamin D has been shown to inhibit antibody secretion and autoantibody production.[60] In vitro, vitamin D inhibits the differentiation of monocytes into dendritic cells and interferes with the stimulatory activity that T cells exert on them.[61] It has been shown that vitamin D is one of the most efficient blockers of dendritic cell differentiation and of interleukin secretion. In vitro vitamin D stimulates phagocytosis and killing of bacteria by macrophages but suppresses the antigen-presenting capacity of these cells and of dendritic cells.[62] Vitamin D has been found to promote the induction of monocytic differentiation to macrophages and modulate macrophage responses, preventing them from releasing inflammatory cytokines and chemokines.[63]

It has been observed that greater intake of vitamin D was associated with a lower risk of RA, as well as lower vitamin D was found associated with higher disease activity.[8] Since lower vitamin D serum levels have been also associated with higher RA disease activity, in a recent study were evaluated serum 25(OH)D3 levels in 64 female RA patients from north Europe (Estonia) and 54 RA patients from south Europe (Italy) during winter and summer and were correlated with the disease activity score (DAS28).[64]

In addition, there may be a higher vitamin D requirement for patients at risk for developing autoimmunity and for those that already have an autoimmune disease such as systemic lupus erythematosus.[65] In fact, the optimal amount of vitamin D to support the immune response may be different from the amount required to prevent vitamin D deficiency or to maintain calcium homeostasis.[66] One review suggest that the optimal plasma 25(OH)D3 concentration lies between 50–80 nmoles/L, other experts suggesting between 75–125 nmol/L.[67]

CONCLUSIONS

Serum vitamin D levels have been found to correlate inversely with the RA disease activity. Greater intake of vitamin D was associated with a lower risk of RA, as well as a significant clinical improvement was strongly correlated with the immunomodulating potential in vitamin D-treated RA patients.

.

REFERENCES

1. Rizzo D. Disorders of skeletal function: rheumatic disorders. In: Porth C, Editor. Pathophysiology: Concepts of Altered Health Status, 7th edition. PA: Lippincott Williams & Wilkins, 2005. 1417-1440.

2. U.S. Department of Health and Human Services. Prevalence of Disabilities and associated health conditions among adults – United States, 1999. MMWR Morb Mortal Wkly Report 2001; 50: 120-125.

3. American College of Rheumatology (ACR), 2010. Retrieved from http://hosted.ap.org/dynamic/stories/U/US_MED_ARTHRITIS_TOLL?SITE=OHRAV&SECTION=HOME&TEMPLATE=DEFAULT.

4. Lee R. Diseases of musculoskeletal system. In: Nelms M, Sucher K, and long S, Editors. Nutrition Therapy and Pathophysiology, 1st edition. Belmont (USA): Thomson Brooks/Cole, 2007. 843-879.

5. Turhanoglu A, Guler H, Yonden Z, Aslan F, Mansurolgu A, Ozer C. The relationship between vitamin D and disease activity and functional health status in rheumatoid arthritis. Rhematology International 2010; 1: 1393-1396.

6. Combs GF. The Vitamins: Fundamental Aspects in Nutrition and Health, 2nd edition. New York: Academic Press, 1998. 155-182.

7. Holick MF. Vitamin D. In: Shils ME, Editor. Modern Nutrition in Health and Disease, 10th edition. Philadelphia: Libbincott Williams and Wilkins, 2006. 377-393

8. Merlino LA, Curtis J, Mikuls TR, et al. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheumatoid 2004; 50: 72–77.

9. Mathieu C, Van Etten E, Gysemans C, et al. In vitro and in vivo analysis of the immune system of vitamin D receptor knockout mice. Journal of Bone Mineral Research 2001; 16: 2057–2065.

10. Hypponen E, Laara E, Reunanen A, et al. Intake of vitamin D and risk of type 1 diabetes: a birth cohort study. Lancet 2001; 358: 1500–1503.

11. Holick MF. Vitamin D deficiency. New England Journal of Medicine 2007; 357: 266–281.

12. Landré-Beauvais A. The first description of rheumatoid arthritis. Unabridged text of the doctoral dissertation presented in 1800. Joint Bone Spine 2001; 68(2): 130–43.

13. Lipsky P. Rheumatoid arthritis. In: Kasper, D; Braunwald, E; Fauci, A; Hausner, S; Longo, D; and Jameson, J, Editors. Harrison's Principles of Internal Medicine, 16th edition. New York: McGraw-Hill, 2005. 2268-2278.

14. Pattison D, Symmons D, Young A. Does diet have a role in the etiology of rheumatoid arthritis? Proceedings of the Nutrition Society 2004, 63: 137-143.

15. Turesson C, O'Fallon W, Crowson C, Gabriel S, Matteson E. "Extra-articular disease manifestations in rheumatoid arthritis: incidence trends and risk factors over 46 years". Annals of Rheumatic Diseases 2003; 62(8): 722–727.

16. Choy E, Panayi G. Cytokine pathways and joint inflammation in rheumatoid arthritis. The New England Journal of Medicine 2001; 344: 907–916.

17. Edwards J, Cambridge G, Abrahams V. Do self-perpetuating B lymphocytes drive human autoimmune disease? Immunology 1999; 97:188-196.

18. Álvarez-Lafuente R, Fernández-Gutiérrez B, de Miguel S, Jover J, Rollin R, Loza E, Clemente D, Lamas J. Potential relationship between herpes viruses and rheumatoid arthritis: analysis with quantitative real time polymerase chain reaction. Annals of the Rheumatic Diseases 2005; 64: 1357-1359.

19. Ferrell P, Aitcheson C, Pearson G, Tan E. Seroepidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. The Journal of Clinical Investigation 1981; 67(3): 681–687.

20. Catalano M, Carson D, Slovin S, Richman D, John Vaughan H. Antibodies to Epstein-Barr virus-determined antigens in normal subjects and in patients with seropositive rheumatoid arthritis. Proceedings of the National Academy of Sciences (USA), Immunology 1979; 76(11); 5825-5828.

21. Balandraud N, Roudier R. Epstein–Barr virus and rheumatoid arthritis. Autoimmunity Reviews 2004; 3(5): 362-367.

22. Plenge RM., Seielstad M, Padyukov L, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis--a genome wide study. The New England Journal of Medicine 2007; 357: 1199-209.

23. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheumatoid 2004; 50: 3085-3092.

24. American Association for Clinical Chemistry, (September 30, 2006). Lab Tests Online for Rheumatoid Factor (RF). Retrieved from: http://www.labtestsonline.org/understanding/analytes/rheumatoid/test.html.

25. American Association for Clinical Chemistry, (January 15, 2005). Lab Tests Online for Cyclic Citrullinated Peptide antibody (CCP). Retrieved from: http://www.labtestsonline.org/understanding/analytes/ccp/test.html

26. American Association for Clinical Chemistry, (December 13, 2004). Lab Tests Online for Antinuclear Antibody (ANA). Retrieved from: http://www.labtestsonline.org/understanding/analytes/ana/test.html.

27. Arnett F, Edworthy S, Bloch D, McShane D, Fries J, Cooper N, Healey L, Kaplan S, Liang M, Luthra H. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheumatoid 1988; 31(3): 315-324.

28. Aletaha D, Neogi T, Silman A, et al. 2010 Rheumatoid Arthritis Classification Criteria. An American College of Rheumatology/European League Against Rheumatism Collaborative Initiative. Arthritis & Rheumatism 2010; 62(9): 2569–2581.

29. O'Dell J. Therapeutic strategies for rheumatoid arthritis. The New England Journal of Medicine 2004; 350(25): 2591-602.

30. Hasler P. Biological therapies directed against cells in autoimmune disease. Springer Seminars in Immunopathology 2006; 27(4): 443-456.

31. Vital E, Emery P. Advances in the treatment of early rheumatoid arthritis. American Family Physician 2005; 72(6): 1002-1004.

32. Rennie K, Hughes J, Jebb, S. Nutritional management of rheumatoid arthritis: a review of the evidence. Journal of Human Nutrition and Dietitian 2003; 16: 97-109.

33. Canter P, Wider B, Ernst E. The antioxidant vitamins A, C, E and selenium in the treatment of arthritis: a systematic review of randomized clinical trials. Rheumatology 2007; 46:1223–1233.

34. Volker D, Fitzgerald P, Major G, Garg, M. Efficacy of fish oil concentrate in the treatment of rheumatoid arthritis. Journal of Rheumatology 2000; 27: 2343-2346.

35. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. Drugs 2003; 63(9): 845–853.

36. Adam O, Beringer C, Kless T, et al. Anti-inflammatory effects of low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. Rheumatology International 2003; 23: 27-36.

37. Little C, Parsons T. Herbal therapy for treating rheumatoid arthritis. Cochrane Database of Systematic; Reviews 2000; 4: CD002948.

38. Tao X, Younger J, Fan FZ, et al. Benefit of an extract of Tripterygium wilfordii Hook F in patients with rheumatoid arthritis: a double-blind, placebo-controlled study. Arthritis & Rheumatism 2002; 46(7): 1735–1743.

39. Canter PH, Lee HS, Ernst EA. Systematic review of randomised clinical trials of Tripterygium wilfordii for rheumatoid arthritis. Phytomedicine 2006; 13(5): 371–377.

40. Funk JL, Frye JB, Oyarzo JN, et al. Efficacy and mechanism of action of turmeric supplements in the treatment of experimental arthritis. Arthritis & Rheumatism 2006; 54(11): 3452–3464.

41. Lantz RC, Chen GJ, Sarihan M, et al. The effect of extracts from ginger rhizome on inflammatory mediator production. Phytomedicine 2007; 14(2–3): 123–128.

42. Martin R. The role of nutrition and diet in rheumatoid arthritis. Proceedings of the Nutrition Society 1998; 57: 231-234.

43. Liao K, Alfredsson L, Karlson E. Environmental influences on risk for rheumatoid arthritis. Current Opinion in Rheumatology 2009; 21(3): 279–283.

44. Leanne MW, Gaboury I, Ladhani M, Zlotkin S. Vitamin D deficiency rickets among children in Canada. Canadian Medical Association Journal 2007; 177(2), 161-166.

45. National Institutes of Health (NIH). Dietary Supplement Fact Sheet: Vitamin D. Maryland, USA. 2008.

46. Scientific Advisory Committee on nutrition (SACN). Update on Vitamin D. London. 2007.
47. Arnson Y, Amital H, Shoenfeld Y. Vitamin D and autoimmunity: new etiological and therapeutical considerations. Annals of the Rheumatic Diseases 2007; 66: 1137–1142.

48. Garcia-Lozano JR, Gonzalez-Escribano MF, Valenzuela A, et al. Association of vitamin D receptor genotypes with early onset rheumatoid arthritis. European Journal of Immunogenetics 2001; 28: 89–93.

49. Maalej A, Petit-Teixeira E, Michou L, et al. Association study of VDR gene with rheumatoid arthritis in the French population. Genes and Immunity 2005; 6:707–711.

50. Fritsche J, Mondal K, Ehrnsperger A, et al. Regulation of 25- hydroxyvitamin D3-1 alpha-hydroxylase and production of 1 alpha 25-dihydroxyvitamin D3 by human dendritic cells. Blood 2003; 102:3314–3316.

51. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB Journal 2001; 15: 2579–2585.

52. Langub MC, Reinhardt TA, Horst RL, et al. Characterization of vitamin D receptor immunoreactivity in human bone cells. Bone 2000; 27: 383–387.

53. Mahon BD, Wittke A, Weaver V, et al. The targets of vitamin D depend on the differentiation and activation status of CD4 positive T cells. Journal of Cellular Biochemistry 2003; 89: 922–932.

54. Lemire JM. Immunomodulatory role of 1,25-dihydroxyvitamin D3. Journal of Cellular Biochemistry 1992; 49: 26–31.

55. Mattner F, Smiroldo S, Galbiati F, et al. Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D(3). European Journal of Immunology 2000; 30: 498–508.

56. Boonstra A, Barrat FJ, Crain C, et al. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. Journal of Immunology 2001; 167: 4974–4980.

57. Van EE. and Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. The Journal of Steroid Biochemistry and Molecular Biology 2005; 97:93–101.

58. Staeva-Vieira TP, Freedman LP. 1,25-dihydroxyvitamin D3 inhibits IFNgamma and IL-4 levels during in vitro polarization of primary murine CD4+ T cells. Journal of Immunology 2002; 168: 1181–1189.

59. Stockinger B. Th17 cells: an orphan with influence 4. Immunology and Cell Biology 2007; 85: 83–84.

60. Linker-Israeli M, Elstner E, Klinenberg JR, et al. Vitamin D(3) and its synthetic analogs inhibit the spontaneous in vitro immunoglobulin production by SLE-derived PBMC. Clinical Immunology 2001; 99: 82–93.

61. Griffin MD, Lutz W, Phan VA, et al. Dendritic cell modulation by 1alpha,25 dihydroxyvitamin D3 and its analogs: a vitamin D receptor-dependent pathway that promotes a persistent state of immaturity in vitro and in vivo. The Proceedings of the National Academy of Sciences Online (US) 2001; 98: 6800–6805.

62. Griffin MD, Lutz WH, Phan VA, et al. Potent inhibition of dendritic cell differentiation and maturation by vitamin D analogs. Biochemical and Biophysical Research Communications 2000; 270: 701–708.

63. Helming L, Bose J, Ehrchen J, et al. 1alpha,25-Dihydroxyvitamin D3 is a potent suppressor of interferon gamma-mediated macrophage activation. Blood 2005; 106: 4351–4358.

64. Cutolo M, Otsa K, Laas K, et al. Circannual vitamin d serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe. Clinical and Experimental Rheumatology 2006; 24: 702–704.

65. Kamen DL, Cooper GS, Bouali H, et al. Vitamin D deficiency in systemic lupus erythematosus. Autoimmunity Reviews 2006; 5: 114–117.

66. Rejnmark L, Jorgensen ME, Pedersen MB, et al. Vitamin D insufficiency in Greenlanders on a westernized fare: ethnic differences in calcitropic hormones between Greenlanders and Danes. Calcified Tissue International 2004; 74: 255–263.

67. Chatfield SM, Brand C, Ebeling PR, et al. Vitamin D deficiency in general medical in patients in summer and winter. International Medicine Journal 2007; 37: 377–382.

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 78-93 (2011)
06-011 © The University Press

UQU Medical Journal

Original Article

Drug-Drug Interactions in Prescribing Practice: *The Challenge and Opportunity for Patient Safety*

Layla Borham[1], Mohammed Garout[2], Alaa Flemban[1], Safaa Al Sanousy[1]
Departments of Pharmacology and Toxicology1 and Community Medicine2, Faculty of Medicine, Umm Al Qura University, Makkah, Saudi Arabia

Correspondence:
Dr. Layla Borham, Pharmacology and Toxicology Department, Faculty of Medicine, Umm Al Qura University, Al Abdyah, Makkah, P.O. 6706, Saudi Arabia.
Tel.: + 966 503 567 843
Fax: + 96625593498
E-mail: borhaml@hotmail.com

Received: February 13, 2011 *Accepted: April 06.2011*

التفاعلات بين الأدوية في الوصفات الطبية التحدي والفرصة لسلامة المرضى

أ.د. ليلى برهام، د. محمد قاروت*، د. آلاء فلمبان، د. صفاء السنوسي

قسم علم الأدوية و السموم و قسم صحة المجتمع وطب الحجيج* بكلية الطب جامعة أم القرى مكة المكرمة – المملكة العربية السعودية. ص. ب. : 7607

الملخص العربي

أهداف البحث: هدفت الدراسة إلى التعرف على التفاعلات المحتملة بين الأدوية التي تحدث للمرضى في بعض المستشفيات السعودية المختارة في مدينة مكة المكرمة بالمملكة العربية السعودية.

وسائل و طرق البحث: تم اختيار ثلاثمائة مريض عشوائياً من المستشفيات التعليمية بمدينة مكة المكرمة. تم تجميع بيانات المرضى وتضم الجنس، العمر، الجنسية، والأمراض المصاحبة لكل مريض. ولتحديد التفاعلات المحتملة بين الأدوية، تم تجميع وتحليل وصفات المرضى والتي تحتوي على دوائين أو أكثر وكذلك سجلاتهم الطبية في خلال فترة أربعة أشهر باستخدام برنامجي Micromedex® لفحص التفاعلات بين الأدوية وبرنامج لإدراج الوصفات الطبية بالمرضى في أثناء إقامتهم بالمستشفى وكذلك عند خروجهم. تم تصنيف التفاعلات بين الأدوية في هذه الدراسة بالكبرى والمعتدلة والخفيفة.

النتائج: وجد أن التفاعلات المحتملة بين الأدوية متكررة في الوصفات الطبية التي تحتوي على أدوية فالمرضى في هذه الدراسة معرضون لخطر إحتمال التفاعلات بين الأدوية خلال الإقامة في المستشفى أو بعد الخروج منها بنسبة 75% و45% على التوالي. وقد كان هناك ارتباط إيجابي بين التفاعلات المحتملة بين الأدوية وبين عدد الأدوية الموصوفة، وكانت الوصفات الطبية التي تحتوي على خمسة أدوية أو أقل والتي تحتوي على أكثر من خمسة أدوية بها تفاعلات محتملة بين الأدوية بنسبة 26.7% و 49% على التوالي. وحظ أن التفاعلات المحتملة بين الأدوية تزيد مع التقدم في العمر وكانت التفاعلات المحتملة بين الأدوية معظمها معتدلة (40%) كبرى (30.3%) بين المرضى البالغين، ، في حين أنها كانت معتدلة (50 ٪) بين المرضى كبار السن.

الخلاصة: إن البرامج المبرمجة على إكتشاف التفاعلات المحتملة بين الأدوية إلى جنب مع المعرفة والخبرة الدوائية، ومعرفة عوامل الخطورة الهامة ذات الصلة بالمريض لإضافة إلى التثقيف الوثيق بين الأطباء المعالجين والصيادلة السريريين، تكون ذات قيمة لخفض عدد التفاعلات الضارة المحتملة ومنع المخاطر المتصلة بالعلاج بالأدوية.

ABSTRACT

Aim:
To evaluate the prevalence of potential Drug-Drug Interactions (DDIs) that occur among patients in selected hospitals in Makkah city, Saudi Arabia.

Methods:
Three hundred (300) patients were randomly selected from two hospitals. Data collected included gender, age, nationality, and co-morbid diseases. To identify potential DDIs, patients' prescription forms with two or more drugs and their medical records were analyzed during a 4-month period. Prescriptions from each patient during hospitalization, and on discharge were included. DDIs were categorized as major, moderate and minor.

Results:
In our study, potential DDIs were found to be frequent among inpatients with multiple medication prescriptions. Around 75% and 45% of patients were exposed to drugs with the risk of potential interactions during hospital stay and on discharge, respectively. There was a positive correlation between total potential DDIs and number of drugs prescribed. Prescriptions with five drugs and less and those with more than five drugs produced a risk of DDIs in 26.7% and 49% of patients, respectively. As age increased, more DDIs were observed among the study population. DDIs were mostly moderate (40%) and major (30.3%) among adult patients, while it was moderate (50%) among senior age group in our study population.

Conclusions:
Computerized programs for detection of DDIs, combined with pharmacological expertise, knowledge of important patient-related risk factors, and close collaboration between treating physicians and clinical pharmacists may be valuable for decreasing the number of potentially harmful drug combinations and preventing the risks related to drug therapy.

***Keywords*:** Soleus, calf, perforator, below-knee amputation.

INTRODUCTION

The expected therapeutic response might be affected by the presence of drug interactions. Although drug-drug interactions (DDIs) constitute only a small proportion of adverse drug reactions, which may cause health problems, they are often predictable and therefore avoidable or manageable.[1] This can be considered as an important risk factor specifically in hospitals, where patients are ill and multiple medications may be prescribed simultaneously.[2]

About 5% of all adverse drug reactions in hospitals are caused by DDI, the majority of which are avoidable.[3,4] Up to 10% of all hospitalized patients have at least one adverse drug reaction after being discharged.[5,6,7] A change of medication, an addition of new drugs duringa hospital stay and a lack of therapeutic or nursing care after discharge are among the most important risk factors for drug related problems. Some studies show that 40–70% of patients at discharge have a potential adverse drug interaction combination.[8,9,10]
Therefore, it is of agreat importance that discharge medication should have the lowest risk of potential DDIs and that doctors should be aware of possibly preventable, drug-related complications.[11]

Drug interaction is the phenomenon which occurs when the effects and/or toxicity of one drug are modified by the prior or concurrent administration of another drug(s). The effect may be an increase or a decrease in the action of either drug, or it may be an adverse effect that is not normally associated with either drug.[12,13,14] Although results may be positive (increased efficacy) or negative (decreased efficacy, toxicity or idiosyncrasy), in pharmacotherapy they are usually undesirable.[15]

Risk of occurrence and severity of potential clinically important DDIs rest upon several factors, including the number of drugs prescribed, duration of treatment, patient age and stages of disease. Patients who require a large number of drugs, longer duration of treatment, and those with physiological aging changes or certain diseases are considered at higher risk for severe drug-drug interactions.[16,17]

Information on the frequency of drug combinations with the potential to induce dangerous drug-drug interactions (DDIs) in patients discharged from the hospitals is scarce.[10]

MATERIAL AND METHODS

Study Population*:* Three hundred (300) patients of both genders at the age of 15 years and above were enrolled. In order to identify potential DDIs, patients' prescription forms that contain two or more drugs as well as their medical records were analyzed during a 4-month period. A written informed consent was obtained from each patient before participation.

Study Design*:* The study was performed using data from the patients' files of medical wards and ICU at Al-Noor and Al-Zaher hospitals in Makkah city, KSA. The hospitals are teaching public hospitals, which are also referral centres for hospital care.

Exposure to potential drug interactions: For each patient exposed to polypharmacy, all pair wise combinations of drugs were analyzed for potential drug interactions. Potential drug interactions were classified according to Hansten and Horn[18], standard drug interaction source using Micromedex® and drug interaction checker. Hansten and Horn classification is internationally accepted and used extensively throughout the world as drug interactions are updated regularly and the classification system gives detailed summaries of clinical outcome, mechanism of action and supporting references.[19]

Drug interactions are categorized as major, moderate and minor depending on the severity of the outcome and the quality of the documentation. Drug interactions that are either well documented with the potential of being harmful, or have a limited documentation with the potential of serious outcome, are classified as 'major drug interaction'. Drug interactions that are less likely to cause harm or less well documented are classified as 'moderate drug interaction'. Drug interactions, regardless of the degree of documentation, with only a limited risk are classified as 'minor drug interaction'.[17,19]

Variables, including main diagnoses, gender, age, polypharmacy and length of stay (LOS) were correlated with the frequency of potential DDIs.

Inclusion Criteria*:* Prescriptions with two or more drugs prescribed were selected once a week, from January to April 2011. All drug groups were accepted. Patients of both genders and aged 15 years and above were included in this study. Prescription forms of all patients during hospitalization and on discharge from hospitals were included.

Statistics:

All analyses were performed by means of the statistical SPSS® program version 16.

RESULTS

The study population was equally chosen from two major hospitals in Makkah city, Kingdom of Saudi Arabia, (150 patients from each hospital). Females constituted 63% (n = 189) and males 37% (n = 111). Moreover, 91.7% (n = 275) of patients were from medical wards and 8.3% (n = 25) were from ICU. Around 20.3% (n = 61) of the study population were from the youth group (from 15 to 24 years old), 51.7% (n = 155) were within the adult age group (between 25 and 64 years old), and 28% (n = 84) were within the senior group (65 years and above) (Table 1, Fig. 1).

Table 1: Demographic data of the study population

300 patients	Nationality		Gender		Department		Age groups (years)		
	Saudi	Non-Saudi	Males	Females	Medical	ICU	Youth 15-24	Adult 25-64	Senior > 65
No. of Participants	203	97	111	189	275	25	61	155	84
% of	67.7	32.3	37	63	91.7	8.3	20.3	51.7	28

Participants

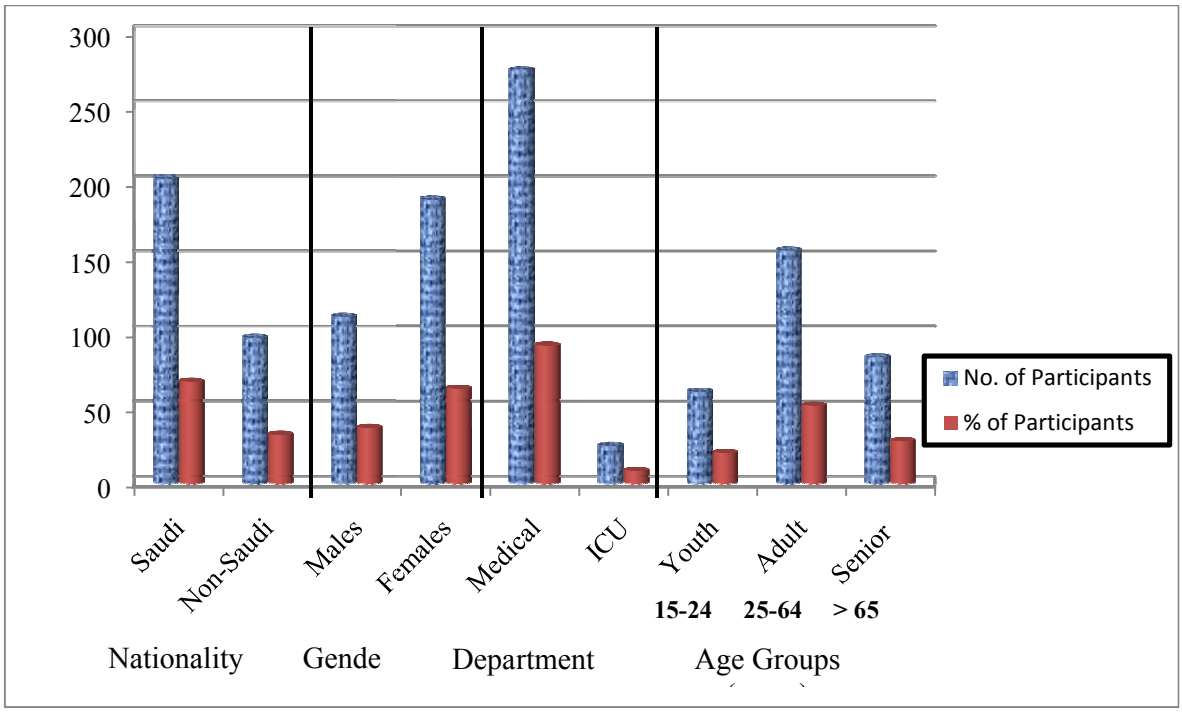

Figure 1. Summary of demographic data of the study population

Psychiatric diseases, acute infections and cardiovascular diseases were among the most frequent medical disorders among the study population (Table 2).

Table 2: Frequency of medical disorders among study population

Medical Disorders	Frequency	%
Rheumatic diseases	1	0.33
Malignancies	1	0.33
Liver diseases	6	2.00
Renal diseases	8	2.67
Epilepsy	11	3.67
Gastrointestinal disorders	17	5.67
Hypertensive heart disease	18	6.00
Diabetes mellitus	21	7.00
Sickle cell disease	22	7.33
Respiratory diseases	26	8.67
Cerebrovascular diseases	54	18.00
Acute infections	56	18.67
Psychiatric diseases	59	19.67

Patients with disturbed liver function tests (LFT) constituted 10.7% (n = 32) of the study population. Whereas, 14.7% (n = 44) of patients had impaired renal function tests (RFT) (Table 3).

Table 3: Distribution and percentage of the LFT and RFT results among study population

300 patients	Liver functions		Renal functions	
	Normal	Disturbed	Normal	Impaired
No. of cases	268	32	256	44
% of cases	89.3	10.7	85.3	14.7

LFT = Liver function tests RFT = Renal function tests

The current study revealed that total number of DDIs increased with the number of prescribed drugs. Prescriptions of five drugs or less carried a potential total DDIs risk in 26.7% (n = 80) of the patients. On the other hand, prescriptions of five drugs or more, showed a risk of potential total DDIs in 49% (n = 147) of patients, most of those DDIs were of moderate and major types (Table 4, Fig. 2).

Table 4: Frequency of individuals at risk of potential DDIs according to the number of drugs prescribed

	Number of patients				
Number of Drugs	No DDIs	Minor	Moderate	Major	Total DDIs (%)
5 drugs and less	56	18	43	19	80 (26.7)
Drugs more than 5	17	10	77	60	147 (49)

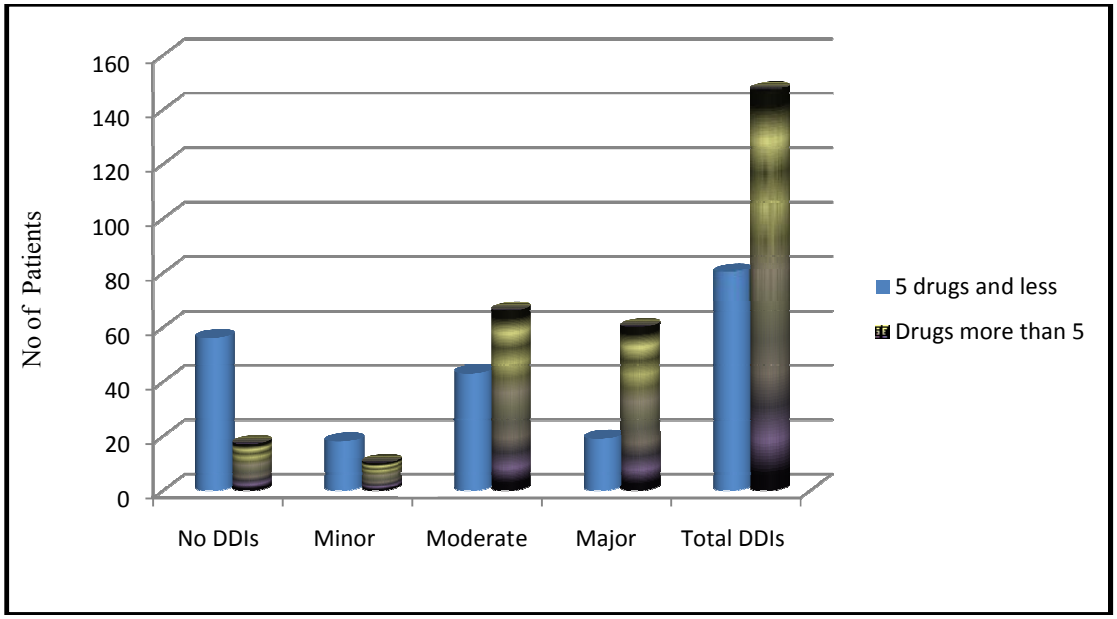

Figure 2. Frequency of individuals at risk of potential DDIs according to the number of drugs prescribed

Up to 75% (n = 225) of patients could have developed or were at risk of developing DDIs during their hospital stay. 82% of cases with potential DDIs were found among Al-Zaher hospital (n = 123/150) as compared to 68% of patients admitted to Al-Noor hospital (n = 102/150). This difference of total DDIs between the two hospitals was statistically significant ($p = 0.005$) (Table 5, Fig. 3).

Table 5: Frequency of individuals at risk of potential DDIs during hospital stay

During stay	DDIs				
	No DDIs	Minor	Moderate	Major	Total DDIs (%)
Al-Noor	48	19	61	22	102 (68)
Al-Zaher	27	9	58	56	123(82)
P-value		0.047*	0.637	0.000***	0.005**

*$P < 0.05$

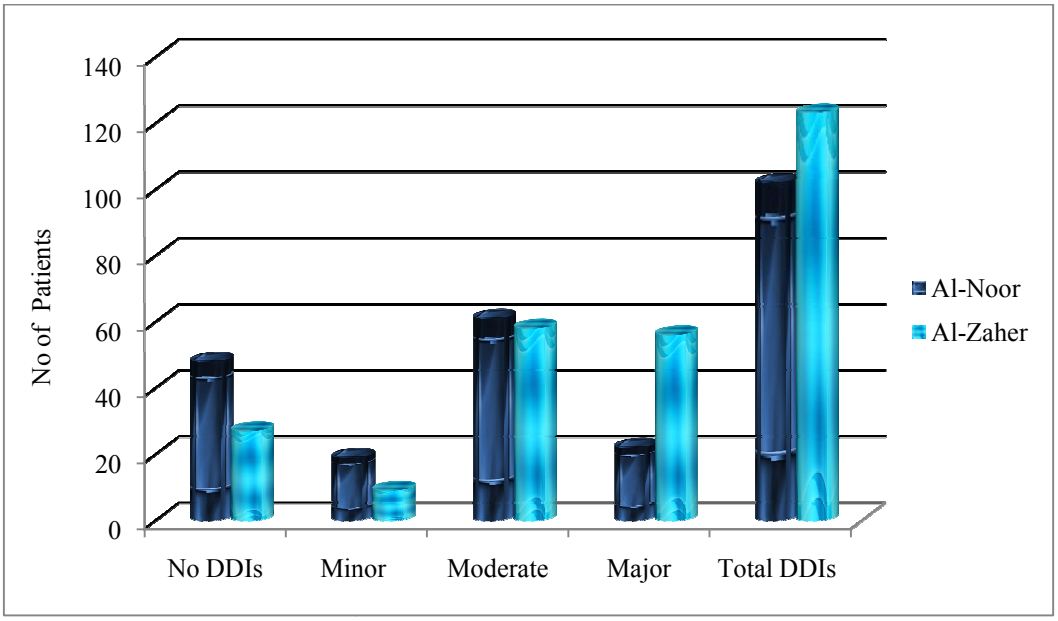

Figure 3. Frequency of individuals at risk of potential DDIs during hospital stay

Meanwhile, up to 45% (n = 135) of patients could have developed or were at risk of developing DDIs on discharge. 69.3% (n = 104/150) of cases with potential DDIs were discharged from Al-Zaher hospital as compared to the 20.7% of patients discharged from Al-Noor hospital (n = 31/150). This difference of total DDIs between the two hospitals was highly statistically significant ($p = 0.000$) (Table 6, Fig. 4).

Table 6: Frequency of individuals at risk of potential DDIs on discharge

On discharge	DDIs				
	No DDIs	Minor	Moderate	Major	Total DDIs (%)
Al-Noor	119	6	23	2	31(20.7)
Al-Zaher	46	6	53	45	104 (69.3)
P-value		0.09	0.000***	0.000***	0.000***

*$P < 0.05$

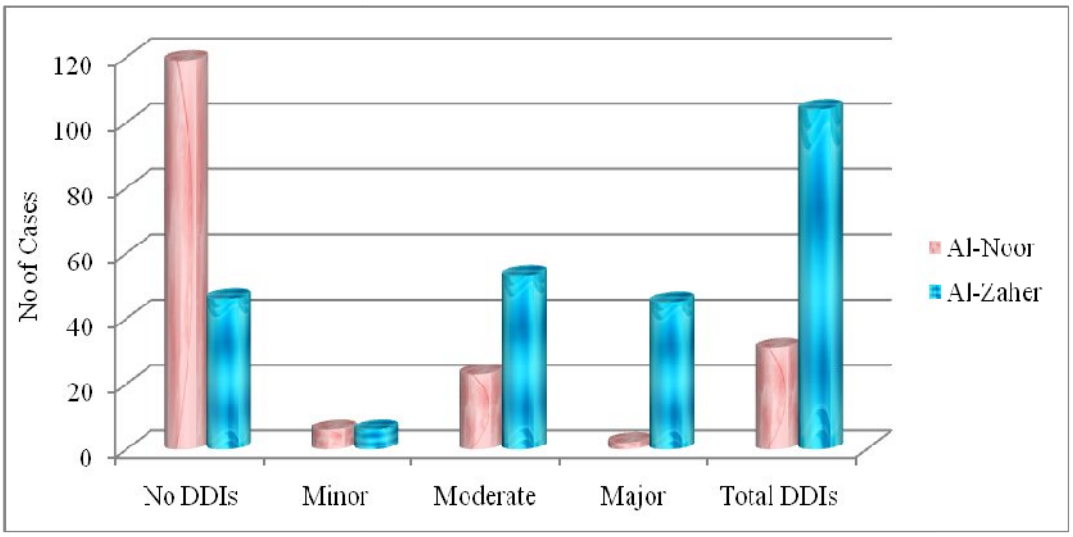

Figure 4. Frequency of individuals at risk of potential DDIs on discharge

As age increased, more potential DDIs were observed among the study population. More cases at risk of moderate and major DDIs were observed among the adult and senior age groups as compared to the young age group (*p*-value of 0.015) (Table 7, Fig. 5). The youth group (15-24 years), accounting for 20.3% of our study population (n = 61), showed equal number of patients (n = 16) with the risk of developing moderate and major DDIs (26.25%). Around 51.7% (n = 155) of patients from the adult group were at risk of developing moderate 40% (n = 62) and major 30.3% (n = 47) DDIs. Meanwhile, 28% (n = 84) of patients belonging to the senior group were at risk of developing 50% (n = 42) moderate DDIs. Contrary to the age, gender showed no significant effect on DDIs among our study population.

Table 7: Frequency of individuals at risk of potential drug interactions DDIs among study population

Number and % of patients					
Age groups (years)	Total of 300 patients	No DDIs	Minor DDIs	Moderate DDIs	Major DDIs
Youth (15-24)	61	18	11	16	16
%	20.3 %	29.5	18	26.25	26.25
Adults (25-64)	155	37	9	62*	47*
%	51.7 %	23.9	5.8	40	30.3
Seniors (65 and above)	84	18	8	42*	16
%	28 %	21.4	9.5	50	19.1
Total No of Patients		73	28	120	79
%		24.3	9.3	40	26.3

*$P < 0.05$ from youth group

Tables 8, 9 and 10 show drug combinations involved in potential major, moderate and minor drug interactions, respectively.

Table 8: Drug combinations involved in potential major drug interactions

Drug Combinations	Potential adverse effect	Frequency (%)
Haloperidol-Promethazine	Prolongation of QT interval	12 (9%)
Aspirin-Enoxaprin	Bleeding	12 (9%)
Rifampin-Isoniazid	Hepatotoxicity	11 (8.3%)
Rifampin-Pyrazinamide	Hepatotoxicity	9 (7%)
Clopidogrel-Omeprazole	Decrease Cardio protection	9 (7%)
Haloperidol-Risperidone	Prolongation of QT interval	9 (7%)
Enoxaprin-Clopidogrel	Bleeding	7 (5.3%)
Lorazepam-Olanzapine	Hypotension , bradycardia, and respiratory or CNS depression	5 (4%)
Morphine-Tramadol	Seizures	5 (4%)
Captopril-Spironolactone	Hyperkalaemia	4 (3%)
Captopril-Potassium Chloride	Hyperkalaemia	4 (3%)

Table 9: Drug combinations involved in potential moderate drug interactions

Drugs Combination	Potential adverse effect	Frequency (%)
Aspirin-Perindopril (ACEI)	Decrease antihypertensive effect	30 (3.9%)
Aspirin-Clopidogrel	Bleeding	29 (3.8%)
Insulin-Aspirin	Hypoglycaemia	28 (3.7%)
Insulin-Captopril	Hypoglycaemia	24 (3.1%)
Aspirin-Amlodipine(CCB)	Decrease antihypertensive effect	19 (2.5%)
Simvastatin-Omeprazole	Myopathy	17 (2.2%)
Aspirin-Heparin	Bleeding	16 (2.1%)
Haloperidol-Benztropine	Anti-cholinergic intoxication	12 (1.6%)
Insulin-Atenolol	Mask physiological response of hypoglycaemia	12 (1.6%)
Perindopril-Enoxaparin	Hyperkalaemia	11 (1.4%)
Phenytoin-Omeprazole	Phenytoin toxicity	10 (1.3%)

Table 10: Drug combinations involved in potential minor drug interactions.

Drugs Combination	Potential adverse effect	Frequency (%)
Aspirin-Omeprazol	Decrease anticoagulants	22 (9.2%)
Aspirin-Atenolol	Decrease antihypertensive effect	15 (6.3%)
Aspirin-Furosemide	Decrease diuretics concentration	15 (6.3%)
Heparin-Clopidogrel	Increase probability of bleeding	13 (5.4%)
Captopril-Amlodipine	Additive hypotensive effect	13 (5.4%)
Ranitidine-Acetaminophen	Potentiate hepatotoxicity	12 (5%)
Clarythromycin-Omeprazol	Increase antibiotic concentration	12 (5%)
Amlodipin-Perindopril	Additive hypotensive effect	11 (4.6%)
Amoxicillin-Clarythromycin	Decrease antibiotic concentration	11 (4.6%)
Ranitidine-Sodium Bicarbonate/ Ca Carbonate	Decrease H_2 blocker concentration	10 (4.2%)
Perindopril/Ca Carbonate	Decrease ACEI level	8 (3.3%)

DISCUSSION

In the current study, three hundred patients have randomly and equally been selected from two hospitals in Makkah city, KSA, of both genders, different age groups and different nationalities. Of the three hundred patients, 75.7% (n = 227) were found to be at risk of developing DDIs. These findings are consistent with the results of previous studies, in which potential DDI were estimated in 40–70% of patients. [9,10,20]

The drug interactions that were established in this study were only potential. This means, it was not known whether these interactions were harmful to the exposed patients or not. Generally, only a small number of patients receiving potentially interacting drugs show clinical signs of a drug interaction.[21] However, individuals respond differently, in which major potential interactions may not produce adverse effects in some patients whereas minor interactions may cause significant adverse effects in others. Drugs with a steep dose-response curve and/or a narrow therapeutic index, and those are metabolized by enzymes susceptible to induction or inhibition are most likely to result in clinically significant interactions.[19,21]

Our data revealed a positive correlation between the number of the prescribed drugs and the increasing age of the patients on one hand, and number of potential DDIs on the other hand. Prescriptions contained five or less drugs produced a risk of DDIs in 26.7% (n = 80) of patients, while 49% (n = 147) of patients having prescriptions enclosed more than five drugs were at risk of developing DDIs. As age increased, more DDIs were observed among the study population, and DDIs were mostly moderate and major in the adult group (40% and 30.3%), and moderate in the senior group (50%).

These results are similar to those of previous studies that showed number of prescribed medications and age of the patients were the major, if not the most important, risk factors for DDIs.[10,22,23] Doubova et al.,[24] found that patients at age of 60 years or older, who were receiving five or more drugs, were at high risk to develop such potential interactions. In addition, various studies have revealed that potential DDIs are frequent when patients received multiple medications prescribed by different physicians,[25,26,27] therefore, prescriptions by a single physician would decrease the risk of inappropriate DDIs.[28] Cruciol-Souza and Thomson[20] reported that along with prescription size, medical specialty and number of prescribers are also clear predictors of potential DDIs.

The present study revealed that the prevalence of total moderate and major potential DDIs among the study population were 40% (n = 120), and 26.3% (n = 79) respectively. These results are higher than those reported by Langdorf et al.,[29] and Cruciol-Souza and Thomson [20] in that the prevalence of the potentially major drug interactions judged to be clinically significant in 25% and 10% of patients respectively.

The most frequent classes of medications that could produce a risk of potential DDIs in our study were NSAIDs, ACE inhibitors, anticoagulants, antipsychotics, hypoglycemic, anticonvulsants, antibiotics and calcium channel blockers. These finding are similar to previous reports which also implicated antibiotics, diuretics, hypoglycemics, calcium-channel blockers,[30] NSAIDs, beta-blockers, steroids, ACE inhibitors, anticoagulants, and anticonvulsants[23,31] in potential drug-drug interactions. Although a number of potential interactions have been identified, not all of them are clinically relevant. It is important for the

expert systems to alert the physicians only to the most important interactions; otherwise they will run the risk of showing too many alerts for the patients on multiple medications.[31]

Basically the clinical management of potential DDIs implies monitoring of symptoms related to possible side effects and laboratory parameters, in order to prevent potentially serious adverse outcomes. In modern medicine, complex therapeutic schemes with multiple drug combinations have become the rule. Therefore, the collaboration between clinicians and clinical pharmacists in the evaluation of drug-drug interactions and getting information that may lead to treatment modification or, at least, to specific patient monitoring in order to identify early potential harmful DDIs, are of paramount importance.[11]

The limitations in this study include the sample size which was not large enough, and that the study focused on potential DDIs while it did not address the question of how many of the detected potential DDIs were known by the physician and if some of the patients were already under close clinical monitoring. Also, the study was not designed to determine how often the potential DDIs identified actually resulted in adverse clinical consequences for patients.

Finally, the present study recommends an essential collaboration between treating physicians and clinical pharmacists to help to prevent and manage the risks related to drug therapy, and a close monitoring of the impact of every given drug or drug combination on each patient.

CONCLUSION

In the present study, the potential drug-drug interactions were frequent among inpatients prescribed multiple medications, as 75% of patients were exposed to drugs with the risk of potential interactions during their hospital stay, while 45% of patients were exposed to drugs with the risk of potential interactions upon discharge. There was a positive correlation between total potential DDIs and the number of drugs prescribed. As age increased, more DDIs were observed among the study population, and DDIs were mostly moderate and major among adult age group, whereas moderate DDIs were observe among the senior age group. Development of alert guidelines and computer-based screening would help physicians to recognize and prevent potentially dangerous drug-drug interactions. This should be combined with pharmacological expertise, as well as the knowledge of important patient-related risk factors to decrease the number of potentially harmful drug combinations. However, in order to appraise the real relevance of such pharmacological expertise, it is necessary to monitor the impact of every given recommendation on each patient. Consequently, adverse outcomes resulting from DDIs can be prevented by making patient- and situation-specific assessments and, whenever appropriate, avoiding concomitant administration by implementing alternative therapeutic strategies or taking precautionary measures such as dosage adjustments and increased monitoring. This may be valuable for decreasing the number of potentially harmful drug combinations, and contribute to an increase in patient safety. A successful DDIs evaluation and prevention will have a positive impact on the medication-use system to improve the quality of patient care and in reducing the occurrence of devastating DDIs in medical inpatients. Finally, population-based studies are needed to assess the prevalence of "real" drug-drug interactions and their clinical consequences to report and avoid them for patient safety.

REFERENCES

1. Cruciol-Souza JM, Thomson JC. A pharmacoepidemiologic study of drug interactions in a brazilian teaching hospital. Clinics. 2006;61(6):515-20.

2. Zwart-van Rijkom JE, Uijtendaal EV, Ten Berg MJ, Van Solinge WW, Egberts AC. Frequency and nature of drug-drug interactions in a Dutch university hospital. Br J ClinPharmacol. 2009;68(2):187-193.

3. Fijn R, Van den Bemt P M L A, Chow M, De Blaey C J, De Jong-Van den Berg L T W, and Brouwers J R B J. Hospital prescribing errors: epidemiological assessment of predictors. Br J ClinPharmacol. 2002;53(3):326–331.

4. Classen DC, Pestotnik SL, Evans RS, Lloyd JF, Burke JP. Adverse drug events in hospitalized patients: excess length of stay, extra cost, and attributable mortality. JAMA. 1997;277(4):301–306.

5. Forster AJ, Clark HD, Menard A, Dupuis N, Chernish R, Chandok N, Khan A and Walraven C. Adverse events affecting medical patients following discharge from hospital CMAJ. 2004;170(3):345–349.

6. Forster AJ, Murff HJ, Peterson JF, Gandhi TK, Bates DW. Adverse drug events occurring following hospital discharge. J Gen Intern Med. 2005;20:317–323.
7. Forster AJ. Can you prevent adverse drug events after hospital discharge? CMAJ. 2006;174(7):921–922.

8. Bonetti PO, Hartmann K, Kuhn M, Reinhart WH, Wieland T. Potential drug interactions and number of prescription drugs with special instructions at hospital discharge. Schweiz Rundsch Med Prax. 2000;89:182–189.

9. Köhler GI, Bode-Böger SM, Busse R, Hoopmann M, Welte T and Böger RH. Drug-drug interactions in medical patients: effects of in-hospital treatment and relation to multiple drug use. Int J Clin Pharmacol Ther. 2000;38:504–513.

10. Egger S, Drewe J, Schlienger RG. Potential drug-drug interactions in the medication of medical patients at hospital discharge. European Journal of Clinical Pharmacology. 2003;58,(11):773-778.

11. Bertoli R, Bissig M, Caronzolo D, Odorico M, Pons M, Bernasconi E. Assessment of potential drug-drug interactions at hospital discharge. Swiss Med Wkly. 2010; 15;140:w13043.

12. Tatro DS. Drug interactions. In: Herifindal ET and Gourley DR. (Eds.) Textbook of Therapeutics, Drug and Disease Management. 7th ed. Lipinicott, Philadeiphia (2000) 35-49.

13. Edward A Hartshorn**.** Drug Interaction: 1. General Considerations. The Annals of Pharmacotherapy: 2006;40 (1):116-118.

14. Hammes JA, Pfuetzenreiter F, Fabrizio da Silveira, Koenig A, Westphal GA. Potential drug interactions prevalence in intensive care units. Rev Bras TerIntensiva. 2008;20(4): 349-354.

15. Streetman DS. Metabolic basis of drug interactions in the intensive care unit. Crit Care Nurs Q. 2000;22(4):1-13.

16. Hansten PD, Horn JR, editors. Hansten and Horn's drug interactions. St. Louis: Facts and Comparisons; 2001.

17. Tatro DS, editor. Drug interaction facts. St. Louis: Facts and Comparisons; 2005.

18. Hansten PD, Horn JR. Drug Interactions and Updates Quarterly. United States: Applied Therapeutics Inc, 2002.

19. Bjerrum L, Andersen M, Petersen G and Kragstrup J. Exposure to potential drug interactions in primary. health care. Scand J Prim Health Care. 2003;21:153-158.

20. Cruciol-Souza JM, Thomson JC. Prevalence of Potential Drug-Drug Interactions and its Associated Factors in a Brazilian Teaching Hospital. J Pharm Pharmaceut Sci. 2006;9(3):427-433.

21. Hartshorn EA. Drug interactions. Fam Community Health. 1982;5:45-57.

22. Geppert U, Beindl W, Hawranek T, Hintner H. Drug interactions in clinical practice. A pilot project for quality assurance in prescribing. Hautarzt. 2003;54:53-57.

23. Riechelmann RP, Tannock IF, Wang L, Saad ED, Taback NA, Krzyzanowska MK. Potential Drug Interactions and Duplicate Prescriptions among Cancer Patients. Journal of the National Cancer Institute. 2007;99(8):592-600.

24. Doubova SV**,** Reyes-Morales H**,** Torres-Arreola L, Suárez-Ortega M. Potential drug-drug and drug-disease interactions in prescriptions for ambulatory patients over 50 years of age in family medicine clinics in Mexico City. BMC Health Services Research. 2007;7:147.

25. Seymour RM, Routledge PA: Important drug-drug interactions in the elderly. Drugs Aging. 1998;12:485-494.

26. Bjorkman IK, Fastbom J, Schmidt IK, Bernsten CB: Pharmaceutical Care of the Elderly in Europe Research (PEER) Group. Drug-drug interactions in the elderly. Ann Pharmacother. 2002;36:1675-1681.

27. Janchawee B, Wongpoowarak W, Owatranporn T, Chongsuvivatwong V: Pharmacoepidemiologic study of potential drug interactions in outpatients of a university hospital in Thailand. J Clin Pharm Ther. 2005;30:13-20.

28. Tamblyn RM, McLeod PJ, Abrahamowicz M, Laprise R. Do too many cooks spoil the broth? Multiple physician involvement in medical management of elderly patients and potentially inappropriate drug combinations. Can Med Assoc J. 1996;154:1177-1184.

29. Langdorf M, Fox J, Marwah R, Montague B, Hart M. Physician versus computer knowledge of potential drug interactions in the emergency department. Acad Emerg Med. 2000; 7(11):1321-1329.

30. Hohl CM, Dankoff J, Colacone A, Afilalo M. Polypharmacy, adverse drug-related events, and potential adverse drug interactions in elderly patients presenting to an emergency department. Ann Emerg Med. 2001;38:666-671.

31. Goldstein JN, Jaradeh IE, Jhawar P, Stair TO. ED Drug-Drug Interactions: Frequency and Type, Potential and Actual, Triage and Discharge . The Internet Journal of Emergency and Intensive Care Medicine. 2005;8 (2).

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 94-101 (2011)
07-012 © The University Press

UQU Medical Journal

Original Article

PATTERN OF ABDOMINAL PAIN AMONG PILGRIMS DURING HAJJ SEASON.

Sameer Bin Abdullah Softa
Department of Surgery, Faculty of Medicine, Umm Al-Qura University, Makkah Almokarramah, KSA.

Correspondence:
Sameer Bin Abdullah Softa, Surgery Department, Faculty of Medicine,
Umm AlQura University, Al Abdyah, Makkah,
P.O. Box.7607, Saudi Arabia.

Received: February 03, 2011 *Accepted: April 06.2011*

دراسة مقطعية لنمط آلم البطن بين الحجيج في مواسم الحج.

د. سمير بن عبد الله صفطة
قسم الجراحة * بكلية الطب – جامعة أم القرى – مكة المكرمة – المملكة العربية السعودية. ص. ب. : 7607

الملخص العربي

مقدمة : إن الوعي والإلمام بهذا العرض يلأمور الأساسية والهامة لكل طبيب خاصة العاملين في طب الحشود كما أن التخطيط الصحي السليم قد يحتاج لمعرفة أنماط آلم البطن الجراحي .

الأهداف : مراجعة طراز آلم البطن الجراحي ورفع الوعي والإلمام بأنماطه لوضع سياسات.

الطريقة : تجمع أكثر من 2 - 3 مليون حاج في منطقة واحدة و في وقت واحد يجعل تقديم الخدمات الصحية تحدي لهم ، هذه دراسة مقطعية لمنطقة المشاعر أثناء موسم الحج شملت (4) مستشفيات تم فيها فحص كل الحالات الطبية لكل مرضى ألم البطن مبنية على الاستبيان والدراسة المسبقة والذين تم تنويمهم بالمستشفيات المعنية.

النتائج : من واقع (250) مريض تمت دراساتهم خلال (4) أيام بمستشفيات في المشاعر لموسم حج 1427 هـ ، أظهرت النتائج أن أكثر الأعمار تعرض لآلام البطن الجراحي الحاد هم الفئة العمرية من (41) أكثر وهذا (73.2 %) واقلهم من الفئة العمرية (12 21) وتمثل (4.8%) من الحالات المماثلة في الظروف العادية نجد أن توزيع الفئات العمرية يختلف كما أن آلم البطن يخالف أيضا المعهود في الحالات العادية حيث التهاب الزائدة هو الأعلى بنسبة بينما الانسداد المعوي في دراستنا هو الأعلى كما أن الدراسة أوضحت أن معظم حالات آلم البطن تم علاجها جراحياً (95%).

الخاتمة: نتائج هذه الدراسة تساعد توجه وضع الخطط والسياسات الصحية للحجيج بصبغة خاصة ولطب الحشود عامة . كما تعتبر أساس للدراسات.

ABSTRACT

Hajj (pilgrimage) is the fifth pillar of Islam, approximately 2-3 million pilgrims gather in one place to perform this ritual of Islam every year.

Abdominal pain is a frequent presentation during Hajj seasons. Varieties of gastrointestinal, chest pelvic and systemic disorders acute life threading condition as well serious medical and surgical conditions encountered causes of these presentations. The awareness of this entity by the clinician is vital to its recognition and crucial to deal with. Planning for health care requires knowledge of the pattern of diseases, complications, and outcome of pilgrims who require hospitalization during the Hajj period.

Objectives:
To elucidate the pattern of acute abdominal pain among pilgrims requiring admissions to hospitals during the Hajj season and to raise the awareness of clinicians for such serious condition.

Methods:
This was a cross-sectional study conducted in hospitals at Almashaer area, one in Arafat and the other one in Mina serving the pilgrims during Hajj season. The record of all abdominal pain cases, together with the diagnosis recorded by various doctors of these two hospitals during Hajj season of 1427H. over four days period.

Results:
A total of 250 patients admitted over four days time tin two hospitals at Almashaer of acute abdominal pain during Hajj season of 1427 H. The largest number of admissions occurred in the age groups above 41 (73.2%) and the lowest incidence was among patients in the age group (12-21),(4.8%) while those with age 82> were only 8 (3.8%). Cholecystitis as a cause of acute abdominal pain were 28 (11.2%) which match previous studies.

When compared to previous studies most common causes of abdominal pain in our study were intestinal obstruction 66 (26.6%),followed by acute appendicitis 52 (20.8%) where non specific abdominal pain is rated lower cause contrary to previous studies and reports which were predominant...

Conclusion:
Findings in this study will be used to improve the health programme and planning to best suit the needs of the pilgrims.

Key Words: *Acute abdominal pain, surgical conditions, Hajj season, Pattern.*

INTRODUCTION

Hajj (pilgrimage) is the fifth pillar of Islam , approximately 2-3 millions pilgrims gathered in one place from more than 140 countries to perform this ritual of Islam every year . This large scale mass gathering of Muslims all over the world of enormous ethnic diversity represents significant challenges for the entire health sector of host country.[1,4]

Abdominal pain is a common symptom that can be attributed to a wide variety of acute and chronic disease processes, many of which represent serious surgical problems. It is one of the most frequent presenting complaints to Emergency Departments.[1,4] The awareness of this entity by the clinician is vital to its recognition and subsequent medical management. Abdominal pain can result from a variety of gastrointestinal, chest, pelvic and systemic disorders. [5,6]

Surgical conditions among the many causes, one of the most common and serious , it represent one quarter of patients visiting health services . For surgical conditions to make a diagnosis and take decision to operate or not is crucial to safe life. It is vital that the physician has an understanding and be familiar with the presentations of common diseases that cause abdominal pain.[7,8] The awareness of this entity by the clinician is vital to its recognition and subsequent medical management and it is of sufficient importance to warrant policy statements by professional organizations.[9]

However, few studies were done to establish the pattern of hospital admission during hajj among them no studies were conducted on surgical acute abdominal pain during Hajj to describe the clinical profile pattern. Therefore a high index of suspicion should be born in mind by physician when patients presenting with acute abdominal pain in order to have early diagnosis, avoid complication and to reduce mortality and cost associated with complicated surgical conditions.

OBJECTIVES

The aim of this study is to elucidate the pattern of acute abdominal pain among pilgrims necessating admission to hospitals during the Hajj season, in order to help health policy makers to plan futuristic services and research.

MATERIAL AND METHODS

This was a cross-sectional prospective study conducted in two hospitals in two different locations in Al-Mashaer for a period of four days. These hospitals are one of other 7 hospitals serving the pilgrims during Hajj. The record of 250 patients was presented with acute abdominal pain as their chief complaint were enrolled in this study. Acute abdominal pain was defined as the onset of any type of abdominal discomfort within the previous 7 days. History and physical examination findings were obtained prospectively and recorded by attending physicians using a standardized data collection questionnaire based pro forma with both open and closed ended questions form. All patients were ordered basic investigations including full blood chemistry and complete blood count, plain x-ray and US of abdomen and pelvis as was clinically appropriate. Children below twelve years were excluded.. Data

collected on paper forms was entered into MS Excel spreadsheet and analysed in SPSS version 12.

RESULTS

A total of 250 patients were admitted to two of seven hospitals at Almashaer during Hajj season of 1427 during a four days period. The largest number of admissions occurred in the age groups above 41 (73.2%) 183 out of the total patients . The lowest incidence was among patients in the age group (12-21),(4.8%) while those with age 82> were only 8 (3.8%). This study also showed that most of the patients presented with abdominal pain were from Middle east 76(30.4%) and African 70(28%) Table 1.

Demographic characteristics of all patients (Table 1)

Characteristics	Numbers & (%)
Age group:	
12-21	12 (4.8%)
22-31	29 (11.6%)
32-41	26 (10.4%)
42-51	69 (27.6%)
52-61	65 (26 %)
62-71	25 (10%)
72-81	16 (6.4%)
82- >	8 (3.2%)
Total	250 (100%)
Geographical regions:	
Arab countries	62 (24.8%)
Middle east	76 (30.4%)
South east Asian	28 (11.2%)
Africans	70 (28%)
Others	14 (5.6%)

More than quarter of the causes of presenting abdominal pain of the patients in this study were intestinal obstruction 66 (26.6%) more than half of them were Africans 38 (54.3%), which included complicated hernias and adhesions. Acute appendicitis 52 (20.8%) of the total patients, most of them were perforated. The other causes were cholecystitis 28(11.2%), perforated peptic ulcer 24(9.6%), pancreatitis 16 (6.4%), ischemic bowel 8 (3.2%). Non-surgical cause was seen in 56 patient (23.2%). 34 (14.4%) 0f the them were labeled as non specific abdominal pain and 22 (8.8%) as renal cause. All non surgical patients were made by clinical resolution of the symptoms or diagnostic laboratory results Tables 2 & 3, In this study no acute abdominal gynaecological pain were reported.

Table 2 . Main causes of abdominal pain.

Causes (Diagnosis)	Numbers (%)
Intestinal obstruction	66 (26.4%)
Appendicitis	52 (20.8%)
Cholecystitis	28 (11.2%)
Perforated ulcer	24 (9.6)
Pancreatitis	16 (6.4)
Ischemic bowels	8 (3.2%)
Renal	22 (8.8%)
Non specific abdominal pain	34 (14.4%)
Total	250 (100%)

Table 3 . Causes of abdominal pain in relation to the geographical regions.

Causes Diagnosis	Intestinal Obstruction	Appendicitis	Cholecystitis	Perforated D.Ulcers	Pancreatitis	Ischemic bowels	Renal	Non-specific	Total No & %
Regions &(%)									
Arab countries	9	17	9	6	4	1	8	8	62(24.8%)
Middle east	11	21	10	9	8	1	10	6	76(30.4%)
Southeast	5	8	3	2	1	2	1	7	28(11.2%)
Asian	38	6	6	7	3	0	1	9	70 (28%)
Africans	3	0	0	0	0	4	3	4	14 (5.6%)
Others									
Total No:& %	66 (26.4%)	52 (20.8%)	28 (11.2%)	24 (9.6%)	16 (6.4%)	8 (3.2%)	22 (8.8%)	34 (14.4%)	250 (100%)

DISCUSSION

Acute abdominal pains continue to be a challenge for practicing surgeons. Acute abdominal pain has been under diagnosed in the past but is now gradually gaining recognition. All previous studies were done on people of certain geographical area but in our study patients were included from all over the world more than 140 country with different racial back grounds that came to Makkah to perform Hajj.

This is the first study conducted in Almashaer Hospitals on the pattern of acute surgical abdominal pain. Previous studies were conducted on pattern of general admission and other medical problems. In our study 216 (86.4%) shown that abdominal pain was serious and genuine and need surgical intervention , in contrary to most of the previous study world-wide, which has shown the non specific abdominal pain with no clear explanation is the common cause.[10,11]

Also in this study more than quarter of the patients (26.4%) presented with abdominal pain were diagnosed as intestinal obstruction and more than half 54.3% of them were Africans. this is contrary to previous studies which reported the nonspecific and acute appendicitis is the commonest pattern of acute abdominal pain. [5.11,12] Similar findings of intestinal obstruction as a common cause of acute abdominal pain followed by appendicitis were shown in a study from Ethiopia[13] The present study has shown 8 patients (3.2%) with serious surgical vascular occlusion and all of them over 80 years of age, which consist with other studies. On the other hand elderly patients in this mass gathering with variety of background can have atypical presentations; This vascular events are more common in this population, and a wide differential diagnosis needs to be considered. [12,14] In our study non-specific abdominal pain reported in 34 (14.4%) patients where previous studies world-wide shown non-specific abdominal pain is the most common diagnosis in patients admitted to hospitals,(25–35% of all patients), and of those patients only a quarter needs surgical intervention.[14,15,16] Diagnosis of non-specific abdominal pain should only be made after thorough assessment, definite pathology excluded and the patient does not return with the same complaint. Our study documents the pattern of acute surgical abdominal pain in Hajj pilgrims admitted in Almashaer two care hospitals.

We are looking forward to conduct another study of larger duration and larger number of patients with acute abdominal pain on pilgrims coming to Makkah and then we will be able to suggest a diagnostic criterion and roadmap for acute surgical abdominal pain management.

CONCLUSION

Intestinal obstruction, acute appendicitis and gastroduodenal perforations were the leading causes of acute admissions for abdominal pain in Mashaer hospitalsl during Hajj season. Non specific abdominal pain is less than other studies shown. In conclusion, our data showed a change in the pattern of acute abdominal pain surgery in Hajj season. Diagnoses that have become more frequent include intestinal obstruction, appendicitis, perforated viscus, while the non specific abdominal pain were rated less in our study contrary to previous studies. In our study non of gynaecological abdominal pain has been reported. The limitations of this study include lack of long-term follow-up, which precludes assessment of morbidity and mortality as well as the long-term outcome.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge my indebtedness to all the medical students of Umm-Alqura surgical clerkship, medical officers, residents and all health professionals at Almashaer Hospitals for their help and cooperation in carrying out this work.

REFERENCES

1. Powers RD, Guertler AT. Abdominal pain in the ED: stability and change over 20 years. Am J Emerg Med 1995; 13: 301-3.

2. Alborzi A, Oskoee S, Pourabbas B, Alborzi S, Astaneh B, Gooya MM, et al. Meningococcal carrier rate before and after hajj pilgrimage: effect of single dose ciprofloxacin on carriage. EMHJ. 2008; 14 (2): 277-82.

3. Madani TA, Ghabrah TM, Albarrak AM, Alhazmi MA, Alazraqi TA, Althaqafi AO, et al. Causes of admission to intensive care units in the Hajj period of the Islamic year 1424 (2004). Ann Saudi Med. 2007; 27(2): l01-5.

4. Al-Maghderi Y, Al-Joudi A, Choudhry AJ, Al- Rabeah AM, Ibrahim M, Turkistani AM. Behavioral risk factors for diseases during Hajj 1422 H. Saudi Epidemiology Bulletin. 2002; 9 (3):19-20.

5. Hawthorn IE. Abdominal pain as a cause of acute admission to hospitals. J. R. College of Surg.Edinb.1992; 37:389-393.

6. Scott C. Sherman, MD and Linda Lee, MD. Strangulated umbilical hernia. The Journal of Emergency Medicine. 2004; 26 (2): 209–211.

7. Laurell H, Hansson LE, Gunnarsson U: Diagnostic pitfalls and accuracy of diagnosis in acute abdominal pain. *Scand J Gastroenterol* 2006, 41(10):1126-31.

8. Flasar MH, Goldberg E: Acute abdominal pain. *Med Clin North Am* 2006, 90:481-503.

9. Nagurney J. T, Brown D F M, Chang Y, Sane S, Wang A C and Weiner J B. Use of diagnostic testing in the emergency department for patients presenting with non-traumatic abdominal pain. The Journal of Emergency Medicine 2003; 25 (4):363-371.

10. Groselj-Grenc M, Repse S, Dolenc-Strazar Z, Hojker S, Derganc M: Interleukin-6 and lipopolysaccharide-binding protein in acute appendicitis in children.*Scand J Clin Lab Invest* 2007, 67(2):197- 206.

11. Datubo-Brown DD, Adotey JM. Pattern of surgical acute abdomen in the University of Teaching Hospital. W Afr.J Med 1990; 9:59-62.

12. Almulhim A.A. Emergency general surgical admission prospective institutional experience in non-traumatic acute abdomen:Implicationfor education,training and service. Saudi Med J. 2006; 27: 1674-1679.

13. Tsegaye S, Osman M. Bekele A. Surgically Treated Acute Abdomen at Gondar University Hospital, Ethiopia. East and Central African Journal of Surgery 2007;12 (1) 53-57.
14. Martinez JP, Mattu A: Abdominal pain in the elderly. *Emerg Med Clin North Am* 2006, 24(2):371-88.

15. McCaig LF, Ly N. National Hospital Ambulatory Medical Care Survey: 2000 Emergency department summary. Hyattsville (Md): National Center for Health Statistics: Department of Health and Human Services & Centers for Disease Control and Prevention.

16. Madani TA, Ghabrah TM, Albarrak AM, Alhazmi MA, Alazraqi TA, Althaqafi AO, et al. Causes of admission to intensive care units in the Hajj period of the Islamic year 1424 (2004). Ann Saudi Med. 2007; 27(2):l'01-5.

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 109-120 (2011)
07-012 © The University Press

UQU Medical Journal

Original Article

Construction of functional bicistronic vector for gene transfer and simultaneous expression of hNkx2.2 and eGFP in human cells

Fasial A. Al-Allaf
Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Makkah, Saudi Arabia

Correspondence:
Fasial A. Al-Allaf
Assistant Professor Medical Genetics
Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, Al-Abdia Campus, P. O. Box 715, Makkah 21955, Saudi Arabia
Tel/Fax: +966 2 5270000 Ext: 4198
Email: fallaf@uqu.edu.sa

Words count: 2753 words

Received: January 25, 2011 *Accepted: April 14, 2011*

بناء ناقل وراثي مزدوج وفعال للتعبير الجيني المتزامن لعامل الاستنساخ ان اكس 2.2 والبروتين الأخضر الفوسفوري في الخلايا البشرية

د. فيصل أحمد علاف
أستاذ مساعد , قسم الوراثة الطبية بكلية الطب بجامعة أم القرى.

الملخص العربي

الهدف: كثير من الأبحاث التي حاولت استخدام سلسلة دخول الريبوسوم الداخلية لبناء النواقل الوراثية المزدوجة واجهت صعوبة في تحقيق تعبير جيني للبروتين الثاني في السلسلة. الدراسة قمنا ببناء ناقل وراثي مزدوج للتعبير الجيني المتزامن يحتوي على سلسلة دخول الريبوسوم الداخلية بين سيسترونية وسلسلة البروتين الأخضر الفوسفوري المعزز.

الطريقة: تم بناء الناقل الوراثي pIRES-2-eGFP المحتوية على سلسلة دخول الريبوسوم الداخلية بين سيسترونية المعززة للتعبير الجيني المستنسخ من الخلايا البشرية. تم تحليل الناقل الوراثي المبني بواسطة التحليل التسلسلي للحمض النووي لتأكيد اندماج السلاسل بالشكل الصحيح. تم اختبار فعالية الناقل وقدرته على التعبير الجيني من سلسلة تحفيز السيتوميقالو فيرس بواسطة الويسترن بلوتينق و التحليل المجهري الفوسفوري.

النتائج: التحليل التسلسلي للحمض النووي للناقل الذي تم بناءه أكد اندماج سلسلة عامل الاستنساخ البشري ان اكس 2.2 في الكاسيت الثنائي وبالتالي بناء الناقل المطلوب بالصورة الصحيحة. تحاليل الويسترن بلوتينق والتحليل المجهري الفوسفوري أكدت ايضا فعالية الناقل وقدرته على التعبير الجيني وإنتاج عامل الاستنساخ البشري ان اكس 2.2 بمقاسه الصحيح والنشاط الحيوي للبروتين الأخضر الفسفوري في نموذج الخلايا المستزرعة.

الإستنتاج: سلسلة دخول الريبوسوم الداخلية تمثل اداة قيمة لإمكانية التعبير الجيني المتزامن والمتعدد السيسترون من نسخة الجين النووي المراسل الناتج من النواقل الجينية والنواقل الوراثية المتعددة الذي تم إنتاجه سوف يكون مفيد للنقل الجيني ولدراسة تخصيص الخلايا الجذعية في المستقبل.

ABSTRACT

Objective: Many researchers using Internal Ribosome Entry Site (IRES) elements for construction of bicistronic gene transfer vector reported difficulties to achieve significant expression of the second cistron. In the present study, we have constructed an expression vector containing a bicistronic cassette composed of the human oligodendrocyte transcription factor 2 cDNA (*hNkx2.2cDNA*), an inter-cistronic sequence, the IRES and enhanced green fluorescent protein (eGFP) sequences.

Methods: The hNkx2.2 cDNA sequence together with an inter-cistronic sequence of 168bp in length was subcloned into the pIRES-2-eGFP mammalian expression vector. Expression of the transgenic proteins from the upstream cytomegalovirus (CMV) promoter was confirmed by Western and fluorescence microscopy analyses.

Results: DNA sequence analysis was performed on nascent vector DNA confirmed the integrity of Nkx2.2 cDNA in the bicitronic cassette and the construction of the desired vector. Analyses of vector containing the cassette clearly confirm the functionality of the produced vector, correct size of the generated Nkx2.2 protein and the biological activity of the eGFP reporter in the cell culture model. Transcription of the bicistronic cassette is driven by a massage from a common upstream CMV promoter and translation of the two cistrons is uncoupled.

Conclusion: IRES sequence provides a valuable tool for simultaneous expression of bicistron from a single mRNA transcript incorporated in the gene transfer and therapeutic vector. The produced bicistronic hybrid vector will be useful for gene transfer and of great value for future stem cells transdifferentiation studies.

Keywords: Diabetes, Miletus, Infection, Bacteria and fungus

INTRODUCTION

Simultaneous expression of multiple genes from a single gene transfer vector is an important requirement in molecular and cellular therapies.[1,4] There are generally three common strategies in which two genes can be co-expressed from a single vector. Firstly, two genes can be fused together in frame to produce a chimeric sequence, ensuring simultaneous expression of both genes in one protein.[5,7] However, this strategy may not work for all combinations of proteins, some of which could result in protein misfolding or mistargeting. Secondly, two transcriptional units can be constructed with separate promoters to drive expression of different genes in the same vector. The major disadvantage of constructing such a dual promoter vector is possible transcriptional interference and/or dissociated gene expression, with a fraction of the transfected cells expressing the selectable marker but not the gene of interest and vice versa.[8,10] To overcome the above shortcomings, the third strategy involve the construction of a bicistronic cassette, in which the two heterologus genes are separated by an element known as internal ribosome entry site (IRES) sequence. Transcription of both genes in the bicistronic cassette is driven by a massage from a common upstream promoter, thus eliminating promoter interferance. As a result, a single messenger unit including the bicistronic transcript of both genes spaced by IRES is produced. Translation initiation of the first cistron at the 5' of IRES is typically mediated by a cap-dependent translation initiation mechanism[11] but the second cistron at the 3' of IRES is translated via cap independent translation initiation, mediated by the IRES element which functions as ribosome-binding sites for internal initiation of translation.

Since both the first and the second genes in a bicistronic cassette are under the control of the 5' upstream promoter, detection of the protein encoded by the second cistron is theoretically the insurance that the first cistron is also being expressed. However, many researchers using IRES elements for construction of bicistronic gene transfer vector reported difficulties to achieve significant expression of the second cistron. In the present study, we have constructed an expression vector containing bicistronic cassette under control of the *CMV* promoter. This bicistronic cassette contains the human oligodendrocyte transcription factor 2 cDNA (hNkx2.2cDNA) and the IRES-eGFP cassette sequences. We then examined the efficiency of this vector with the aim to use it in future gene transfer studies for stem cells transdifferentiation and stem cells based gene therapy.

MATERIAL AND METHODS

Plasmid Construction and Cloning

The hNkx2.2cDNA insert was excised from pBatl2-Nkx2.2 plasmid by digestion with XhoI/BamHI using BamHl buffer from NEB in a total volume of 200µl reaction mix topped with 3 drops of oil and incubated at 37°C for 14 hrs. The tube was incubated at 65°C for 20mins to inactivate the endonucleases. Digested DNA was then cleaned with PCR purification column (Qiagen) and eluted in 40µl sterile DPEC treated H_2O to ensure removal of enzymes and salts. Approximately, 5µg of the pIRES-2-eGFP plasmid DNA was also digested with XhoI/BamHI enzymes, heat inactivated at 65°C for 20 mins and column purified to remove the short XhoI-BamHI flanking sequence. Purified digested vector and

insert DNA were mixed together in 1:10 ratio and ligated using T4 DNA ligase for overnight at 16°C. Ligated mix was purified again using the Qiagen column and eluted in 30µl TE buffer. Only 2ul of eluted ligation mix was used to tranform Top 10 competent cells. Transformation was performed according to invitrogen recommendations and 300µl of serially diluted SOC media containing transformed bugs was plated on *Kan* resistance plates. Colonies were allowed to grow for overnight and then subcultured on *Kan* and *Amp* resistance plates. Only *Kan* resistance colonies were screened further by insert-vector PCR method [12] using the forward primer 5'-CAC CAA AAT CAA CGG GAC TT-3' and the reverse primer 5'-TTT GTG TTG GTC AGC GAC AT-3' which anneals to the 5' and 3' restriction/ligation site. Colonies producing an amplicon 244bp were inoculated for overnight culture and the integrity of the hNKx2.2-insert in the pNKx2.2-IRES-eGFP clones was examined by restriction digestion and DNA sequence analysis.

Gene transfer into cultured cells

The human embryonic kidney epithelial (HEK 293), the Hela, and hfMSCs cells were cultured with Dulbecco's modified Eagle's medium (Invitrogen or Sigma, UK) supplemented with 10% fetal calf serum (FCS, Sigma). All cells were incubated at 37°C with 5% CO_2 in a humidified incubator. Cells were seeded into a $10cm^2$ dishes at 60-70% confluence with a density of $5x10^6$ cells and transfected the following day with 10µg of the expression vector DNA using FuGene6 (Roche, UK) according to the manufacturer's instructions. Forty-eight hours later, cellular expression of fluorescent proteins was examined with a fluorescence microscope (type). Mock transfected cells were used as controls. All transfection experiments were repeated at least three times. Approximately 48 hours post-transfection, HEK 293T cells were analysed for cellular expression of eGFP using fluorescence microscopy.

Western blot analysis of protein expression

Forty eight hours after transfection, cells were harvested by scrubbing and re-suspended in $30µl/10^6$ cells ice-cold lysis buffer (10mM Tris pH 7.5, 1mM EDTA, 1% NONIDET P-40 (Sigma, UK), protease inhibitor cocktail at indicated dilutions (Roche, UK)). Cell suspensions were then centrifuged at 13,000×*g* for 20 mins at 4°C. The protein content of cell lysate supernatants was assayed by the bicinchoninic acid method using a commercial assay kit from Pierce with bovine serum albumin as a standard. Five milligrams protein of each lysate were combined with equal volumes of Laemmli sample buffer, boiled for 5 min and were separated on a 13% or 11% SDS-PAGE gel for NKx2.2, and β-actin protein detection, respectively. Gels were then blotted onto PVDF membranes with a Hoefer apparatus (200 mA). After 1 h incubation at room temperature in blocking solution (5% dried skimmed milk in PBS-Tween-20 0.1%), membranes were incubated overnight at 4°C with specific primary antibodies, diluted as specified below with blocking solution. After three 5-min washes in PBS-Tween-20, blots were incubated at room temperature for 1h with peroxides-conjugated goat anti-rabbit antibody (Abcam) or rabbit anti-mouse polyclonal antibody (Dako, UK) diluted 1:1000 in blocking solution. Following the final wash detection on autoradiography hyper-films was performed after inducing a chemiluminescence reaction with the Amersham Supersignal detection kit. Primary antibodies used in this study were anti-NKX2.2 (polyclonal, 1:2000; Research Diagnostic, USA) and anti-GFP (polyclonal, 1:2000; abcam, UK) and anti-beta-tubulin (1:2000; Sigma, UK).

RESULTS

Structure of the bicistronic cassettes and proof of clonality

We placed the hNkx2.2cDNA sequence, at the 5' of IRES element (Figure 1-A). The hNkx2.2cDNA and the 168bp downstream spacer sequences were derived from the pBatl2-Nkx2.2 plasmid which was a kind gift of Dr Huseyin Mehmet, Imperial College London, UK. The pIRES2-eGFP expression vector was chosen because it contains the IRES of the encephalomyocarditis virus (ECMV) which has high translation efficiency compared to other IRES sequences including those from hepatitis A and C viruses, poliovirus, human rhinovirus, and foot-and-mouth disease virus.[13,16] In addition to its high efficiency, ECMV IRES possess a broad tissue tropism.[17] which makes it the most widely used in gene transfer protocols.[18,20] This vector also permits high level of plasmid DNA production and the multi-cloning site offer the possibility of convenience cloning. The eGFP incorporated in this vector is a red-shifted variant [21,22] of wild-type GFP which has been optimised for brighter fluorescence and higher expression in mammalian cells. Moreover, sequences flanking the eGFP have been converted to a Kozak consensus translation initiation site [23] to further increase the translation efficiency in eukaryotic cells. The initiator AUG codon of the eGFP is located 12 bases downstream of the IRES element. Such a short distance is nevertheless sufficient to greatly affect the eGFP expression. The eGFP was used as a marker for easy, rapid and less expensive detection and monitoring of expression.

DNA fragment containing the *hNkx2.2cDNA* was subcloned into the pIRES2-eGFP (Clontech) mammalian expression vector and its integrity was confirmed by PCR colony screening method (Figure 1-B) and DNA sequence analyses (Figure 1-C).

The correct 244bp fragment size was found in 8 colonies (Figure 1-B), indicating Nkx2.2cDNA insertion in the desired orientation. The other twelve screened colonies showed no PCR product and therefore excluded from further screening. The ligation mixture was also examined by insert-vector PCR[12] as positive control to confirm the physical presence of the expected recombinant plasmid containing the hNKx2.2cDNA in correct orientation. The PCR positive colonies were then grown individually in LB growth media under kanamycin selection. DNA sequence analysis was performed on nascent vector DNA which also confirmed the integrity of Nkx2.2 cDNA in the bicitronic cassette and the construction of the desired mammalian expression vector (Figure 1-C).

Analysis of hNKx2.2 expression by Western blotting

Analysis of gene expression was performed by Western blotting and fluorescence microscopy after cells transfected with bicistronic construct containing 5'-Nkx2.2-IRES-eGFP-3' cassette. Western analysis ensures that the expressed proteins of a correct mature sizes and the fluorescence microscopy permits accurate assessment of gene transfer and expression at a single cell level.

Immunoprobing with anti-hNKx2.2 antibody (Figure 2-A) shows the mature form of the hNKx2.2 protein from cell extracts transfected with the pNkx2.2-IRES-eGFP construct. The hNKx2.2 is migrating as one band of apparent molecular masses of 32kDa (lane 2). No signals were detected from the mock transfected negative controls (lane 1) or from extract of cell transfected with pIRES-2-eGFP mother plasmid (lane 3). Immunoprobing of the membrane with anti-β-actin antibody in Figure 2-B shows the production of β-actin protein of

the expected molecular mass of approximately 47kDa from cells. No other proteins were recognised from cell extract immunoprobed with these antibodies.

In summary, the data provided above show that the produced vector is functional and the generated proteins of correct sizes.

Analysis of eGFP expression by fluorescence microscopy

Fluorescence microscopy analysis was carried out for the detection of cellular expression of eGFP approximately 48 hours post-transfection. Transfected human kidney epithelial (HEK 293T) cells were able to express high levels of eGFP compared to mock transfected control as shown in Figure-3. Transfection efficiency of cells was estimated to be more than 90%.

Figure 1. Diagram of the constructed 5'-NKx2.2-IRES-eGFP-3' bicistronic expression cassettes and the clonality analysis. A) Schematic diagram of the constructed expression vector showing the orientation of 5'-NKx2.2-IRES-eGFP-3' transcriptional unit and the position of the upstream CMV promoter. B) Insert-vector PCR analysis for screening and selection of the desired clone. Amplicon size of 244bp generated from lane 3, 4, 5, 6, 7, 8, 9, 10, 14, indicate clonality and integrity of Nkx2.2 cDNA in the bicistronic cassette. The ligation mixture was used as positive control. Clone at lane 10 was used in the subsequent analysis. C) Sequence analysis at the ligation site from purified plasmid DNA proof construction of 5'-NKx2.2-IRES-eGFP-3' bicistronic expression cassettes.

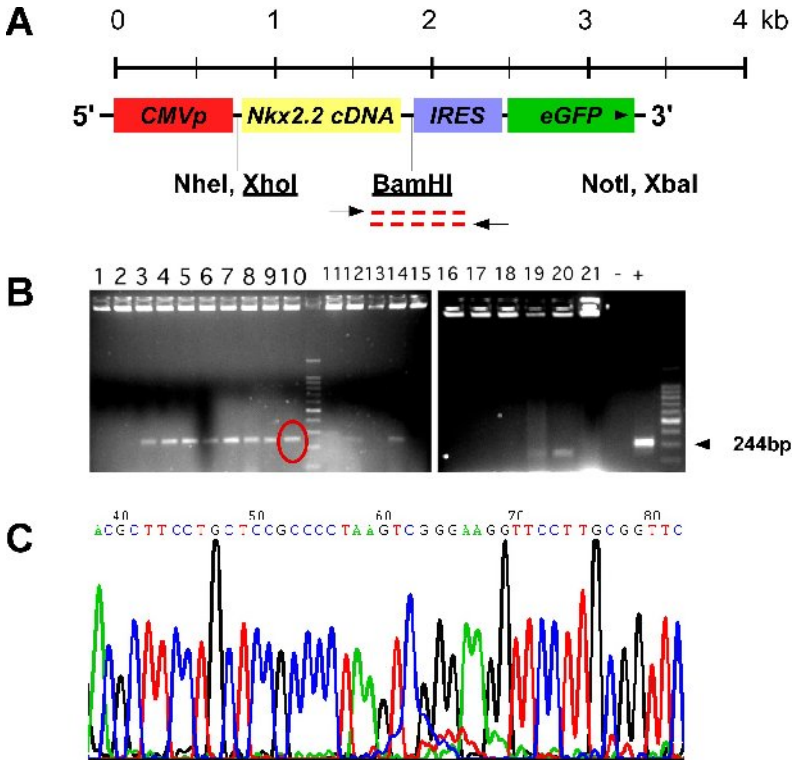

Figure 1.

Figure 2. Expression of NKx2.2 protein from HEK 293 cells transfected with pNKx2.2-IRES-eGFP constructed vector. 5µg of HEK 293 cellular extracts transfected with 10µg DNA of the pNKx2.2-IRES-eGFP construct were loaded into lane of 13% (A) and 11% (B) SDS-PAGE gel for NKx2.2, and β-actin protein detection, respectively. **A)** Immunoblotting with anti-hNKx2.2 antibody shows the mature form of the hNKx2.2 migrating as one band of apparent molecular mass of 32kDa (lane 2). No signals were detected from the mock transfected negative controls (lane 1) or from extract of cell transfected with pIRES2-eGFP mother plasmid (lane 3). Positive control is extract of primary culture of rat oligodendrocyte precursor CG4 cells (+). **B)** Immunoblotting of the membrane with anti-β-actin antibody shows the production of β-actin protein of the expected molecular mass of approximately 47kDa from cells. No other proteins were recognised from cell extract immunoprobed with these antibodies.

Do not forget a and b

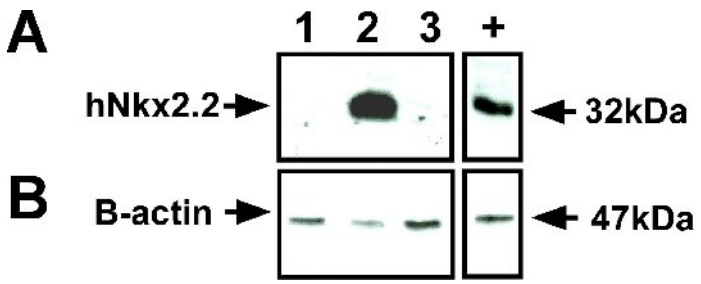

Figure 2.

Figure 3. Fluorescence microscopy analysis of IRES-mediated eGFP expression from pNKx2.2-IRES-eGFP bicistronic construct in mammalian cells.
A) Phase contrast photo of transfected HEK 293T cells. B) High levels of eGFP expression which confirms functionality of the bicistronic vector.

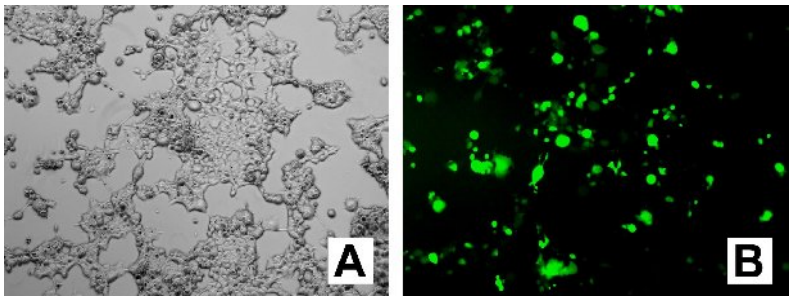

Figure 3.

DISCUSSION

When an IRES is used for the construction of a multi-cistronic cassette in gene transfer and expression vectors, it has been frequently observed that the DNA sequence subcloned at the 3' to the IRES element is poorly expressed compared to the sequence subcloned at the 5' of the IRES.[24,28] In most of the reported articles this phenomenon is attributed to the low efficiency of IRES mediated translation initiation compared to cap dependent translation. This is possibly due to the following; firstly, a higher affinity of translation factors for the cap structure than for the IRES element, therefore, transcription factors may become less available for the internal initiation of translation.[27] Secondly, the IRES may requires additional host *trans*-acting factors, which modulate its function in a cell or tissue type specific manner [(29,31)]. Thus, high levels of IRES mediated internal translation initiation can be anticipated depending on the nature of the IRES sequence and its tissue tropism.[13] Another possible explanation for this phenomenon is the length and possibly the nature of the ICS sequence flanking the IRES element at both its 5' and 3' ends. [13, 17]

In this work, an IRES derived from the ECMV genome was used to mediate eGFP marker gene translation initiation. We have successfully constructed bicistronic cassette containing the *hNkx2.2cDNA* and the *IRES/eGFP* elements. We then analysed the expression levels of the respective transgenes systematically at the DNA and protein levels. We showed functionality of the produced vector and the correct size of the transgenic proteins.

Expression efficiency of the eGFP was obtained when the ICS was 168bp in length. However, the expression levels of eGFP become completely undetectable at a spacer length of 450bp (data not shown). These results are in agreement with data published by Attal et al.[32] who found that IRES elements from ECMV as well as from poliovirus function optimally when about 100 nucleotides were added after the termination codon of the first cistron (luciferase). These authors also showed that IRES elements become totally inefficient when added after a 300-500 nucleotide spacer.[32] In a similar study using the IRES elements from poliovirus and from SV40, respectively in bicistronic cassettes containing the firefly luciferase gene as the first cistron and the *CAT* gene as the second cistron, Attal et al.[25] also showed that the expression of the second cistron was undetectable when the spacer fragment was 500 nucleotides. To exclude the effect of tissue tropism, we have also transfected our construct into Hela and hfMSCs cells and obtained similar results.

The produced bicistronic hybrid vector will be useful for simultaneous gene transfer and expression of the hNKx2.2 and the marker eGFP genes and of great value for stem cells transdifferentiation studies. The human *NKx2.2* gene encodes a homeobox protein which acts as a nuclear transcription factor involved in the morphogenesis of the central nervous system[33] and in the control of expression of myelin genes as *Nkx2.2* is able to drive gene expression of myelin proteolipid protein, an OL-specific marker. Specifically, cells hosting a proteolipid protein promoter-GFP reporter construct showed fluorescence when transfected with an Nkx2.2 encoding vector.[33] In addition to its role in neural development and differentiation, Nkx2.2 is also critical for the early pancreatic endocrine development and the following differentiation into pancreatic β cells.[34] as Nkx2.2 knockout mice are completely devoid of insulin expression.[35] In 2005, Zhang *et al.*[36] used transfection methods and expression plasmids to deliver *Nkx2.2* gene inside neural stem cells and accomplished a transient expression up to 12 days with a 60-80% efficiency of transfection. Accurate

assessment of gene transfer and expression following transfection may require a marker gene to evaluate levels and longevity of expression. In the present study, transfection of HEK 293 cells permit up to 90% transfection and eGFP expression.

We are also aware that transdifferentiation of stem cells by gene transfer may require long-lasting expression of the transgenic NKx2.2 in the relevant cell model, therefore, future work will focuses on subcloning the 5'-NKx2.2-IRES-eGFP-3' into an integrated virally based gene transfer vector system to ensure permanent expression of the integrated transgenes.

CONCLUSIONS

In conclusion**,** we have overcome the previously reported problem of insignificant second cistron expression and have produced a functional 5'-hNKx2.2-IRES-eGFP-3' expression cassette containing a 168bp spacer..

ACKNOWLEDGEMENT

The author would like to thank Miss Lia Zambitti for her valuable technical help and carry over the project to the next step. The author would also like to thank Dr Hussain Mehmet for providing all the plasmids, constructs, cDNA, and working environment to carry out this work.

REFERENCES

1. Chen WS, Villaflores OB, Jinn TR, Chan MT, Chang YC, Wu TY. Expression of recombinant human interferon-gamma with antiviral activity in the bi-cistronic baculovirus-insect/larval system. Biosci Biotechnol Biochem. 2011;75(7):1342-8.

2. Licursi M, Christian SL, Pongnopparat T, Hirasawa K. In vitro and in vivo comparison of viral and cellular internal ribosome entry sites for bicistronic vector expression. Gene Ther. 2011 Jun;18(6):631-6.

3. Ali Z, Schumacher HM, Heine-Dobbernack E, El-Banna A, Hafeez FY, Jacobsen HJ, et al. Dicistronic binary vector system-A versatile tool for gene expression studies in cell cultures and plants. J Biotechnol. 2010 Jan 1;145(1):9-16.

4. Ho SC, Bardor M, Feng H, Mariati, Tong YW, Song Z, et al. IRES-mediated Tricistronic vectors for enhancing generation of high monoclonal antibody expressing CHO cell lines. J Biotechnol. 2011 Oct 17.

5. Holt JR, Johns DC, Wang S, Chen ZY, Dunn RJ, Marban E, et al. Functional expression of exogenous proteins in mammalian sensory hair cells infected with adenoviral vectors. J Neurophysiol. 1999 Apr;81(4):1881-8.

6. Hoque AT, Liu X, Kagami H, Swaim WD, Wellner RB, O'Connell BC, et al. Construction and function of a recombinant adenovirus encoding a human aquaporin 1-green fluorescent protein fusion product. Cancer Gene Ther. 2000 Mar;7(3):476-85.

7. Kollen WJ, Mulberg AE, Wei X, Sugita M, Raghuram V, Wang J, et al. High-efficiency transfer of cystic fibrosis transmembrane conductance regulator cDNA into cystic fibrosis airway cells in culture using lactosylated polylysine as a vector. Hum Gene Ther. 1999 Mar 1;10(4):615-22.

8. Cullen BR, Lomedico PT, Ju G. Transcriptional interference in avian retroviruses--implications for the promoter insertion model of leukaemogenesis. Nature. 1984 Jan 19-25;307(5948):241-5.

9. Emerman M, Temin HM. Genes with promoters in retrovirus vectors can be independently suppressed by an epigenetic mechanism. Cell. 1984 Dec;39(3 Pt 2):449-67.

10. Emerman M, Temin HM. Quantitative analysis of gene suppression in integrated retrovirus vectors. Mol Cell Biol. 1986 Mar;6(3):792-800.

11. Kozak M. Adherence to the first-AUG rule when a second AUG codon follows closely upon the first. Proc Natl Acad Sci U S A. 1995 Mar 28;92(7):2662-6.

12. Al-Allaf FA, Tolmachov O, Themis M, Coutelle C. Coupled analysis of bacterial transformants and ligation mixture by duplex PCR enables detection of fatal instability of a nascent recombinant plasmid. J Biochem Biophys Methods. 2005 Aug 31;64(2):142-6.

13. Borman AM, Le Mercier P, Girard M, Kean KM. Comparison of picornaviral IRES-driven internal initiation of translation in cultured cells of different origins. Nucleic Acids Res. 1997 Mar 1;25(5):925-32.

14. Gallardo HF, Tan C, Sadelain M. The internal ribosomal entry site of the encephalomyocarditis virus enables reliable coexpression of two transgenes in human primary T lymphocytes. Gene Ther. 1997 Oct;4(10):1115-9.

15. Ramesh N, Kim ST, Wei MQ, Khalighi M, Osborne WR. High-titer bicistronic retroviral vectors employing foot-and-mouth disease virus internal ribosome entry site. Nucleic Acids Res. 1996 Jul 15;24(14):2697-700.

16. Saiz JC, Lopez de Quinto S, Ibarrola N, Lopez-Labrador FX, Sanchez-Tapias JM, Rodes J, et al. Internal initiation of translation efficiency in different hepatitis C genotypes isolated from interferon treated patients. Arch Virol. 1999;144(2):215-29.

17. Borman AM, Bailly JL, Girard M, Kean KM. Picornavirus internal ribosome entry segments: comparison of translation efficiency and the requirements for optimal internal initiation of translation in vitro. Nucleic Acids Res. 1995 Sep 25;23(18):3656-63.

18. Azzouz M, Martin-Rendon E, Barber RD, Mitrophanous KA, Carter EE, Rohll JB, et al. Multicistronic lentiviral vector-mediated striatal gene transfer of aromatic L-amino acid decarboxylase, tyrosine hydroxylase, and GTP cyclohydrolase I induces sustained transgene expression, dopamine production, and functional improvement in a rat model of Parkinson's disease. J Neurosci. 2002 Dec 1;22(23):10302-12.

19. Morgan RA, Couture L, Elroy-Stein O, Ragheb J, Moss B, Anderson WF. Retroviral vectors containing putative internal ribosome entry sites: development of a polycistronic gene transfer system and applications to human gene therapy. Nucleic Acids Res. 1992 Mar 25;20(6):1293-9.

20. Pizzato M, Franchin E, Calvi P, Boschetto R, Colombo M, Ferrini S, et al. Production and characterization of a bicistronic Moloney-based retroviral vector expressing human interleukin 2 and herpes simplex virus thymidine kinase for gene therapy of cancer. Gene Ther. 1998 Jul;5(7):1003-7.

21. Cormack BP, Valdivia RH, Falkow S. FACS-optimized mutants of the green fluorescent protein (GFP). Gene. 1996;173(1 Spec No):33-8.

22. Yang TT, Cheng L, Kain SR. Optimized codon usage and chromophore mutations provide enhanced sensitivity with the green fluorescent protein. Nucleic Acids Res. 1996 Nov 15;24(22):4592-3.

23. Jang SK, Krausslich HG, Nicklin MJ, Duke GM, Palmenberg AC, Wimmer E. A segment of the 5' nontranslated region of encephalomyocarditis virus RNA directs internal entry of ribosomes during in vitro translation. J Virol. 1988 Aug;62(8):2636-43.

24. Attal J, Theron MC, Houdebine LM. The optimal use of IRES (internal ribosome entry site) in expression vectors. Genet Anal. 1999 Nov;15(3-5):161-5.

25. Attal J, Theron MC, Puissant C, Houdebine LM. Effect of intercistronic length on internal ribosome entry site (IRES) efficiency in bicistronic mRNA. Gene Expr. 1999;8(5-6):299-309.

26. Brocard M, Paulous S, Komarova AV, Deveaux V, Kean KM. Evidence that PTB does not stimulate HCV IRES-driven translation. Virus Genes. 2007 Aug;35(1):5-15.

27. Brown EA, Zajac AJ, Lemon SM. In vitro characterization of an internal ribosomal entry site (IRES) present within the 5' nontranslated region of hepatitis A virus RNA: comparison with the IRES of encephalomyocarditis virus. J Virol. 1994 Feb;68(2):1066-74.

28. Romero-Lopez C, Berzal-Herranz A. The functional RNA domain 5BSL3.2 within the NS5B coding sequence influences hepatitis C virus IRES-mediated translation. Cell Mol Life Sci. 2011 May 20.

29. Dobrikova EY, Grisham RN, Kaiser C, Lin J, Gromeier M. Competitive translation efficiency at the picornavirus type 1 internal ribosome entry site facilitated by viral cis and trans factors. J Virol. 2006 Apr;80(7):3310-21.

30. Lourenco S, Costa F, Debarges B, Andrieu T, Cahour A. Hepatitis C virus internal ribosome entry site-mediated translation is stimulated by cis-acting RNA elements and trans-acting viral factors. FEBS J. 2008 Aug;275(16):4179-97.

31. Ray PS, Das S. La autoantigen is required for the internal ribosome entry site-mediated translation of Coxsackievirus B3 RNA. Nucleic Acids Res. 2002 Oct 15;30(20):4500-8.

32. Attal J, Theron MC, Rival S, Puissant C, Houdebine LM. The efficiency of different IRESs (internal ribosomes entry site) in monocistronic mRNAS. Mol Biol Rep. 2000 Mar;27(1):21-6.

33. Qi Y, Cai J, Wu Y, Wu R, Lee J, Fu H, et al. Control of oligodendrocyte differentiation by the Nkx2.2 homeodomain transcription factor. Development. 2001 Jul;128(14):2723-33.

34. Shiroi A, Ueda S, Ouji Y, Saito K, Moriya K, Sugie Y, et al. Differentiation of embryonic stem cells into insulin-producing cells promoted by Nkx2.2 gene transfer. World J Gastroenterol. 2005 Jul 21;11(27):4161-6.

35. Furuta H, Horikawa Y, Iwasaki N, Hara M, Sussel L, Le Beau MM, et al. Beta-cell transcription factors and diabetes: mutations in the coding region of the BETA2/NeuroD1 (NEUROD1) and Nkx2.2 (NKX2B) genes are not associated with maturity-onset diabetes of the young in Japanese. Diabetes. 1998 Aug;47(8):1356-8.

36. Zhang X, Cai J, Klueber KM, Guo Z, Lu C, Qiu M, et al. Induction of oligodendrocytes from adult human olfactory epithelial-derived progenitors by transcription factors. Stem Cells. 2005 Mar;23(3):442-53.

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 102-108 (2011)
07-012 © The University Press

UQU Medical Journal

Original Article

Role of White Cell Count and Neutrophil Differential in Diagnosis of Acute Appendicitis

Jamal T. Hamdi
Department of Surgery, Umm Al-Qura University, Makkah, KSA.

Correspondence:
Jamal T. Hamdi
Associate Professor of Surgery
P.O.Box:8343, Faculty of Medicine, Umm Al-Qura University
Makkah
Mobile: 0509506820
e.mail: jthamdi@uqu.edu.sa

Received: April 11, 2011 *Accepted: May 16.2011*

دور عدد كرات الدم البيضاء والعدلات التفاضلية في تشخيص التهاب الزائدة الدودية

د.جمال حمدي
قسم الجراحة-,كلية الطب – جامعة أم القري – ص. ب: 8343 - مكة المكرمة- المملكة العربية السعودية

الملخص العربي

التهاب الزائدة الدودية الحاد هو أكثر حالة جراحية حادة في البطن تحتاج الى تشخيص دقيق لالتهاب الزائدة الدودية ، مما يؤدى إلى ارتفاع معدلات استكشاف البطن السلبية خاصة في الإناث ، وكذلك تأخر التشخيص وانفجار الزائدة الدودية عند آخرين. تناول هذه الورقة تقييم دقة التشخيص من مزيج من عدد كرات الدم البيضاء والنسبة المئوية للعدلات التفاضلية على تشخيص التهاب الزائدة الدودية الحاد.

المنهجية: دراسة استطلاعية أجريت على مائة مريض أزيلت منهم الزائده الدودية. تم قياس عدد كرات الدم البيضاء والعدلات التفاضلية لكل مريض.تم حساب الحساسية والنوعية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية لكل اختبار بمفرده وبالاشتراك مع الاختبار الأخر على قيم مختلفة ومتنوعة.

النتائج :كان هناك 46 من الذكور و 54 من الإناث , مع 80 ٪ من المرضى في نطاق 10 الي 30 عاما. وعثر على الزائده الدوديه ملتهبة في 80،4 ٪ من الذكور ، و 64،8 ٪ من المرضى من الإناث. وكان خصوصية كرات الدم البيضاء 100% إذا تم تعيين القيمة في (> 16 × 10^9 / لتر) ، والعدلات التفاضلية 93 %إذا تم تعيين القيمة > 85 ٪ . الحساسية والنوعية والقيمة التنبؤية الإيجابية والقيمة التنبؤية السلبية والكفاءة لاختبار كرات الدم البيضاء > 10 × 10^9 / لتر ، والعدلات التفاضلية > 75 ٪ التي هي الحدود العليا من الاختبارات العادية تم قياسها علي حده، بالاضافه الي مزيج من الاختبارين.

مناقشة: في هذه الدراسة كرات الدم البيضاء > 10 × 10^9 / لتر والعدلات التفاضلية > 75 ٪ أعطت أعلى كفاءة 75 ٪ لكل اختبار علي حده. الجمع بين الاختبارين أدي إلي كفاءة 83 ٪ ، وحساسية من 93،1 ٪ ، والقيمة التنبؤية الإيجابية من (84.8 ٪) ، وهو مستوي معقول لتشخيص التهاب الزائدة الدودية الحاد ، وتجنب عمليات الاستكشاف السلبية.

ABSTRACT

Acute appendicitis is the most common severe surgical condition of the abdomen. The accurate diagnosis of appendicitis is difficult, resulting in a higher incidence of negative laparotomies especially in females, as well as delayed diagnosis and rupture of appendix in others. The aim of the present work was to assess the diagnostic accuracy of the combination of white cell count (WCC) and neutrophil differential (ND) on the diagnosis of acute appendicitis.

Methodology: A prospective study was carried out on one hundred consecutive patients. The white cell count (WCC) and neutrophil differential (ND) tests were done for every patient. The sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of the individual test were assessed at different values. The SN, SP, PPV, NPV, and efficiency of WCC>10×10^9/L, and ND>75% which are the upper limits of normal tests, were measured, as well as the combination of the two tests.

Results: There were 46 males and 54 females with 80% of the patients in the range 10 to 30 years. The acutely inflamed appendix was found in 80.4% of male, and 64.8%of female patients. The specificity of the WCC was 100 if the value was set at (>16×10^9/L), and of ND was 93 if the value was set as >85%.

Conclusion: In this study WCC at cut off >10×10^9/L and ND>75% gave the highest efficiency of 75% for each test on its own. The combination of the two tests gave an efficiency of 83%, a sensitivity of 93.1%, and positive predictive value of (84.8%), which is reasonably accurate to diagnose acute appendicitis, and avoid negative laparotomy.

***Keywords*:** White cell count, neutrophil differential, specificity, sensitivity.

INTRODUCTION

Acute appendicitis is the most common severe surgical condition of the abdomen[1]. The overall mortality rate for appendicitis is less than 1%, but it increases to 3% if the appendix is ruptured and approaches 15% in the elderly.[2] The diagnosis of appendicitis is more difficult in the extremely young and the elderly, resulting in a higher incidence of delayed diagnosis and rupture in these populations. Because a ruptured appendix can be associated with increased morbidity and mortality, it is felt that a certain number of negative laparotomies is acceptable (approximately 15% in the United States). However, negative laparotomies are twice as common in young women as in men (20% vs. 9%, respectively). [3, 4]

Combining various signs and symptoms into a scoring system may be more useful in predicting the presence or absence of appendicitis. The Alvarado score, originally described in 1986, is the most widely reported scoring system for acute appendicitis [5] (Table 1). The Alvarado score combines patient symptoms, physical examination results, and laboratory values to assign a score from 0 to 10.

Table 1: **Alvarado score in acute appendicitis.**

		Value
Symptoms	Migration	1
	Anorexia-acetone (in the urine)	1
	Nausea-vomiting	1
Signs	Tenderness in right lower quadrant	2
	Rebound pain	1
	Elevation of temperature (>37.3°C measured orally)	1
Laboratory	Leukocytosis (>10,000/mm^3)	2
	Shift to the left (>75% neutrophils)	1
Total score		10

Wang [6] reported a positive likelihood ratio of 9.8 for both elevated white blood cells(WBC) count and left shift, but the diagnostic value of the WBC count remains controversial[7].Many authors found a relatively unimpressive positive likelihood ratio (LR) between 1.59 and 2.7 and a negative ratio between 0.25 and 0.50 for the WBC count in appendicitis. [8, 9, 10, 11] Nearly over 50% of all emergency appendectomies were performed on normal or mildly inflamed appendix. This figure rises to 60% if we consider females alone. [12, 13] These patients would definitely benefit from conservative treatment with observation and antibiotics, if we can identify them preoperatively.[14,15] Hence the recent trend for more conservative treatment and more diagnostic accuracy of non inflamed appendix to avoid negative appendectomies. This paper tries to assess the diagnostic accuracy of the combination of white cell count (WCC) and neutrophil differential (ND) on the diagnosis of acute appendicitis.

MATERIAL AND METHODS

This was a prospective study which was carried on one hundred consecutive patients admitted with the diagnosis of acute appendicitis through the emergency room of a district hospital and subjected to surgery for that diagnosis. The white cell count (WCC) and neutrophil differential (ND) tests were done for every patient prior to surgery. The histopathology of the removed appendix was taken as the golden standard for the presence or the absence of acute appendicitis. Different values of WCC and ND were assessed against the presence or absence of acute appendicitis. The sensitivity (SN), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of the individual test were assessed at different values including the upper normal limit of each test and for the combination of the two tests.

RESULTS

A total on one hundred patients were included in this study. There were 46 males and 54 females. The range of age was from 7 years to 45 years, with 43% between 10 to 20 years, and 37% between 20 to 30 years, accordingly, 80% of the patients were in the range 10 to 30 years. According to the histopathology records; acute appendicitis either perforated or non-perforated was found in 72% compared to normal appendix in 28%. The acutely inflamed appendix was found in 37 males (80.4% of all males), and 35 females (64.8%of all females).

Table (2) shows the mean values of WCC and ND in both acutely inflamed appendix and normal appendix groups. Using t-test there is a significant difference ($p<0.05$) between the two groups in both WCC and ND.

Table 2: showing the mean values of WCC and ND in both appendicitis and non-appendicitis groups.

Test	Total	Appendicitis	Non-appendicitis
WCC($\times 10^9$/L	10.8	11.7	8.5
ND (%)	72%	77%	59%

The specificity of the WCC was 100 if the value was set at (>16×10^9/L), but the sensitivity was only 19.4 at that set value, which means that if you suspect acute appendicitis and WCC>16×10^9/L, then it is very likely that the appendix is inflamed, but only a small proportion of acute appendicitis present at that level.
The specificity of the ND was 93 if the value was set as >85%,but the sensitivity at that value was only 33.3,which means that if you suspect acute appendicitis and ND>85% then it is likely that the appendix is inflamed , but only a third of acute appendicitis can be detected at that level(Table 3).

Table 3: showing Sensitivity and Specificity to WCC (>16×10^9/L) and ND (>85%)

Test	Sensitivity	Specificity
WCC>16×10^9/L	19.4	100
ND>85%	33.3	93

Table(4) shows the sensitivity(SN), Specificity(SP),positive predictive value(PPV), negative predictive value(NPV),and efficiency of WCC>10×10^9/L which is considered as the upper limit of normal WCC, and ND>75% which is considered also as the upper limit of normal neutrophil percentage. The combination of these two upper normal limits of WCC and ND is also shown in the table. The relative efficiency of these two tests is 83, comparing with a figure of 75 for each test on its own.

Table 4: showing SN, SP, PPV, NPV and efficiency of WCC>10×10^9/L and ND>75% and the combination of the two tests

Test	SN	SP	PPV	NPV	Efficiency
WCC>10×10^9/L	76.4	71.4	87.3	54	75%
ND>75%	79.2	64.3	85	54.6	75%
WCC+ND	93.1	57	84.8	76.2	83%

DISCUSSION

This was a prospective clinical study on 100 consecutive patients with appendicectomy operations. Appendiceal inflammation proved by histopathology in 72%, with negative laparotomy in 28% and perforation in 9%.These figures are compatible with other studies with laparotomy ranging from 11-76% and perforation rate ranging from 8-29%.[3,15,16] The diagnostic accuracy was 80.4%for males, but only 64.8%for females, which is compatible with other studies.[15,16,17]

The mean values for WCC and ND of the appendicitis group were 11.7×10^9/L and 76.9%.In non-appendicitis group the values were 8.49×10^9/L and 59.3% respectively. There was a significant difference between the mean values of the groups, which reflects clinical values of WCC and ND in the diagnosis of acute appendicitis.

The diagnostic accuracy of any test could be improved by changing the cut off values above which the test is considered positive. By lowering this level the sensitivity of the test increases, but at the expense of the specificity of the test. If the cut off is elevated then the sensitivity detected by the test decreases, while the specificity increases. The optimal discriminating point is that one which gives the highest efficiency.

In this study WCC at cut off $>10\times10^9$/L and ND>75% gave the highest efficiency of 75% for each test on its own. The combination of the two tests gave an efficiency of 83%, which is reasonably good for any test or combination of tests. Also this combination has a sensitivity of 93.1%, which means that the false negatives are very low relative to the true positives. Also the positive predictive value is reasonably good (84.8%), which means that the false positives are reasonably low relative to true positives.

The clinical application of this data means that if you apply this combination of tests on clinically suspected cases of acute appendicitis, then you can pick up the vast majority of true acute appendicitis. Good clinical acumen would compensate for the low specificity and reduce the rate of negative appendicectomy operations.

ACKNOWLEDGEMENT

Special thanks and gratitude to Dr Ala Hasan; my ex-resident for his great help in the conduction of the study.

REFERENCES

1. Liu CD, McFaden DW. Acute abdomen and appendix. In: Greenfield LJ, (ed.) Surgery: Scientific Principles and Practice, Philadelphia: Lippincott-Raven, 1997: 1246-1261.

2. Yeh B: Does this adult patient have appendicitis?. Ann Emerg Med 2008 ;52: 301-303.

3. Velanovich V, Satava R: Balancing the normal appendectomy rate with the perforated appendicitis rate. Am Surg 1991; 52: 264-269.

4. Colson M, Skinner KA, Dunnington G: High negative appendectomy rates are no longer acceptable. Am J Surg 1997; 174: 723-727.

5. Alvarado A.: A practical score for the early diagnosis of acute appendicitis. Ann Emerg Med 1986; 15: 557-564.

6. Wang LT, Prentiss KA, Simon JZ, et al: The use of white blood cell count and left shift in the diagnosis of appendicitis in children. Pediatr Emerg Care2007; 23: 69-76.

7. Raftery AT: The value of the white blood cell count in the diagnosis of acute appendicitis. Br J Surg 1976; 63: 143-144.

8. Andersson RE: Meta-analysis of the clinical and laboratory diagnosis of appendicitis. Br J Surg 2004; 91: 28-37.

9. Cardall T, Glasser J, Guss DA: Clinical value of the total white blood cell count and temperature in the evaluation of patients with suspected appendicitis. Acad Emerg Med 2004; 11: 1021-1027.

10. Kessler N, Cyteval C, Gallix B, et al: Appendicitis: evaluation of sensitivity, specificity, and predictive values of US, Doppler US, and laboratory findings. Radiology2004; 230: 472-478.

11. Birchley D.: Patients with clinical acute appendicitis should have pre-operative full blood count and C-reactive protein assays. Ann R Coll Surg Engl 2006; 88: 27-32.

12. Rao PM, Rhea JT, Novelline RA, Mostafavi AA, McCabe CJ. Effect of computed tomography of the appendix on treatment of patients and use of hospital resources. N Engl J Med 1998; 338(3): 141-146.

13. Bijnen C, van den Broek W, Bijnen A, Ruiter P, Gouma D. Implications of removing a normal appendix. Digestive Surgery 2003; 20(3): 215-221.

14. Liu K, Ahanchi S, Pisaneschi M, Lin I, Walter R. Can acute appendicitis be treated by antibiotics alone? Am Surg 2007; 73(11): 1161-1165.

15. Hamdi J. Is there a Place for Conservative Treatment of Acute Appendicitis? JKAU: Medical Sciences 2010; 17 (1) : 11-17.

16. Seetahal S, Bolorunduro O, Sookdeo T et al. Negative appendectomy: a 10-year review of a nationally representative sample The American Journal of Surgery 2011; 201: 433–437.

17. Sakorafas G, Mastoraki A, Lappas C, Sampanis D, Danias N, Smyrniotis V. Conservative treatment of acute appendicitis: heresy or an effective and acceptable alternative to surgery? European Journal of Gastroenterology & Hepatology.2011 23(2):121-127.

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 121-125 (2011)
07-012 © The University Press

UQU Medical Journal

Brief Communication

Accreditation Is It a Fashion or Necessity?

Suliman Abdulaziz Jastaniah
Fellowship (trauma),Canada,Department of surgery,Umm-Alqura faculty of medicine,Makkah, KSA.

Elbagir Ali A. Elfaki
Department of Surgery,Faculty of Medicine,Umm-Alqura University,Makkah,KSA.

Correspondence:
Dr. Suliman Abdulaziz Jastaniah. FRCSE. FACS.
Associate Professor of surgery

Umm Alqura University - Medicine
P.O.Box 1821 , Jeddah 21441
Saudi Arabia
sa_jastaniah@hotmail.com

Professor. Elbagir Ali A. Elfaki MBBS. FRCS. FRCSI. FACS.
Professor of surgery,Umm-Alqura faculty of Medicine,Makkah

Saudi Arabia Mobile: 0558803253
e.mail: albagirali@hotmail.com

Received: March 02, 2011 *Accepted: March 27, 2011*

الاعتماد الاكاديمي هل هو موضة ام ضرورة ؟

سليمان عبد العزيز جستنية * الباقر علي احمد الفكي
قسم الجراحة- كلية الطب- جامعة أم القرى- مكة المكرمة- المملكة العربية السعودية مستشفى النور التخصصي-مكة المكرمة

الملخص العربي

الهدف الرئيسي من هذه الورقة هوالتقديم لمفهوم الاعتماد الأكاديمي كليات الط ب.ناقشاهمي ة الاعتم اد الاك اديمي و التعريف والأغراض ،والفوائد ومعايير وإجراءات اعتماد المؤسسات الطبيةمع خارطة طريق الاعتماد الا ك اديمي لكون ه ضرورة وتطرقتنا باختصارالقض ايا للنق اش بش أن اعتم اد المؤسس ات الطفيق ة للمع ايير القياس ية العالمي ة وذل ك لك ون اهمي ةالتعل يمالطب يوتنط وي ه نفوالق مطق مونها الع ام عل ى التط وير والتح ديثلمبدالاعتم اد الأك اديمي ومبادئ ضمان جودة التعليم والاعتماد.

ABSTRACT

It is argued that accreditation is one of the influential mechanisms for assessing the performance of healthcare organizations and improving the quality and safety of healthcare services. Its aim is to encourage continuous improvement of quality rather than simply maintaining minimal levels of performance. It is also described as the public recognition emanating from the achievement of specific standards by a health care which is demonstrated after an independent external assessment of the organization's performance. Accreditation process involves evaluating programs in light of their own training models and goals and judging the degree to which a program has achieved those goals and objectives. It is the processes by which the programme's outcomes should be reached; rather it evaluates a programme's success in achieving outcomes and goals that are consistent with its stated mission (including religious mission, if relevant). Accreditation is viewed as an important process in the continuing development of quality health service management curricula and preparing competent practitioners for the health care field.

Introduction and Background

Medicine is becoming increasingly globalized, as manifested by the worldwide growth in the number of medical schools.[1] Presently, as of 2010, there were 2,200 recognized and operating medical schools (public and private) in 177 countries or territories listed in the International Medical Education Directory (IMED).[2] Within this listed medical institutes, more than 300 located in the 22 countries of EMRO region. Relatively little is known about the characteristics or the quality of these institutions. In a world with this expansion in medical schools and rapidly changing technology, certifying an adequate process of education is challenging.

There is significant variation in the structure and quality of undergraduate medical education around the world. Their graduates are educated in countries with diverse educational systems, including variations in teaching traditions, curricular models, instructional methods, clinical opportunities, assessment principles, and available resources[3]. A rising urgency of accountability and accreditation for medical education developed in the late 20th century, also as a result of individuals and societal expectations with the rise in the numbers of physicians who migrate from their native countries to other parts of the world for medical education and graduate training opportunities. Accreditation is viewed as an important process in the continuing development of quality health service management curricula and preparing competent practitioners for the health care field 4. The need for starting this process has come both from a self felt requirement of the rapid growth of medical schools and an external initiative.[5]

Along with global expectations the concept of accreditation is becoming a necessity and important issue for learners, educators, regulatory bodies and the public in general. Accreditation processes can encourage institutional improvement and help promote high-quality education experiences.[6, 7]

What is accreditation and what its purpose?

Accreditation is a process by which a group of peers and medical education experts systematically review every facet of a medical institution programme and its contributing elements, and determine whether the quality of the education provided to the students meets the high standards expected. It is a pprocedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks 8. Accreditation is also known as a process of quality assurance aiming to fosters programme improvement and continuously upgrade the educational and service quality. It is fundamental to be based on standards which is a statement of excellence, eveloped by peers, against which conformity of the agency is evaluated. The standards for accreditation are set by a peer review board whose members include faculty from various accredited colleges and universities and perhaps accredited bodies. These standards can be international, regional, national or sub-national (in countries with large numbers of medical schools).[9]

The basic characteristics of accreditation its prevailing sense of volunteerism but strongly recommended, strong tradition of self-regulation, reliance on evaluation techniques and primary concern with quality.

Purpose and benefits of accreditation:

An important factor in realizing a successful career is choosing a reputable institute. Institutes that have been through the accreditation process are more likely to offer degrees those employers, clients and recruiters recognize. By accreditation the institute will: ensure the accountability of educational institutions and provide public confidence in educational processes. Also institutional strengths can be identified, and strategies can be put in place to ensure strengths are maintained. Any problems or issues requiring action can be addressed. Accreditation decisions are usually limited to a fixed and stated period of time, after which the institution or programme is required to engage with a more or less rigorous re-accreditation process.[10]

Types of Accreditation:

Two recognized types:

1- Institutional accreditation: Most accreditation is institutional, meaning an entire college is accredited. This gives credibility to the college as a whole. This type of accreditation can be given by either a “regional accreditation agency” or a “national accreditation agency.”

2- Specialized and programmatic accreditation: Certain specialized professional programs are accredited independently of their parent institution. There are specialized accrediting bodies for these programs.

What is the difference between program accreditation and institutional accreditation?
Program Accreditation: refers to the accreditation of academic courses such as liberal arts, sciences, education, commerce, law, engineering, nursing, etc.

Institutional Accreditation: refers to the accreditation of the school, college, universities or institution as a whole. Only when all the programs of an institution are accredited may that particular institution be considered an accredited institution.

Road map and accreditation process:

The accreditation process requires educational programs to provide assurances that their graduates exhibit general professional competencies that are appropriate for entry to the next stage of their training, and that serve as the foundation for life-long learning and proficient medical care. In order for potential institute to proceed with the accreditation process smoothly, they must meet the general standards set by the peer review accreditation and other accepted global rules for accreditation. Each institute is typically assessed using the following pathway: First of all the institute should be legible for accreditation and evaluated in terms of the appropriateness and adequacy of:

- Mission, objectives and Goals.
- Student Requirements for Admissions.
- Services Available to Students.
- Quality of Education.
- Reputation of Faculty.

Necessity of accreditation:

Most of us if not all have learned important material from unaccredited programmes over the course of our career. This is not to say that so called unaccredited programms do not have educational value.

But on the regional and global movement of health professionals, the complexity of international labour markets and trade agreements make render such procedure as accreditation as a mandatory element of any educational program.. In addition, the demands of different regulatory bodies for well defined graduates competences, safe doctors and fitness-to-practice add much pressure on all health professionals' education institutes and governments to start initiating and sustaining national and institutional systems of accreditation as soon as possible. All these factors began to pressure institutions to prove their worth in clear ways.

CONCLUSION

The accreditation is expected to help today doctors to cope with the explosion in medical and scientific knowledge and technologies by acquiring the abilities of lifelong self learning.[11] For institution accreditation is expected to initiate and promote culture of external evaluation and self-review, and to assist in resource mobilization. With globalization (manifested by expansion in trade in health services accreditation and in numbers of migrating doctors) can be a tool for international recognition and categorization of medical schools.[12]

REFERNCES

1. Boulet J, Bede C, McKinley D, Norcini J. An overview of the world's medical schools. Med Teach. 2007;29:20–26.

2. FAIMER“ foundation of advancement of international medical education and research, Philadelphia19104-2685,USA.2011. boulet@faimer.org.

3. The Commission (2010). Commission on Education of Health Professionals for the 21st Century. Education of health pro-fessionals for the 21st Century: a global independent commission. Lancet. 2010 ;375:1137-1138.

4. Hallock JA, McKinley D, Boulet J. Migration of doctors for undergraduate medical education. Med Teach. 2007;29:98–105.

5. Sukkar M Y. Accreditation of medical schools in the Sudan. Khartoum Medical Journal 2008; 1,1 , 49-50

6. Karle H. International trends in medical education: Diversification contra convergence. Med Teach. 2004;26:205–206.

7. Zanten M, Norcini JJ, Boulet JR, Simon F..(Overview of accreditation of undergraduate medical education programmes worldwide. Med edu. 2008 Sep;42(9):930-7.

8. ISO/IEC Guide 2:1996).

9. World Health Organization, World Federation of Medical Education. Accreditation of medical education: report of a technical meeting, Schaefergarden, Copenhagen, Denmark, 4-6 October 2004, PP 1-25

10. Vlãsceanu, L., Grünberg, L., and Pârlea, D., 2004, Quality Assurance and Accreditation: A Glossary of Basic Terms and Definitions (Bucharest, UNESCO-CEPES) Papers on Higher Education, ISBN 92-9069-178-6.

11. World Health Organization, World Federation of Medical Education. Accreditation of medical education: report of a technical meeting, Schaefergarden, Copenhagen, Denmark, 4-6 October 2004, 1-25.

12. World Health Organization Eastern Meditteranean Regional Office, Accreditation of hospitals and medical education institutions: A. Hospitals. Cairo, 2004.

Umm Al-Qura Univ. Medical J. Vol. 2, No.4, pp. 126-131 (2011)
07-012 © The University Press

UQU Medical Journal

A Case Report

CONGENITAL EPULIS: *A CASE REPORT*

Osama A Bawazir
Umm-Alqura Faculty of Medicine, Consultant Pediatric surgery and Pediatric urology, King Faisal Specialist Hospital and Research Centre, Jeddah, KSA.

Correspondence:
Dr. Osama A Bawazir, MBBS. FRCSC, FRCS(I, Edin, Glas), FAAP. FACS
Associate Professor of surgery

Umm Alqura University - Medicine
P.O.Box 7607 , Makkah
Saudi Arabia
e.mail: obawazir@yahoo.com
Mobile: 00966-503660669

Received: November 01, 2011 *Accepted: November 28, 2011*

الورم اللثوي الخلقي: تقرير حالة

د. اسامة باوزير
أستاذ مساعد
جامعة أم القرى، كلية الطب، والجراحة استشاري أمراض الأطفال وجراحة المسالك البولية للأطفال، مستشفى الملك فيصل التخصصي ومركز الأبحاث، جدة، السعودية.

الملخص العربي

يعد الورم اللثوي الخلقي او ورم نيومان او الـ ورم الحبيبي الخلقي لحديثي الـ ولادة من الاورام النادرة التي تنشأ من خلايا حرف اللثة وهو ورم حميد يحدث في حديثي الولادة بنسبة 1:9 (الاناث:الذكور) وجز هنا حالة مسجلة لـ ورم لثوي خلقي بالجانب الايسر لحرف الفك العلوي لطفلة سعودية تبلغ من العمر ثلاث أيام لاحظت والدتها صعوبة الرضاعة والتغذية الفمية دون انسداد في التنفس تم استئصاله جراحيا بالتجلخ د لطبيعة الـ ورم بالتشخيص النسيجي كما قمنا بعرض الادب الطبي لهذه الاورام عند حديثي الولادة مع عرض وسائل التشخيص ونسبة حدوثه مع الاهتمام بالتشخيص التفريقي

ABSTRACT

Congenital epulis , Neumann's tumor or congenital granular cell tumor (CGCT), of the newborn is a rare gingival tumor that occurs along the alveolar ridge.This benign condition, seen more frequently in females, with multiple Epuli occurring in م only 10% of cases. In this article we present a case report of an otherwise healthy Saudi female neonate with congenital epulis noticed at birth on the left anterior maxillary ridge intervening with feeding and was not cause any respiratory obstruction. We also present a review of the literature and an estimation of the incidence of Congenital Epulis of 0.006% is reported.

INTRODUCTION

Medicine The term congenital epulis of the newborn refers to a rare gingival tumor that most commonly occurs along the alveolar ridge of the maxilla in newborn. Congenital epulis is a rare lesion found on the alveolar process of a newborn child, diagnosed soon after birth. Usually without associated abnormalities of the teeth or additional congenital malformations. The characteristic features of congenital epulis are a pedunculated, flesh-pink coloured tumour with a predominant occurrence on the anterior maxillary alveolar ridge in a female newborn[8] depend on their size they interfere with normal feeding and potentially compromise airway and respiration. The lesion has a site predilection for the anterior maxillary alveolar process and a 9:1 sex predilection for females. Once diagnosed the traditional management of the lesion has been surgical excision under general anesthesia.

Neumann is credited in documenting the first case congenital epulis. In 1871 he described a red smooth-surfaced bilobed tumor resembling a polyp that was attached by a stem to the gums on the left jaw's upper edge of a normally built/shaped newborn. He portrayed the tumor as being composed of large coarse-grained cells with numerous blood vessels that was separated from the overlying oral mucosa by a loosely defined boundary1. Since then, multiple cases have been reported, primarily in the pathologic, dental, and otolaryngologic literature 2–4. Two recent articles in the otolaryngologic literature report the in utero sonographic findings of larger lesions. In one case,the patient had hydramnios; in the other case, the authors also describe the postnatal computed tomographic (CT) findings.[3,4] It is normally diagnosed at birth, but there are some cases in which diagnosis can be made on the third trimester pre-natal.[5] The recommended treatment for congenital epulis is prompt surgical excision due to interferences with feeding, respiration or adequate closure of the mouth.[6] Although some cases of congenital epulis have been reported in the literature 6., it is important to allow pediatric dentists to be aware of this congenital tumor and its presentation, differential diagnosis, treatment and histopathology.[7]

CASE

A full term newborn Saudi girl , product of unevntfull pregnancy to 34 years old mother with history of smoking during the pregnancy (1 pack/day). This newborn noticed at birth to have a soft tissue mass protruding from her mouth. The mass did not seen on antenatal ultrasound which was done on the 32 weeks. At birth the baby weight was 2900g. On examination

pedinculated firm mass measure 1 x 1.5 cm., fix to the anterior alveolar ridges toward the left side with no ulceration or bleeding ,no other oral or congenital abnormality was found, and the adjacent tissues were normal in appearance Fig 1. The mother reported that the baby have feeding problems with no airway obstruction or respiratory distress. Differential diagnosis of teratoma, neuroectodermal tumour, haemangioma and fibroma with a provisional diagnosis of congenital granular cell tumour (CGCT). Nasogastric feeding was instituted and 3 days after birth, the infant was operated on under general anesthesia with oral intubation and cautary was use. Blood loss was insignificant. The area of the resection was left open for closure by secondary intention Fig 2. Uneventful post operative course,patient start oral feed in first post operative day and discharged on the second post operative day, the gingiva re-epithelized completely within 2 days Fig 3. Histopathology examination of the tumor confirmed the diagnosis of a congenital GCT,

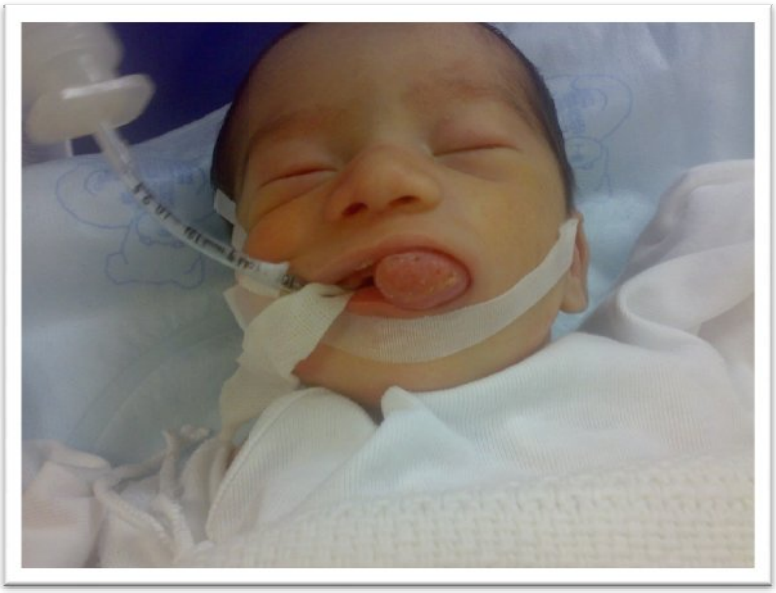

Figure 1. PRE-OPERATIVE
Smooth, pink-colored soft tissue mass on the alveolar ridge to the left of the alveolar ridge, with no other anomalies could be observed.

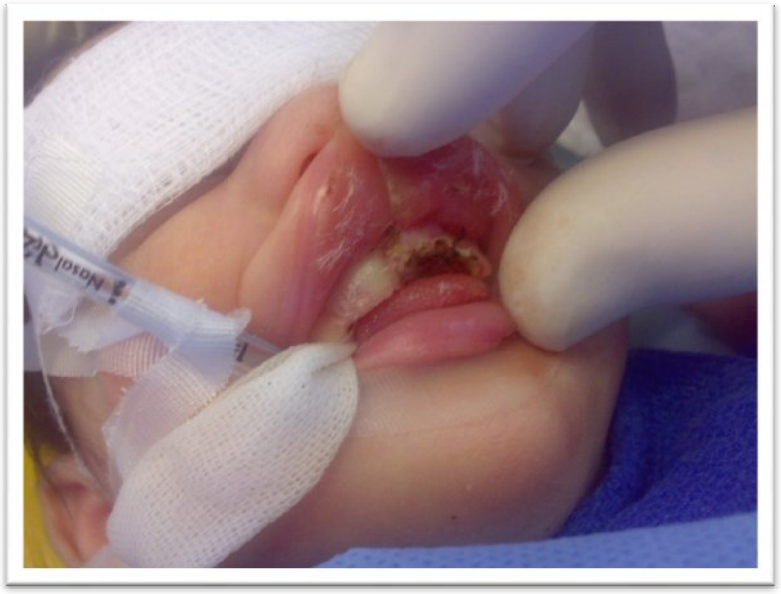

Figure 2. POST - OPERATIVE
Immediate post operative appearance,showing good haemostasis and cosmosis.

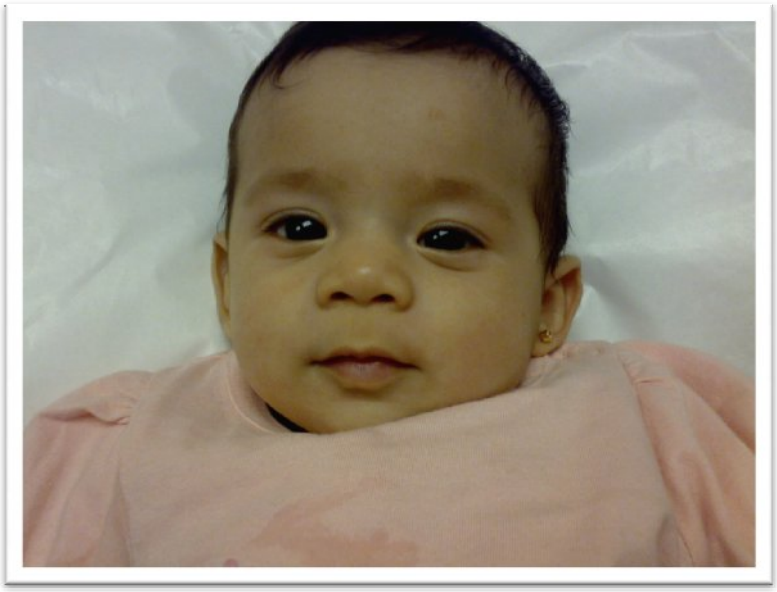

Figure 3. Post-operative clinical image 8 months after surgery. vivid looking .

DISCUSSION

Congenital epulis has been reported with an 9:1 female and 3:1 maxillary alveolar site predilection, with a Caucasian predisposition.[8] Zucker and Buenecha found only 167 cases reported before 1993-9. Some antenatal events have been described associated with CE. Pellicano etal.[10] have reported that the tumor may obstruct the fetal mouth and cause polyhydramnios, a medical condition describing an excess of amniotic fluid in the amniotic sac. Post-natally, feeding and respiration problems and also interference with mouth closure have been reported.[11] In the present case, the lesion was not interfering with feeding and breathing, which could be attributed to lesion size, as already described by Kannan and Rajesh.[12] It is important to stress that clinicians should know differential diagnoses of growths in the oral cavities of newborns, including hemangioma, lymphangioma, fibroma, granuloma, rhabdomyosarcoma and osteogenic and chondrogenic sarcomas, as treatment modalities will be different for each case.[2,13] In the present case, the clinical diagnosis of congenital epulis was further confirmed by the histopathology of the fibrotic mass removed from the patient . which showed a stratified squamous mucosa and a prominent branching fibrovascular network. In our case, the lesion was interfering with feeding and was not interfering with breathing, which could be attributed to lesion size, as already described by Kannan and Rajesh.[12] Our case,is not associated with any other congenital abnormalities. Perhaps our case has an atypical presentation of a CE, which is a female Caucasian neonate diagnosed with a pedunculated mass in the maxillary alveolar ridge. In our case the tumor was not diagnosed by ultrasoundon the 25th week of gestation, suggesting that it may have developed later. There have been reports of prenatal diagnosis and in those cases, in which the tumor was detected late in gestation.[14] The treatment adopted in this case was the surgical excision under general anesthesia ,using cautery as cutter and haemostatic measures. It is important to stress that clinicians should know differential diagnoses of growths in the oral cavities of newborns, including teratoma, hemangioma, lymphangioma, fibroma, granuloma, rhabdomyosarcoma and osteogenic and chondrogenic sarcomas, as treatment modalities will be different for each case,and team work is mandatory. The presentation of congenital epulis can be impressive due to size and appearance, although in our case the lesion was relatively small, a considerable apprehension by the mother observed. Therefore, surgical intervention should be performed as soon as possible to benefit both infant and family well-being.

CONCLUSION

CGCT is relatively common in Caucasia newborns but appears to be much less common in colored or black newborns, and the occurrence of more than one lesion is rare. The tumor is often misdiagnosed and cause anxiety to the family and pediatrician. The family of an infant with CGCT should be assured of the benign nature and the simple early excision is the treatment of choice for this condition with good cosmetic result. Recurrences of the tumor and damage to future dentition have not been reported, suggesting that radical excision is not warranted. Simple excision of the tumor is the treatment of choice with good cosmetic result.

REFERENCES

1. Neumann E: Ein fall von kongenitaler Epulis. Arch Heilkd 1871, 12:189-190
2. Anderson PJ, Kirkland P, Schafler K, Moss AL: Congenital gingival granular cell tumor. J R Soc Med 1996, 89: 53-54.
3. Kim ES, Gross TL. Prenatal ultrasound detection of a congenital epulis in a triple X female fetus: a case report. Prenatal Diagn. 1999;19: 774–776
4. Koch BL, Myer C 3rd, Egelhoff JC: Congenital epulis. AJNR Am J Neuroradiol 1997, 18:739-741
5. Kópers AM, Andriessen P, van Kempen MJ, van der Tol IG, Baart JA, Dumans AG, et al. Congenital epulis of the jaw: A series of five cases and review of the literature. Pediatr Surg Int 2009;25:207-10.
6. Stavropoulos F, Guelmann M, Bimstein E, Katz J. Congenital epulis of the newborn: A case report. Quintessence Int 2007;38:e1-4.
7. Diniz MB, Giro Elisa MA, Zuanon Angela CC, Costa CA, Hebling J. Congenital epulis: A rare benign tumor in the newborn. J Indian Soc Pedod Prev Dent 2010;28:230-3.
8. Olson JL, Marcus JR, Zuker RM. Congenital epulis. J Craniofac Surg 2005;16:161-4.
9. R. M. Zucker and R. Buenecha, "Congenital epulis: review of the literature and report of a case," Journal of Oral and Maxillofacial Surgery, vol. 51, pp. 1040–1043, 1993.
10. Pellicano M, Zullo F, Catizone C, Guida F, Catizone F, Nappi C. Prenatal diagnosis of congenital granular cell epulis. Ultrasound Obstet Gynecol 1998;11:144-6.
11. Merrett SJ, Crawford PJ. Congenital epulis of the newborn: A case report. Int J Paediatr Dent 2003;13:127-9.
12. Kannan SK, Rajesh R. Congenital epulis-congenital granular cell lesion: A case report. J Indian Soc Pedod Prev Dent 2006;24:104-6.
13. Lapid O, Shaco-Levy R, Krieger Y, Kachko L, Sagi A. Congenital epulis. Pediatrics 2001;107:E22.
14. Martelli-Jïnior H, Mesquita RA, de Paula AM, Pкgo SP, Souza LN. Peripheral odontogenic fibroma (WHO type) of the newborn: A case report. Int J Paediatr Dent 2006;16:376-9.

INSTRUCTIONS FOR AUTHORS

The preferable mode of submission of manuscripts is online via the Journal's online submission and review system on the website: www.uqumedicalju.com. On this system the author after submitting his/her manuscript may track the progress of the editorial processing. This system is user friendly and will ask you to register after which you will have access as an author.

REVIEW PROCEDURE

Submitted manuscripts are reviewed for originality, significance, adequacy of documentation, reader interest and composition. Manuscript not submitted according to instructions will be returned to the author for correction prior to beginning the peer review process. Revised manuscripts are judged on the adequacy of responses to suggestions and criticisms made during the initial review after which they are sent to selected Reviewers for assessment and evaluation. All accepted manuscripts are subject to editing for scientific accuracy and clarity by the office of the Editor.

FORMAT REQUIREMENTS

Manuscript should be written in English. Both the American and British style of writing and spelling will be acceptable. The acceptable file format is Word. Please do not submit your manuscripts in PDF format. Manuscripts should be typed using ***New Times Roman font and point 12 without any formatting.*** Number pages consecutively, beginning with the title page. Type the page number in the upper right-hand corner of each page.

Title Page
The title page of the manuscript should include:

Type of the manuscript (Original article, case report, review etc.)
Title of the manuscript
Author/s' names (first name, middle initial and last name)
Authors' affiliation (department, institution)
Authors' addresses and
Email (for the corresponding author)

Abstracts
Provide on a separate page a structured abstract of not more than 300 words for original article and an unstructured abstract of no more than 200 words for other submission types. The structured abstract should consist of four paragraphs labeled Objective, Methods, Results and Conclusion. They should briefly describe, respectively, the problem being addressed in the study, how the study was performed, the salient result and what the authors conclude from the results. The unstructured abstract is in the form of one paragraph covering these headings.

Introduction
State the purpose of the article and summarize the rationale for the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported. Clearly mention the objective(s) of the study in this section without any sub-heading.

Methods
Describe your selection of the observational or experimental subjects (patients or laboratory animals, including controls) clearly identify the age, sex and other important characteristics of the subjects. Identify the methods, apparatus study design, sampling method, sample size, inclusion/exclusion criteria wherever applicable without adding any sub-headings. Give references to established methods if necessary.

Results
Present your results in logical sequence in the text, tables and illustrations. Do not repeat in the text all data in the tables or illustrations emphasize or summarize important observations.

Discussion
Emphasize the new and important aspects of the study and conclusions that follow from them. Do not

repeat in detail data or other material given in the introduction or the results section. Include in discussion section the implications of the findings and their limitations including implications for future research. Relate the observations to other relevant studies.

Conclusion
Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by data. State new hypothesis when warranted but clearly label them. Such

Acknowledgements
Persons who have contributed intellectually to the paper but whose contributions do not justify authorship may be named and the function or contribution described.

References
References should be cited in the Vancouver style in consecutive numerical order at first mentioned in the text and designated by the reference number in superscript. References appearing in a table or figure should be numbered sequentially with those in text.

> **Vancouver style of references:**
> Snowdon J. Severe depression in old age. Medicine Today. 2002 Dec;3(12):40-47.
>
> Skalsky K, Yahav D, Bishara J, Pitlik S, Leibovici L, Paul M. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. BMJ. 2008 Mar 29;336(7646):701-4.

Illustrations
Illustrations should clarify and augment the text. The selection of sharp, high-quality illustrations is of paramount importance. Photographs including all types of images should be prepared as .jpg uncompressed files at a resolution of 300 dpi. Figures of inferior quality will not be acceptable.

SUBMISSION FORMAT

Original article: maximum 3000 words excluding title page and a structured abstract of 250 words and references with no more than three tables or figures and 40 references Short Reports / Short Communications / Special Communications / Case reports: maximum 1250 words excluding title page and an unstructured abstract of 150 words and references with no more than two tables or figures and 10 references. It should not have more than six authors

Case Report: Abstract; Introduction; Case Report; Discussion and Conclusion.

Short Report: Abstract; Introduction; Patients Methods and Results; and Conclusion.

Special Communication: Abstract; Introduction; Methods and Results; and Conclusion.

Letters to the Editor: maximum 300 words if it is in reference to a recent journal article, or 400 words in all other cases. It must have no more than five references and one figure or table and may not be signed by more than three authors.

Review article: maximum 4000 words excluding title page and an unstructured abstract of 150 words and references with no more than five tables or figures and 60 references.

[Detailed instructions can be found on the Journal website.]

UQU Medical Journal
Vol. 2 No.4 (July 2011)

UQU Medical Journal is the official publication of the Faculties of Medicine, Umm al Qura University, Makkah.

"You are invited to send manuscripts describing original research in clinical and basic medical sciences; review articles are by invitation. While the original articles, case reports, short reports, reviews and letters to the editor constitute the regular features of the journal, high quality articles of concurrent interest may be included from time to time.

Please type your manuscripts in a plain format using New Times Roman font, pt. 12 and in double space. Tables and figures should be kept simple. Detailed instructions can be found elsewhere in this journal. It is worthwhile to read these instructions to avoid unnecessary delay.

Send your manuscripts using our online system where it will be possible for you to track the progress of the editorial processing."

www.uqumedicalju.com

www.uqumedicalju.com

مجلة جامعة أم القرى
الطبيـــة

مجلة علمية محكمة نصف سنوية

البحوث :

المجلد (٢) العدد (٤) رجب ١٤٣٢هـ / يوليو ٢٠١١ ردمد: ١٦١٥٨-٤٧٣٢