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The Majmaah Journal of Health Sciences (MJHS) enters its second year of publication and I believe the aims that we set for the MJHS continue to be valid and to a certain extent; achieved. The mission of the MJHS is to promote basic, translational and clinical research with the overall goal to improve the health status in the Middle East region. The vision is clear: to become a prominent journal of the highest quality in this region. The main objective of MJHS is to educate and inform, to awaken a critical spirit and foster observational skills and to facilitate the expression of the abilities and preoccupations of the health professionals.

For most professionals, what is important is their work, such that we are aware that there may be great interest in reading the journal but less in publishing in it. I strongly encourage our readers and professionals from the academic, clinical and scientific community to utilize this platform to share their research findings by publishing in MJHS.

If you believe, you have the ability to critically review manuscripts that are submitted for possible publication in the MJHS, I encourage you to consider sharing your knowledge and talent with us. You should know that reviewers are not obligated to review articles whenever requested. If you are preoccupied, it is acceptable to decline the invitation without negative consequences.

MJHS is appreciative of its energetic team members. These team members have devoted themselves diligently to make the timely publication of MJHS a reality. I am confident that with their continuous diligence, MJHS will achieve our goals of recognition in the near future.

The role of Editor-in-Chief has been a learning experience for me thus far, and I continue to learn each day. I am also extremely grateful to the Editors for their valuable input and their hard work and to the Editorial Board for their wisdom, guidance, and commitment.

Prof. Mohammad Othman Al Rukban

Editor in Chief

Majmaah Journal of Health Sciences

Vice Rector, Academic Affairs, Majmaah University

Potential Therapies to Promote Tissue Revascularization and Remodeling after Ischemic Stroke "A Systematic Review"

*Raid Saleem Al Baradie[1]

ABSTRACT

INTRODUCTION: After analysis of current reviews and research into current clinical tries, a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischaemic stroke sufferers long-term recovery and outcome. **METHOD**: Only those articles that provide original research were reviewed, such as primary research articles, cohort studies, and animal model studies and phase 1 clinical trial. Multiple electronic databases using a variety of outlined search terms were used to gather suitable papers. The sources used were Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus etc. **RESULT**: The result of this review shows that the number of pre-clinical animal model studies for potential therapies after stroke outnumber clinical human trials on a ratio 2.6:1 (pre-clinical animal : clinical human). Therapies after TIA to prevent major stroke recurrence appear to be more advanced, with all potential therapies in phase 1 clinical trial for safety and efficacy. **DISCUSSION**: There are many trials which has shown potential for better stroke recovery and results from those studies support the safe and effective use of many chemical derivatives like 2-growth factor therapy initiated 24 to 48 hours after stroke onset, therapy using adenovirus-expressing heparin-binding epidermal growth factor which significantly enhanced recovery after ischaemic stroke, human umbilical mesenchymal stem cells transplantation for ischemic stroke, and monocyte-derived multipotential cells which are potentially effective candidate for use in cell transplantation therapy for cerebral ischaemia.

Key words: Ischaemic stroke, Transient ischaemic attack (TIA), Tissue remodeling, Tissue revascularization, Neuroprotection.

الملخص

مقدمة : بعد تحليل عدة بحوث ودراسات سريرية، يمكن اقتراح مجموعة من العلاجات تنفذ على مراحل لمعالجة التغيرات الفيسيولوجية المرضية في الدماغ والتي قد توفر نتائج أكثر إيجابية في التجارب السريرية ، والتغلب على بعض الصعوبات التي تواجه تجارب العلاجات الحالية, وتحسين شفاء الذين يعانون من السكتة الدماغية على المدى الطويل . **المنهج:** تم استعراض ومراجعة البحوث الأصلية فقط (مثل مقالات البحوث الأساسية ودراسات الزمر و دراسات التجارب علي الحيوان و التجارب السريرية) . تم تجميع الاوراق المناسبة من قواعد بيانات إلكترونية متعددة باستخدام مجموعة متنوعة من مصطلحات البحث الواردة. وكانت المصادر المستخدمة هي (Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus, etc) **النتائج:** اوضح الاستعراض ان دراسة العلاجات المحتملة لما بعد السكتة الدماغية علي حيوانات التجارب فاقت الدراسات السريرية على الإنسان بنسبة ٢,٦ الي ١ , كما ظهر تطور كبير في العلاجات التي تعطي بعد حالات نقص الدم المؤقت للمخ لمنع تكرار السكتة الدماغية الكبرى . **المناقشة:** هناك العديد من التجارب التي أظهرت إمكانات لتحسين الشفاء من السكتة الدماغية ونتائج هذه الدراسات تدعم امكانية الاستخدام الآمن والفعال للعديد من المشتقات الكيميائية مثل عامل النمو ٢ اذا بدأ العلاج به بعد ٢٤ إلى ٤٨ ساعة من الإصابة بالسكتة الدماغية ، والعلاج باستخدام الهيبارين المرتبط بعامل نمو البشرة والذي عزز بشكل كبير التحسن بعد السكتة الدماغية ، و زرع الخلايا الجذعية من الحبل السري البشري للسكتة الدماغية الناتجة من نقص التروية الدموية، و لوحيدات الخلايا الناشئة من الخلايا متعددة الامكانات و التي يحتمل أن تكون فاعلة عند استخدامها في زراعة الخلايا كعلاج للسكتة الناتجة من نقص التروية.

INTRODUCTION

Cerebrovascular accident (CVA) or stroke is a neurological disorder caused by altered cerebral circulation, defined as an 'acute neurological dysfunction of vascular origin with sudden or at least rapid occurrence of symptoms and signs corresponding to the involvement of focal areas in the brain [1]. Those over the age of 65 are most at risk, but 25% of strokes occur in people under the age

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of 65. Certain nationalities, for example south Asian, African or Caribbean are pre-disposed to conditions such as diabetes and heart disease, which are two major risk factors for stroke.2 Other risk factors include high blood pressure, high cholesterol and diabetes, all of which could be due to unbalanced diet and a lack of exercise, but also age, gender and family history. Smoking can double the risk of having a stroke as it narrows arteries and makes the blood more likely to clot [2].

There are two major mechanisms causing brain damage in stroke; ischemia and hemorrhage. Ischemic stroke represents about 80% of all strokes, the blood supply to the brain is interrupted, and the brain cells are deprived of the glucose and oxygen they need to function sufficiently. The effects of ischemia can be fairly rapid as the brain does not store glucose, the main energy substrate, and is incapable anaerobic metabolism [3]. Ischemic stroke is a complete entity with multiple etiologies and variable clinical manifestations. Hickey [4] approximated that 45% of ischemic strokes are caused by small or large artery thrombus, 20% are embolic in origin, and others have unknown cause.

Normal cerebral blood flow is approximately 50 - 60ml/100g/min and varies in different parts of the brain. Cerebral auto-regulatory mechanisms compensate for reduced CBF in response to ischemia by local vasodilation, opening collaterals, and increasing oxygen and glucose extraction from blood. When CBF is reduced below 20ml/100g/min, electrical silence ensues and synaptic activity is greatly diminished in an attempt to preserve energy stores. CBF less than 10ml/100g/min is irreversible neuronal injury [5].

Understanding the ischemic cascade has led to the concept of a therapeutic time window, or timed approach for treatment possibilities. There is a core zone of severe ischemia with dead cells and blood flow below 10 – 25%. This is surrounded by an area of hypoperfused tissue, known as the penumbra, in which the cells may remain viable for several hours. This is because the penumbral zone is supplied with blood by collateral arteries anastomosing with branches of occluded vascular system. If reperfusion is not established during the early hours, the cells here will also die since collateral circulation cannot maintain the neuronal demand for oxygen and nutrients [5].

Krupinski *et al*. [6] demonstrated an increase in capillary density, active angiogenesis around infarcts, more developed in the penumbra in post-mortem brains of patients who had survived acute ischemic stroke for up to several weeks. These recent developments in the understanding of pathophysiological events following acute ischemic stroke suggest an important role for angiogenesis, which, through new blood vessel formation, results in collateral circulation and may influence positively on the medium to long-term recovery of patients. Future treatment regimens focus on optimization of this process in the penumbra.

Currently a thrombolytic drug called alteplase is used to dissolve the clot, but has proven to be effective only within the first hour after stroke onset. Tissue plasminogen activator (tPA) was approved in 1996 by the U.S. Food and Drug Administration (FDA) as a thrombolytic drug with a 3 hour window for those with severe stroke (NIHSS score > 22) [7]. However, it remains a limited option due to its narrow therapeutic time window and the related risks of intracranial hemorrhage. Aspirin will be given regularly as an anti-platelet medication to prevent further clotting. Anticoagulants such as heparin and warfarin are given to prevent further clotting7. Medication to control blood pressure and cholesterol may also help.

The level of brain damage after a stroke can vary greatly depending on what vessels are affected, but can be widespread and long-lasting. Although many people recover their former independence after extensive rehabilitation with teams of specialists including physiotherapists, psychologists, occupational and speech therapists and specialist nurses and doctors, approximately 90% of stroke survivors are left with some residual deficit [8].

Due to the complexity of physiological regulation of blood vessel formation, involving numerous critical growth factors expressed differentially in time, space and concentration, ongoing therapeutic efforts using single agents and aimed at treatment of ischemic vascular disease are of limited potential. Optimization of therapeutic treatments might involve a complex series of interventions beginning with administration

of a cocktail of drugs within the first few hours of illness to reduce inflammation, but at the same time maintaining neuronal viability with neurotrophins and stimulating growth factor-induced angiogenesis. Perfusion pressure in the penumbra region could be increased using thrombolytic therapy, and susceptible neurons could be protected from apoptosis by viral transfer of genes such as Bcl-2 [9]. With angiogenic therapies there is a tendency for inflammation and vascular permeability, so therapies to prevent this, perhaps by inducing blood vessel maturation, will be analyzed.

After analysis of current reviews and research into current clinical tries, a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischemic stroke sufferers long-term recovery and outcome.

METHODS

Male and female adults were included, and they must have a mean age of 60 or older after ischaemic stroke onset or following a transient ischaemic attack (TIA). Animal models, such as mice and monkeys, will also be included as many potential therapies have not been through stage 1 clinical trials yet. The intervention to ischemic stroke will be therapies that focus on inducing and sustaining angiogenesis, revascularisation and vascular remodelling, also to induce neuroprotection, inflammation. The use of novel combination therapies will be reviewed. Medications to prevent inflammation and vascular permeability following angiogenic therapies will be analyzed. Therapies to prevent major stroke recurrence after transient ischemic stroke will also be analyzed. The aim is to review current potential therapies to be able to suggest a novel combination therapy which is phased to tackle the pathophysiological events in the brain over time may be suggested which may provide more positive results in clinical trials, overcome some of the limits that current therapies in trial are facing, and improve ischemic stroke sufferers long-term recovery and outcome.

The interventions to ischemic stroke will be compared to other potential therapies. As they are not in clinical use it would not be appropriate to compare interventions to currently used thrombolytic therapy for ischemic stroke, tissue plasminogen activator (tPA) and alteplase. Only articles that provide original research are used, such as primary research articles, cohort studies, and animal model studies and phase 1 clinical trials.

Inclusion Criteria

For inclusion, trials need to satisfy the following criteria:

- Articles published since the year 2005
- Adult participants (>18 years) males or females with a mean age of 60 years or older or animal models
- All nationalities are included
- Articles are freely available
- Trials must be primary and randomized
- Pre-clinical and any phase of clinical trial included

Exclusion Criteria

- Peer reviews, surveys, questionnaires
- Any previous haemorrhagic event (including haemorrhagic stroke)
- Previous cardiovascular conditions
- Any known intracerebral pathology other than stroke
- Any other serious medical condition which is likely to affect outcome assessments

Sources of Information

Google Scholar, PubMed, Science Direct, Web of Science, Scientific Web Plus

Search Strategy

Make use of multiple electronic databases using a variety of outlined search terms to gather suitable papers. Review papers related to the topic of cerebral angiogenesis and current and future therapeutic targets for ischemic stroke were used to identify relevant primary studies.

DISCUSSION

The 90 day risk of stroke after a transient ischemic attack (TIA) has been reported as being as high as 17% [7], the stroke recurrence rate has been calculated at 15% within 2 years, and as high as 30% within 5 years [28]. Anti-platelet therapies are still the most

successful method to prevent secondary stroke. Neuroprotection is a key target for stroke treatment and the therapies with current potential are outlined in table 1. As well as for protection against secondary stroke, chronic statin treatment has also been suggested for decreasing stroke severity and improve outcome.

The effects of intravenous versus intra peritoneal administrations of rosuvastatin in a pre-clinical mouse model of focal cerebral ischemia at different dosages were studied [10]. The results of this study showed that i.v rosuvastatin at relative pharmacologically relevant concentrations up to 4 hours after an event shows significant signs of neuroprotection; in this study a dosage of as low as 0.02mg kg^{-1} achieved neuroprotection. The protective effects extended to 5 days after ischemia and were accompanied by improved functionality based on a few simple functionality tests, namely the wire hanging test, pole test, and Bederson score. Intraperitoneal administration only appeared effective within 1 hour of ischemia, and at a high dose of 2mg kg-1, which is a restrictive time window. It has been demonstrated that the protective effects may be mediated by up-regulation of endothelial-derived nitric oxide synthase (NOS) in the vascular wall and direct activation of endothelial nitric oxide synthase (eNOS) via protein-kinase Akt. The increased availability of eNOS seems to improve endothelium function, increase cerebral blood flow, inhibit platelet aggregation and exert anti-inflammatory effects, all of which enhance neuroprotection [10]. A safe, efficacious formulation of intravenous statins should be developed in order to move statin treatment in to the phases of human clinical trials.

An epidemiological inverse association has been found between high-density lipoprotein (HDL) cholesterol levels and cerebrovascular events [29]. A study in 2010 tested timing and dosage of intravenous HDL administration compared with saline control. There were positive pre-clinical results with HDL showing neuroprotection up to 5 hours after stroke onset at higher dosages in a rat model of embolic stroke. This model of embolic stroke is suggested to be the closest to human pathophysiology, as there are majorities of ischemic strokes, which involve an embolic mechanism. The results may possibly be attributed to the ability of HDL to protect the blood-brain barrier and limit neutrophil recruitment as shown by immunostaining [18].

Table 1: Novel therapies to induce neuroprotection and combat apoptosis following ischemic stroke

Study	Result
Prinz et al [10], 2008	Intravenous rosuvastatin given at pharmocologically relevant concentrations up to 4 hours after an event can be neuroprotective of focal brain ischemia in an animal model. Development of intravenous statin formulation is warranted for phase 1 acute stroke trials with statins in humans.
Dávalos et al [11], 2012	Citicoline appears not to be efficacious in the treatment of moderate-to-severe acute ischaemic stroke.
Ehrenreich et al [12], 2009	Erythropoietin showed no favourable effects on functional outcomes of ischemic stroke.
Zhao et al [13], 2007	Functional restoration in chronic brain ischemia can be attained using haematopoietic growth factor.
Yang et al [14], 2012	VEGF reduced ischemic neuronal danger with a therapeutic time window within the first 3 h of transient MCAO and may be useful in the treatment of acute ischaemic stroke in humans.
Zhao et al [15], 2011	Postischemic treatment with intravenous Tf-VEGF-PLs promoted neuroprotection and angiogenesis and vascular remodelling.
Emerich et al [16], 2010	Intracerebral implantation of an alginate provides therapeutic sustained delivery of VEGF in a rodent model of stroke provides anatomical and neurological protection.
Pathipati et al [17], 2009	Delayed, chronic central treatment of unilateral stroke with rGH in adult rat was associated with slightly more rapid recovery of motor function and spatial memory.
Lapergue et al [18], 2010	Administration of HDL is neuroprotective when performed up to 5 hours after stroke onset.

Breakdown of the blood-brain barrier (BBB) is a key step associated with ischemic stroke and its increased permeability causes extravasation of plasma proteins and circulating leukocytes, including polymorphonuclear neutrophils, which may use their associated matric metalloproteinases to contribute further to BBB breakdown and basal lamina degradation. In addition to reverse transport of excess cholesterol from tissues back to the liver, HDL particles exert anti-inflammatory, antioxidant, anti-elastase, and anti-thrombotic effects that may protect endothelial cells from acute injury [30].

Lapergue *et al* [18] demonstrated by immunostaining that HDL was taken up by endothelial cells and glial cells, but not neurons, which suggests a beneficial role on astrocyte and cerebral endothelial cell function, and prevent ischemia induced BBB disruption. Many further studies are required to confirm whether HDL based therapies are applicable, efficacious, and effective in human ischemic stroke.

There have been some recent studies which showed no positive results for therapies to induce neuroprotection. Ehrenreich *et al* [12] used recombinant human erythropoietin (EPO) in a small early phase clinical trial on 522 human patients, which suggests pre-clinical studies showed more positive results. Wang *et al* [31] demonstrated that EPO enhanced functional recovery in rats, possibly due to increased levels of vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF). This is a clear example to prove that animal models do not always relate well to human therapies. EPO is a hematopoietic cytokine that promotes proliferation and differentiation of erythroid progenitors and the survival of maturing erythroid cells [31] in theory it represents and ideal compound for neuroprotection in brain disease acting as anti-apoptotic, antioxidant and anti-inflammatory amongst other things [12]. Recombinant human EPO or a placebo control was given intravenously over 30 minutes within 6 hours of stroke, and repeated 24, then 48 hours thereafter. The use of one of the only recommended treatments for stroke, namely rtPA, was allowed and stratified for in this study. Although the EPO group showed a slight reduction in infarct lesion size compared with the placebo group, neither primary outcome BARTHEL index on day 90 or any other outcome showed any favorable protective effects of stroke outcome in patients. In fact mortality rates were increased in the EPO group at 16.4% compared with 9.0% in the placebo group. Due to the unsuccessfulness of this clinical trial, strict adherence to comprehensive safety protocols would be necessary for any further EPO trials, as well as more balance in the stroke severity of those enrolled on to the study and an exclusion of those in receipt of rtPA treatment.

It has been recently suggested that therapies with the potential to enhance endogenous brain plasticity and repair could reduce brain damage and improve functional recovery in animal models of stroke, even when administered several hours after the onset of the ischemic event [32]. Citicoline is a naturally occurring endogenous compound and an essential precursor in the synthesis of phosphatidylcholine, a key cell membrane phospholipid. It is increasingly recognized as a neuroprotectant that may be able to act through the stages of ischemic damage inhibiting different stages of the ischemic cascade. This is most probably due to its anti-apoptotic effects, its ability to synthesize and repair membranes, inhibit free fatty acid release, decrease free radical formation, and favor the synthesis of nucleic acids, proteins and neurotransmitters [32]. Citicoline is approved in some countries for the treatment of ischemic stroke, the drug required more testing for efficacy in pooled analysis in an international, randomized, multicenter, placebo-controlled study. A very recent phase 3 clinical trial of citicoline compared with a placebo after having received 2000mg of citicoline daily for 6 weeks in 2298 patients has shown not to be efficacious in the treatment of ischemic stroke. This confirms extensive studies previous to this, which report similar safety profiles compared with placebo, and previous failures to prove efficacy. The International Citicoline Trial on acute Stroke (ICTUS) trial deems citicoline safe but not efficacious in the treatment of ischemic stroke [11]. A paper yet to be published online from 'Nature' seems to be set to draw attention to the fact that rodent models, which have seen positive efficacious results following citicoline treatment after ischemic stroke models, metabolize citicoline differently when it is administered in different ways (intravenously or orally) from human models. This therefore suggests that perhaps if a clinically relevant method of administration of citicoline to humans following stroke, for example in a liposomal formulation, it may still provide the

potential neuroprotective effects seen in pre-clinical models, and improve functional outcome in humans following ischemic stroke [33].

Growth factors make up one of the most studied therapies for stroke treatment. It has previously observed that stem cell factor (SCF) and granulocyte-colony stimulating factor (G-CSF), the growth factors that regulate hematopoiesis, have neuroprotective and functional effects on acute cerebral ischemia [13]. In this study the combined administration of both factors at 14 weeks (3.5 months), which is entering the chronic phase after ischemic stroke onset, resulted in improved functional outcomes and reduced infarction size in pre-clinical rat models.

According to Zhao *et al* [13] this was the first evidence that pharmaceutical intervention as late as 3.5 months after stroke can improve neurological deficits and reduce penumbra and focal infarct size. Pathipati [17]used an infusion of rat growth hormone on rats 4 days after induction of ischemic stroke for 6 weeks, followed by 6 weeks to allow for restorative processes to occur. The results showed a slightly more rapid recovery of motor function and spatial memory compared with the buffer treated group. However, the most recent work on growth factor treatment to provide neuroprotection following ischemic stroke is on vascular endothelial growth factor (VEGF).

VEGF is a potent pro-angiogenic peptide, its administration has been considered as a potential neuroprotective strategy following cerebral stroke [16]. There have been previous studies, which confirm the ability of VEGF to promote neuroprotection, promote neurogenesis and cerebral angiogenesis [34], and therefore improve functional outcome and recovery following ischemic stroke through multiple mechanisms.

Previous studies are all pre-clinical animal models, the majority with rat models. Due to the poor permeability of the BBB, in previous studies, in which the majority are pre-clinical rat models, the models have been subjected to intra cerebral injections or craniotomies to administer the VEGF, both very invasive procedures.

Emerich *et al* [16] demonstrated a successful method of prolonged VEGF delivery to the brain by injection of the drug directly to the striatum of a rat model using hydrogels and alginate scaffold. The results showed VEGF was still viable and detectable around 2 weeks after the hydrogel delivery. This is promising to provide chronic VEGF treatment, therefore increase the scope of anatomical and neurological recovery and result in an improved outcome following ischemic stroke.

However, the invasive nature of delivery poses obvious ethical, safety and methodological issues for the relation of VEGF treatment for neuroprotection from pre-clinical models to a safe, efficacious human treatment of ischemic stroke. A study by Zhao *et al* [15] aimed to achieve enhanced delivery of VEGF to the ischemically disordered parts of the brain; the penumbral region and the infarct cortex. The results indicate that brain-specific expression of exogenous genes, such as VEGF, is possible with the combined use of a cationic gene therapy system such as stabilized liposomes, and a brain targeting ligand. In this case transferrin-targeted coupled liposomes (Tf-PLs) are used, as transferrin, the iron-transporting protein, has free access across the intact BBB as it is a key carrier of essential nutrients to the brain. The intravenous administration of VEGF-loaded Tf-PLs also achieved high VEGF expression in the ischemic cerebral tissues, and seemed to induce neovascularization. Although the full mechanisms for this delivery method are still under investigation, Zhao *et al* [15] believe that Tf-VEGF-PLs hold promise for efficient, non-invasive, and specific brain-targeting gene delivery, and advanced developments in ischemic stroke treatment.

The most recent study into VEGF focuses on the dose response and time window, the results of which may help to bring about safe, efficacious human clinical trials of VEGF for the treatment of stroke targeted to neuroprotection. A dose-response relationship was established indicating the mid-dose level was the most efficacious, which was set at 2.5ng/µl relative to rabbits weighing 2.5–3.0kg. A therapeutic time window for the neuroprotective effects of VEGF in a rabbit model was defined as being within 3 hours of the MCAO ischemic stroke model onset [14]. The future of VEGF therapy to treat ischemic stroke should focus on the multi-potential aspects of VEGF; it's promotion of angiogenesis and brain plasticity leading to improved neurological function. There

is a need to define how these properties can come together for the development of new therapies, it is also important to consider any unfavorable side effects [35].

Inflammation is a key contributor in the pathogenesis and progression of stroke. Inflammatory biomarkers such as high-sensitivity C-reactive protein have been identified as predictors of initial stroke and for prognosis predictions after stroke [36]. Some of the most important studies on therapies to target the inflammation stage if the ischemic cascade are outlined in table 3. Statins have proven to be therapeutic in preventing secondary stroke following TIA, and they have also been suggested to play a role in treatment due to their anti-inflammatory properties. In a small phase 1 clinical trial in a small group of 55 patients received 20mg of lovastatin daily for 90 days or no treatment, however after a 90 day follow-up using the NIHSS and the BARTHEL index, no significant improvement was shown in the lovastatin group [19].

Table 2: To show novel anti-inflammatory therapies to target the pathophysiological stage of inflammation following ischemic stroke.

Study	Result
Zare *et al* [19], 2012	Lovastatin therapy shows no role in improved stroke recovery over 90 days.
Jung *et al* [20], 2010	High dose nitrite therapy within 24hrs after ischemia reduced inflammatory cytokine levels and caspase activity in sub-acute period.
Herz *et al* [21], 2012	Anti-inflammatory effects, neural plasticity, promoting effect of VEGF accompanied by immunosuppressive effects were observed.

A pre-clinical trial on rats on nitrite therapy showed more successful results. NO is known to mediate, proliferation and differentiation of brain cells and VEGF-induced angiogenesis, all of which improve functional recovery following ischemic stroke. This trial was especially interested in measuring the anti-inflammatory effects of NO therapy, and found that inflammatory cytokine levels were reduced as was caspase activity [20]. RT-PCR was performed to measure the mRNA of cytokine-related genes, IL-1β IL-6, TNF-a, and Fas-L, all of which are usually raised in the ischemic brain, were reduced in nitrite-treated rats. Western blotting measured reduced expression of PARP, a family of proteins involved in DNA repair and programmed cell death and caspase-3 which is involved in cell apoptosis.

VEGF has also shown to have ant-inflammatory properties; in a study by Herz *et al* [21] VEGF mechanisms providing neurological recovery were accompanied by a profound immunosuppressive effect and anti-inflammatory mechanisms. However, there were negative side effects associated with administration of VEGF in the acute phase such as hemorrhage and vascular permeability [21]. These may be associated with the angiogenic and neovascularization properties of VEGF, as vessels that are developed rapidly can often be described as 'leaky', or permeable, the new vessels haven't had a chance to mature.

Table 3 concentrates on therapies to induce angiogenesis, tissue revascularisation and remodelling. An early study on neurotrophic factors, which have been shown to promote neurogenesis after cerebral ischemia, used heparin-binding epidermal growth factor-like growth factor (HB-EGF) injected into the lateral ventricle in the ischemic side 3 days after ischemic onset in rats, and again on the 6th and 7th day [23]. The study shows improved functional recovery by inducing neurogenesis and angiogenesis. However, during the searches, the results of this study didn't seem to lead to any further developed studies which have been published as of yet.

A practical idea demonstrated by Levy *et al* [27] was intracranial stent deployment. Patients with contraindications to rtPA treatment, or those who failed to improve following 1 hour of rtPA treatment were assigned to receive the self-expanding stents (SES) intracranialy within 8 hours of stroke onset. This was a small-scale phase 1 clinical trial on humans to evaluate the safety and efficacy of primary stent deployment for revascularisation following acute ischemic stroke, with encouraging 1-month follow-up results and the conclusion that it may be a valuable addition to stroke treatment. Due its successful potential in this small scale trial, the therapy should advance through the clinical trial phases in bigger

patient groups to assess the best candidates for this type of treatment, as it is more invasive than intravenous therapies.

Following on from the unsuccessful trial of EPO on neuroprotection therapy after ischemic stroke, EPO plus β-human chorionic gonadotropin (β-hCG) was used in an effort to induce neurogenesis. β-hCG is in the same growth factor family as nerve growth factor and is able to cross the BBB. The time window of B-E therapy suggests it acts as a restorative rather than neuroprotective therapy, as the therapy remains effective when administered up to 48 hours after stroke onset and continued for 9 days [22]. This was a small patient sample, therefore not representative of the population as a whole, although the inclusion and exclusion criteria would have maintained some consistency. A placebo-controlled, double-blind, phase IIb trial of B-E therapy had been initiated at the time of publication, the results of which are unpublished as of yet.

Table 3: Novel therapies to induce angiogenesis, tissue revascularization and tissue remodeling.

Study	Result
Cramer *et al* [22], 2010	Results support the safety of this sequential, 2-growth factor therapy initiated 24 to 48 hours after stroke onset.
Suqiura *et al* [23], 2005	Gene therapy using Ad-HB-EGF significantly enhanced recovery after ischaemic stroke in rats.
Lin *et al* [24], 2011	There were therapeutic benefits of hUMSC transplantation for ischemic stroke.
Hattori *et al* [25], 2012	MoMCs are a potentially effective candidate for use in cell transplantation therapy for cerebral ischemia.
Yu *et al* [26], 2010	NSCs and collagen type 1 implantation facilitated structural and functional recovery of neural tissue following ischaemic injury.
Levy *et al* [27], 2009	Primary intracranial stenting for acute stroke may be a valuable addition to stroke treatment.

One of the most studied methods of revascularisation and remodelling is by the use of stem cells. The transplantation of pluripotent stem cells hold the greatest potential, however, the use of human embryonic stem cells is controversial and sparks many ethical issues from pro-life groups and others alike. As a result, adult stem cells are the favorable option, although there has been a lack of sufficient adult human tissue and appropriately differentiated cells to provide therapeutic potential.

A stem cell, which appears to show potential, was neural stem cells (NSCs), as, studied by Yu *et al* [26], due to its importance in basic research of neural development as well as their broad multi-potential properties for stem-cell based therapies for neurological diseases such as stroke. In a pre-clinical rat model, NSCs were taken from 14-day old embryos in gestating female rats, and transplanted back into the female rat subjects following middle cerebral artery occlusion to replicate human ischemic stroke and 4 hours of observation. The issue as to whether the transplanted cells which differentiate into neurons and proliferate are capable of acquiring full neuronal functionality and synaptic contact [26]. Yu *et al* [26] suggested that an ideal cerebral remodelling in stroke should be composed of a biodegradable material strong enough to resist damage, be permeable to diffusion of nutrients, enable normal cellular processes and facilitate extracellular matrix formation; collagen was used in this study. The results show that in the group administered with NSCs and collagen type 1, the majority adopted a neuronal fate, and demonstrated significant improvements in structural and functional neurological function following ischemic injury.

The use of neural stem cells has been preceded by more modern sources of stem cells. Human umbilical mesenchymal stem cells (hUMSCs) from the Wharton's jelly of the umbilical cord can be cultured in abundance in vitro. They are less controversial to use and aren't associated with many ethical issues as the umbilical cord isn't required once a new baby is born, the cord would usually be discarded so is often donated by new mothers to harvest the stem cells. Although the hUMSCs are multipotent, it has been shown that they are able to differentiate into neural cells when cultured with neuronal-condition medium (NCM) for 6 days24. In the pre-clinical model by Lin *et al* [24], both treated and untreated hUMSCs were grafted into the infarct cortex of rats in 2 injections

to observe the differences, and compared with a control group, which received only phosphate-buffered saline (PBS). Therapeutic benefits were demonstrated in both the NCM-treated and non-treated hUMSC groups, there appeared to be no major significant difference in revascularisation, motor function or metabolic activity of cortical neurons between the treated untreated groups. HUMSCs are abundant and easily accessible, therefore were said to be a promising and reliable source for the future. Lin (2010) confirmed that additional research was required to define the therapeutic potential for chronic treatment of ischemic stroke.

In the most recent study, Hattori *et al* [25] used monocyte-derived multipotential cells (MoMCs) in a pre-clinical rat model, which have the potential to differentiate in to mesenchymal, neuronal and endothelial lineages. Similar significant improvements in revascularisation as shown by microvessel-like structures in the penumbra, and angiogenesis as demonstrated by increased numbers of angiogenic factors being expressed (Hattori, 2012). These are adult stem cells, so are a safe, abundant and readily available in the adult peripheral blood system; they are without ethical issues, and due to them being transplanted by autologous methods they are without the immunological complications of other stem cell transplant methods. Hattori [25] suggests that further studies should determine optimal therapeutic timing and recommended dosages, as well as an appropriate method of autologous transplantation to maintain therapeutic levels in the penumbra and ischemic cortex.

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Prostate Cancer Contribution to Elevated PSA levels: A Screening Study

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ABSTRACT

OBJECTIVE: To investigate the causes of elevated prostate specific antigen (PSA) levels in patients with lower urinary tract symptoms (LUTS) through transrectal ultrasound guided biopsy (TRUS biopsy), regardless of prostate volume. **METHODS:** Retrospective study was conducted between January 2008 and December 2009 to find the cause of elevated PSA levels in sixty six (N=66) patients from the outpatient department in Guryyte General Hospital, AlJouf. Age of the patients was between 52 and 90 years and PSAlevel ranged between 5 ng/ml and 26 ng/ml . All patients were presented with some degree of lower urinary tract symptoms (LUTS) and high PSA levels for age. They underwent TRUS biopsies performed by a Radiologist and Urologist **RESULTS:** Out of the total sixty six patients, twenty two (33%) of them with a PSA range between 5.1ng/ml and 26 ng/ml were found to have Benign Prostatic Hyperplasia (BPH) and Prostatitis. Eighteen patients (29.5%) with a PSA level between 9.3 ng/ml and 25.6 ng/ml were diagnosed as adenocarcinoma of the prostate. Sixteen patients with PSA between 12ng/ml and 26 ng/ml were diagnosed as BPH. Ten patients with PSA between 9.3ng/ml and 16 ng/ml were found to have Prostatitis (15.15%). **CONCLUSION:** Benign prostate hyperplasia and chronic prostatitis are the two major causes of elevated serum PSA in our cohort followed by prostate cancer. With regards to the PSA level in chronic prostatitis and in prostate cancer, no significant difference was noted. The conclusion reached is that TRUS biopsy is mandatory to determine the exact cause of an elevated PSA level.

الملخص

الاهداف: معرفة اسباب ارتفاع مستوي مولد المضادات (الانتيجين) الخاص بالبروستات للمرضي الذين يعانون من اعراض في الجهاز البولي السفلي , وذلك بأخذ خزعة باستخدام الموجات فوق الصوتية عبر المستقيم وبغض النظر عن حجم البروستات. **المنهج:** اجريت الدراسة ما بين يناير 2008 وديسمبر 2009 لمعرفة اسباب ارتفاع مستوي مولد المضادات (الانتيجين) الخاص بالبروستات على66 من مرضي العيادات الخارجية بمستشفى القريات العام بالجوف , اعمار المرضي كانت بين 52 الي 90 سنة و مستوي مولد المضادات الخاص بالبروستات تراوح بين 5 نانو جرام / ملم الي 26 نانو جرام / ملم . كل المرضي كانوا يشتكون من اعراض في الجهاز البولي السفلي مع ارتفاع في مستوي مولد المضادات مقارنة بالعمر , وقد تم أخذ خزعة باستخدام الموجات فوق الصوتية عبر المستقيم بواسطة اخصائي الاشعة واخصائي المسالك البولية. **النتائج:** 22 مريض (33%) من اجمالي 66 مريض والذين يتراوح مستوي مولد المضادات لديهم من 5.1 نانو جرام / ملم الي 26 نانو جرام / ملم وجد ان لديهم ورم حميد بالبروستات و التهاب بالبروستات. 18 مريض (29.5) والذين يتراوح مستوي مولد المضادات لديهم من 9.3 نانو جرام / ملم الي 25.6 نانو جرام / ملم وجد ان لديهم ورم سرطاني بالبروستات 16 مريض والذين يتراوح مستوي مولد المضادات لديهم من 12 نانو جرام / ملم الي 26 نانو جرام / ملم وجد ان لديهم ورم حميد بالبروستات. 10 مرضي والذين يتراوح مستوي مولد المضادات لديهم من 9.3 نانو جرام / ملم الي 16 نانو جرام / ملم وجد ان لديهم التهاب بالبروستات (15.15%). **الإستنتاج:** الورم الحميد للبروستات والالتهاب المزمن للبروستات اهم سببين لارتفاع مستوي مولد المضادات الخاص بالبروستات في زمرة هذه الدراسة يتبعه سرطان البروستات (لم يلاحظ دلالة احصائية), خلصت الدراسة الي ان أخذ خزعة باستخدام الموجات فوق الصوتية عبر المستقيم من الضروريات لتحديد اسباب ارتفاع مستوي مولد المضادات الخاص بالبروستات

INTRODUCTION

Prostate cancer is the most common malignancy among men and second cause of death after lung cancer. Statistically, if men live long enough, they will eventually develop prostate cancer. Prostate cancer differs from most other cancers in that, with the exception of metastatic disease, stage at diagnosis has a small impact on overall survival [1]. Several studies investigated the ability of new biomarkers to improve prostate cancer diagnosis reducing unnecessary biopsies and to discriminate between aggressive and slow-growing cancers avoiding overtreatment.

Recently, Prostate cancer antigen 3 (PCA3) and phi (prostate health index) have been proposed as useful tools in prostate cancer patient care [2,3]. The investigated alternative methods for predicting the prostate volume, and prostate- specific antigen (PSA)

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was propounded as a feasible proxy for predicting prostate volume [4,5]. It has been reported that serum PSA is inversely correlated with body mass index (BMI) [6,7]. Many urologists are more labile to perform extended prostate biopsy rather than the conventional sextant biopsy. The studies reported previously are on the comparison of sextant with extended biopsies. It has been suggested the extended prostate biopsy to improve the cancer detection rates compared to standard sextant prostate biopsy [8]. Transrectal ultrasound guided prostate biopsy is a recommended diagnostic method with the minimum of 6-10 systematically taken, peripherally directed biopsy samples [9].

A higher number of biopsy samples increases the degree of prostate cancer detection. Schroeder *et al* proved that the prostate DRE has a low predictive value in detection, at which point a substitution with a more sensitive test was proposed [10]. A study of 76,693 men aged 55 – 74 in the United States found no decrease in prostate cancer mortality in men who received annual testing compared with usual care in 10 and 13 years of follow-up [11,12]. In addition to prospective trials, a number of investigators have explored the impact of the PSA testing through ecological analyses, comparing the rates of PSA testing indifferent geographic areas to rates of prostate cancer diagnoses and mortality [13,14,15]. The detection of low-risk tumors that may not clinically progress during lifetime. However, preoperative tools (such as PSA and DRE) lack accuracy to avoid many negative biopsies and to predict confined PCa at radical prostatectomy (RP) [16]. Ultrasonography, especially transrectal ultrasonography (TRUS), is consideredto be the gold standard for measurement of prostate volume [17]. Despite its accuracy, however, routine measurement of prostate volume by TRUS is clinically not feasible owing to its cost and invasiveness [4]. Prostate specific antigen (PSA) blood test, together with an abnormal digital rectal examination (DRE), improve the ability to detect prostate cancer. Carvalhal *et al* suggest the use of the prostate DRE in patients with low PSA values, as prostate cancer was established in 14-30% of the cases with PSAT values between 1 and 4 ng/ml, with abnormal DRE findings [18]. Prostate cancer, benign prostate hypertrophy (BPH) and inflammation (chronic prostatitis) are well known reasons for high PSA levels.

METHODS

Between January2008 and December 2009, a total number of sixty six patients aged between 52 and 90 years, complaining of lower urinary tract symptoms (LUTS) attended our urology Clinic at Guryyte general hospital, Aljouf, Guryyte city, KSA. Urological assessment was performed including thorough medical and urological history, physical examination, routine investigations, ultrasound of urinary bladder included prostate volumes and PSA serum levels. Their PSA were between 5.1 ng/ml and 26 ng/ml. All patients were prepared for TRUS biopsies after obtaining an informed consent . Anticoagulants and aspirin were stopped one week before the procedure. All patients were instructed to take Ciprofloxacin 500 mg twice daily and Metronidazole 500 mg three times daily one day before the procedure as well (laxative) suppositories.

On the day of the procedure, a pain killer in the form of Diclofenac sodium 75 mg was given intramuscularly 30 minutes before patients were placed in the left lateral side with the knees flexed to the chest, local Lidocaine gel 5% was applied prior to digital rectal examination. Ultrasound probe was used to delineate suspicious areas and guide the biopsy needle through the prostatic tissue.

Generally, complications were at low rate. In our study Three patients developed acute prostatitis with fever , they were admitted to urology department for I.V antibiotics. Two patients developed hematuria which subsided three days later.

RESULTS

TRUS biopsies were performed and sent to histopathology department. Among the study population (N=66) the highest percentage of TRUS biopsies belong to BPH + Prostatitis group (33%) followed by other groups (Table 1). The significant PSA levels were found in BPH (12-26 ng/ml) followed by adenocarcinoma (9.3- 25.6 ng/ml), BPH + Prostatitis (5.1-26) and Prostatitis (9.3 – 16 ng/ml).

DISCUSSION

Serum PSA elevation is relatively common in chronic prostatitis, prostate cancer and benign prostate hyperplsia. Prostatitis may cause a rise in PSA levels which could lead to false positive screens and referral

for biopsy to investigate possible prostate cancer. Prostatitis is the most common cause of falsely elevated serum PSA level. Prostatitis is a common condition affecting many if not most men over a period in their lives. It can occur virtually at any age. There appears to be a minimal relationship between prostatitis and prostate cancer, as well as most patients who have prostate cancer demonstrate some kind of prostatitis on biopsy, both diseases are so common that it is difficult to prove a causal interrelationship. Subclinical histological prostatitis , an unusual form of chronic prostatitis simulates adenocarcinoma as it can cause high serum PSA level. Prostate volume and inflammation are the most important factors contributing to serum PSA elevation in men without clinically detectable is the most common malignancy in males, substantially causing an elevated PSA level. Cancers of <1 ml volume usually do not elevate PSA , and 16% of normal men who have PSA>4 ng/ml and 19% of prostatic cancers have normal PSA .

Table 1: The percentage of TRUS biopsies and PSA levels of the study groups

TRUS biopsies samples	Number of patients (N=66)	Percentage (%)	PSA ng/ml
BPH + Prostatitis	22	‘33%	5.1-26
Adeno-carcinoma	18	29.5%	9.3-25.6
BPH	16	24.5%	12-26
Prostatitis	10	15.15%	9.3-16

Conclusion

Benign prostatic hyperplasia and chronic prostatitis are the major causes of elevated serum PSA levels followed by prostate cancer. The PSA level in chronic prostatitis and in prostate cancer, no significant difference was noted. This renders TRUS biopsy as mandatory to determine the exact cause of elevated PSA.

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Inverse correlation of Interferon-gamma and CD8+ T Lymphocytes in Tuberculosis Patients

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ABSTRACT

BACKGROUND & OBJECTIVES: Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. All over the world TB is one of the leading causes of mortality and morbidity. Interferon-gamma (IFN-γ), at the site of MTB infection, activates macrophages to kill intracellular mycobacteria. CD8+ T cells kill the cells infected by viruses and other intracellular microbes. Diagnostic significance of IFN-γ has been reviewed in latent and active TB. Aim of this study was to determine association between IFN-γ and CD8+ T-cells in the blood of pulmonary TB-patients. **METHODS:** Study consisted of 54-TB-patients and 38 healthy subjects. Level of IFN-γ was determined by ELISA and percentage of CD8+ T-cells by flowcytometry. **RESULTS:** Level of IFN-γ and percentage of CD8+ T-lymphocytes were high in the blood of TB patients compared to control. **CONCLUSION:** TB patients had high level of IFN-γ compared to healthy controls. IFN-γ and CD8+ T cells were negatively correlated with duration of treatment of tuberculosis. There was no difference in percentage of CD8+ T cells in TB patients after 3 months of chemotherapy compared to controls.

Key words: Interferon gamma, tuberculosis, CD8+ T lymphocytes

الملخص

المقدمة والاهداف: الدرن هو مرض معدي يسببه بكتريا عصيات الدرن. وهو احد الاسباب الرئيسية للمراضة والوفاة في كل العالم. الانترفيرون جاما ينشط كريات الدم البيضاء لقتل البكتريا داخل الخلية في حالات الاصابة بالدرن . خلايا تي المناعية من نوع سي دي 8 لها القدرة علي قتل الخلايا التي تحتوي علي فيروسات او جراثيم . تم مراجعة الاهمية التشخيصية للانترفيرون جاما في حالات الاصابة بالدرن الكامن او النشط. هدفت الدراسة لتحديد العلاقة بين الانترفيرون جاما و خلايا تي المناعية من نوع سي دي 8 في دم مرضي الدرن الرئوي. **المنهج:** شملت الدراسة علي 54 مريض بالدرن و 38 شخص سليم. تم تحديد مستوي الانترفيرون جاما بطريقة الاليسا و تحديد نسبة خلايا تي المناعية من نوع سي دي 8 بواسطة مقياس الخلايا . **النتائج:** وجد ان مستوي الانترفيرون جاما و نسبة خلايا تي المناعية من نوع سي دي 8 اعلي في دم مرضي الدرن بالمقارنة بالأشخاص السليمين. **الإستنتاج:** مستوي الانترفيرون جاما اعلي في دم مرضي الدرن بالمقارنة بالأشخاص السليمين. مستوي الانترفيرون جاما و نسبة خلايا تي المناعية من نوع سي دي 8 تتناسب تناسبا عكسيا مع مدة علاج الدرن. لا توجد فروق في نسبة خلايا تي المناعية من نوع سي دي 8 في مرضي الدرن بعد 3 شهور من العلاج بمضادات الدرن بالمقارنة بالأشخاص السليمين.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by mycobacteria, mainly Mycobacterium tuberculosis [1]. All over the world TB is one of the leading causes of mortality and morbidity. In 2010, 8.8 million people had TB and 1.4 million died of TB [2]. Pakistan ranks eight among 22 high burden tuberculosis countries in the world [3] and in 2001, government of Pakistan declared TB as national emergency. The emergence of multi-drug resistant TB (MDR-TB) is another growing concern in the country [4].

Mycobacterium tuberculosis (Mtb) is large, non-motile, rod shaped intracellular bacterium, also known as acid-fast bacilli (AFB) [5]. Clinically tuberculosis may manifest as pulmonary or extra-pulmonary TB that may affect any body organ. Smear-positive pulmonary TB is the most infectious form and is diagnosed by microscopic examination or sputum culture. Smear-negative pulmonary TB is diagnosed by findings on chest x-ray or on treatment failure after standard antibiotics [6].

More than 90% of Mtb infected individuals do not develop disease and the organism remains dormant but in about 10% of individuals, there is reactivation of latent bacteria that proceeds to TB infection. Cell mediated immunity is considered vital in preventing

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spread of Mtb, whereas macrophages, dendritic cells, CD4+ T and CD8+ T cells are also essential in controlling TB [7].

Interferon-gamma (IFN-γ) is a soluble protein secreted by a number of cells which activates macrophages to kill intracellular mycobacteria and at the site of Mtb infection it is the major cytokine [8]. After disrupting IFN-γ gene, mice subjected to sub-lethal dose of intravenous Mtb progressed to disseminated TB [9]. IFN-γ knockout mice developed neither mature granulomas nor protective immunity after infection with a virulent Mtb strain [10].

Lymphocytes particularly CD4+ T cells secrete cytokines which can modify/amplify specific immune response. Based on cytokine secretion CD4+ cells are further divided into Th1 or Th2. Th1 cells produce IFN-γ, IL-2 whereas Th2 cells produce IL-4 [11]. Macrophages kill internalized Mtbs when activated by TNF-α and IFN-γ [12]. CD8+ T cells kill the cells that contain foreign antigens such as viruses and intracellular microbes [13].

There can be significant increase in CD8+ T cells with a notable reduction in CD4+ T cells during TB infection [14]. Number of CD4+ and CD8+ T cells varies during anti-tuberculosis treatment (ATT) but there is no significant change in the number of these cells between chronic TB patients and healthy controls [14].

Diagnostic significance of IFN-γ in latent and active TB has been reviewed [15]. Enzyme linked immunosorbent assay (ELISA) of IFN-γ provides information about the level of cytokine at a given point of time [16]. The present study was carried out to determine the level of IFN-γ and percentages of CD8+ T cells in the blood of pulmonary TB patients.

Materials and Methods

Study design: It was a cross sectional analytical study

Period & Place of Study: The study was performed during December 2011–November 2012, in the Department of Immunology, University of Health Sciences (UHS) Lahore after approval of Ethical Committee and Advanced Studies & Research Board of UHS and Shalimar Hospital Lahore. Written, informed consent was obtained from participants. According to the formula sample size was 385 but due to budget constraints, sample size was limited to 92; 54 patients and 38 healthy controls (Kukreja et al., 2001).

The subjects were from both genders between 15–70 years of age. Newly diagnosed TB patients not on anti-tuberculosis treatment (ATT) were labeled as Group-I and TB patients on ATT as Group-II. Each group consisted of suspected TB/diagnosed TB patients on clinical grounds and confirmed TB patients. Pregnancy, Hepatitis B, Hepatitis C, immunoproliferative disorder, malignancy, allergy, immunosuppressive therapy and TB patients on ATT for more than 3 months were excluded. Clinically suspected TB patients had clinical history, findings on chest x-ray, non-responsiveness to antibiotics and good response to ATT within two months. Sputum positive for ZN staining, sputum culture for AFB, tissue biopsy for granuloma/AFB or Mtb nucleic acids by molecular methods were considered as confirmed TB patients [5,6]. For controls subjects without history of chest infection in last three weeks were included.

Five ml of venous blood was drawn aseptically from anterior cubital vein and added into 3 vacutainers (BD); two containing ethylene diamine tetra acetic acid (EDTA) and one with gel. Blood samples were transported to the Department of Immunology, UHS. Complete blood count (CBC), total leukocyte count (TLC) and differential leukocyte count (DLC) was measured by Sysmex analyzer XT1800i.

Gel vial was centrifuged and plasma was separated and stored at -80°C for IFN-γ detection by ELISA technique (DIACLONE diagnostics, France). Assay was performed according to manufacturer's directions who claimed no cross reactivity with other autoantigens and detection limit was between 0.25-400pg/ml.

Out of 54 TB patients, 39 subjects were selected randomly for immunophenotyping. Fluorescein isothiocyanate (FITC) tagged MoA CD8, phycoerythrin (PE) tagged MoA CD3, allophycocyanin (APC) tagged MoA CD4, and peridinin-chlorophyll-protien (PerCP) tagged MoA CD45 (DIACLONE, France) was used. Lyse-wash method using whole blood was performed. CD4/CD8 cells ratio was analysed by FACS Calibur

4-color analyser (BD, USA). 100µl of whole blood was added to 2 FACS tubes. 10µl each of CD3, CD4, CD8, and CD45 was added to one tube and isotype control to other. Tubes were mixed and incubated in dark at room temperature for 15 minutes. Two ml of BD FACSLyse was added to each tube. Tubes incubated in dark for 12 minutes, centrifuged at 250g for 10 minutes and supernatant was discarded. Pellet was re-suspended and cells were washed twice by adding 2ml of sheath fluid, mixed, centrifuged and supernatant was discarded. Cells were re-suspended in 0.5ml of sheath fluid with 2% paraformaldehyde. Fluorescence attributable to FITC, PE, APC and PerCP labeled monoclonal antibodies were determined using excitation by 15mW, 488ηM, argon-ion blue laser. Machine was calibrated and fluorescent signal compensation was performed using Cell Quest Pro software (BD) and Calibrite beads (BD). Data was acquired, templates were designed for two parameter dot-plot representing forward angle light scatter-side scatter (FALS-SS), and SS-CD45. Former dot-plot was used as indicators of satisfactory sample preparation while the latter to identify lymphocyte population. Lymphocytes (CD45 brightest population with lowest side scatter) in SS-CD45 dot-plot were gated and data for CD3+CD4+ and CD3+CD8+ cells was acquired. CD4+, CD8+ cells were analysed using two parameters dot-plot with log FITC fluorescence (CD3) on X-axis and log PE or APC fluorescence (CD4 or CD8) on Y-axis. Isotype control was run for each sample and fluorescence channel boundary was selected above which no more than 1% of control cells were detected and below this all stained cells were considered negative.

Statistical Analysis

Data was analyzed using SPSS-16.0, mean ± SD for quantitative variables, frequencies and percentages for qualitative variables and two-independent sample t test was applied to observe group mean differences of T cell percentages and IFN-γ levels. Pearson correlation was applied to observe correlation between IFN-γ levels, mean T cell percentages and duration of ATT, and p≤0.05 was considered as statistically significant.

Results

Among 54 TB patients, 25 (46.30%) were males and 29 (53.70%) were females, while in control group, 18 (47.30%) were males and 20 (52.63 %) were females. Mean age of TB patients and controls was 27.8 and 26.6 years respectively (Table 1). 27 (50%) patients had confirmed TB that included 24 (44.44%) ZN positive sputum and 3 (5.56 %) biopsy/granuloma positive while 27 (50%) were clinically diagnosed TB patients. 11 (20.37%) TB patients were on ATT while 43 (79.62%) were newly diagnosed. The results of CBC and ESR (erythrocyte sedimentation rate), CD4+cells and their comparison between TB patients and controls were made and described previously Hussain et al [17] and Afzal et al [18].

The mean±SD of IFN-γ was high in TB patients (48.69 ±4.20) pg/ml compared to controls (12.99±5.7) pg/ml and there was significant difference (p=0.002). Percentages of CD8+ cell were also high in TB patients but not statistically significant (Table-3).

The mean ± SD of IFN-γ was high in newly diagnosed TB patients (59.68±28.78) pg/ml compared to TB patients on ATT (36.85±24.76) pg/ml and there was significant difference (p=0.001). Mean ± SD of CD8 cells was not significantly different between newly diagnosed TB patients and TB patients on ATT (Table 4).

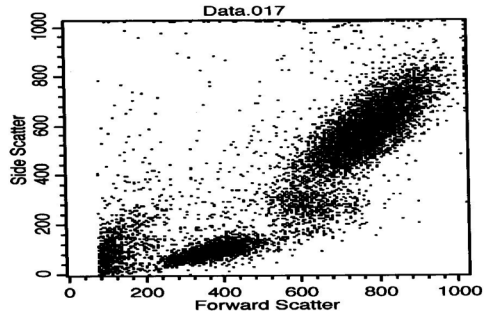

Figure 1: Distribution of different blood cells

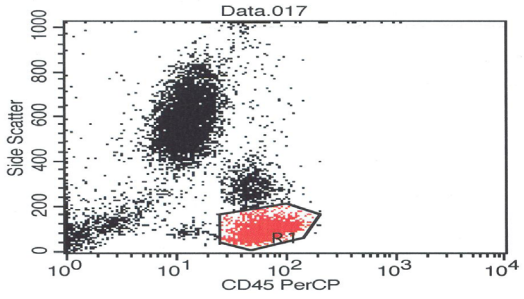

Figure 1: Lymphocytes selected from different cells of the blood

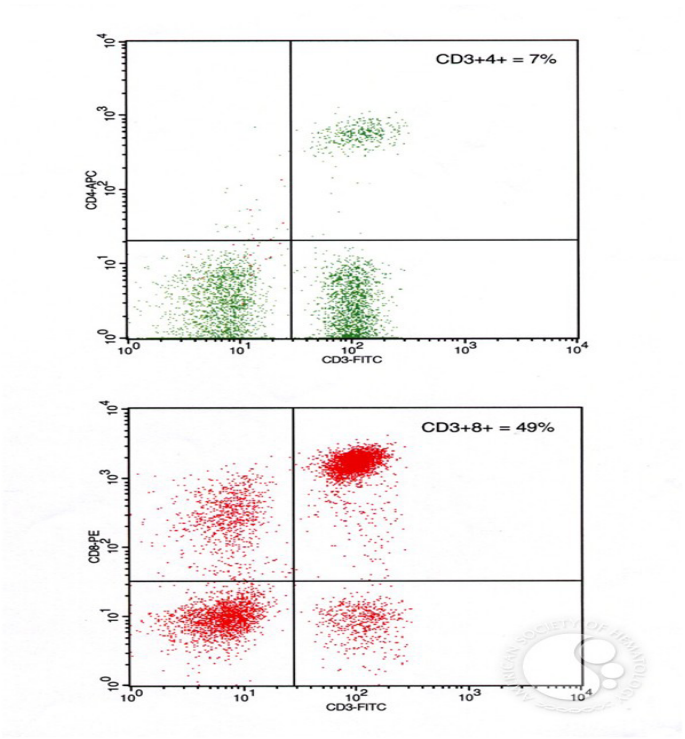

Figure 1: Upper: T lymphocytes i.e. CD3 and CD4 cells = 7% Lower: T lymphocytes i.e. CD3CD8 cells = 49%

The level of IFN-γ and percentage of CD8+ T cells decreased with the duration of treatment. By applying Pearson's correlation test, an inverse correlation was observed between mean percentage of CD8+ T cells and mean level of IFN-γ with the duration of ATT (r=-0.433, p=0.021) (Figure-1).

Discussion

Hematological parameters, percentages of CD4+ T, CD8+ T cells and level of IFN-γ in TB patients and controls have been described and discussed elsewhere [17, 18]. There is inverse correlation between the level of IFN-γ and the percentage of CD8+ T cells with the duration of ATT. It probably indicates that CD8+ T cells are good source of IFN-γ [19-21]. It has been documented that IFN-γ and tumor necrosis factor-α play role in the induction of inducible nitric oxide synthase and nitric oxide [22, 23]. In an earlier work, specific mycobacterial antigens like ESAT6 and CFP-10 were used to stimulate IFN-γ production [24]. Although the current study did not use such specific antigens, but significantly high level of IFN-γ has been observed in TB patients compared to controls.

There is compelling evidence that CD8+ T cells are critical in different ways for protective immunity against TB: first, the adoptive transfer and cell depletion studies in vivo have demonstrated that CD8+ T cells are involved in controlling Mtb infection. Second, CD8+ T cell lines and clones can recognize Mtb antigens in vitro, lyse Mtb-infected macrophages in an antigen specific manner, and restrict the growth of Mtb in macrophages [25,26], and third, beta-2 macroglobulin deficient mice as well as TAP-deficient mice failed to express MHC class I molecules and succumbed to Mtb infection [27,28]. Therefore, CD8+ T cells can contribute to the immune response against Mtb infection by at least three possible pathways: the release of IFN-γ, lysis of infected targets, and a direct antimicrobial activity. The present study provides indirect evidence that human CD8+ T cells release IFN-γ.

Table 1: Age and Gender Distribution

Subjects	Mean Age (years)	Male	Female	Total
TB Patients	27.8	25 (46.30%)	29 (53.70%)	54
Controls	26.6	18 (47.30%)	20 (52.63%)	38

The in vitro study of Cho 2000 et al [29] also demonstrated that human MHC class I restricted CD8+ T cells specific for Mtb protein epitopes contribute to host defense by the release of IFN-γ, by lysing infected target cells, and by directly killing the intracellular bacteria. Claudia 2006 et al [30] also documented that CD8+ T cells control Mtb infection in healthy house hold contacts of TB patients.

Table 2: Mean CD8+ T lymphocyte Percentages and mean IFN-γ level in TB patients and Controls

Parameter	TB patients Mean ± SD	Controls Mean ± SD	p Value
IFN-γ (pg/ml)	48.69 + 4.20	12.99 ± 5.7	0.002*
CD8+ T cell%	32.0±9.8	30.2±7.2	0.356
* Statistically significant p value ≤0.05			

Gariby et al 2008 [31] documented Mtb specific CD8+ T cells secreted IFN-γ and decreased percentage of CD8+ T cells in TB patients compared to latent TB

infection subjects which returned to normal after 4-month therapy. It is different from the current study because they included active and latent TB patients while in the current study TB patients were compared with controls.

The study of Nadia 2009 et al [32] showed that majority of Mtb specific CD8+ T cells secreted IFN-γ when stimulated by Mtb antigens but they reported ex-vivo percentages of CD8+ T cells were significantly lower in TB patients as compare to the subjects with latent TB infection which return to normal after 4-month therapy. This discrepancy is probably due to fact that they made TB patients into two groups; active and latent TB patients while in the current study this categorization was not made and TB patients had been compared with controls. Iukary T et al (2012) [33] also suggested levels of interferon-gamma increases after treatment of latent tuberculosis infection in high-transmission setting. Further WHO (2011) [34] made recommendations on the use of commercial TB interferon-gamma release assays in low and middle income countries.

Table 3: Mean CD8+ T lymphocyte percentages and Mean IFN-γ in newly-diagnosed TB patients and TB Patients receiving ATT

Parameter	Newly Diagnosed TB patients Mean ± SD	TB patients on ATT Mean ± SD	p value
CD8+ T cell%	34.01 ± 9.3	30.8 ± 10.0	0.319
IFN-γ (pg/ml)	59.68 ± 28.78	36.85 ± 24.76	0.001*
* Statistically significant p value ≤0.05			

The results of current study are in partial agreement with study of Virginie et al [35]. They documented that patients of active pulmonary TB had increased frequency of Mtb-specific IFN-γ producing CD8+ T cells as compared to patients suffering from latent TB. In the current study, categorization of TB patients into active and latent was not done and TB patients were compared with healthy controls.

Conclusion

The level of IFN-γ and CD8+ T cells decreases with the advancement of duration of TB treatment.

Acknowledgement

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Conflict of Interest

There is no conflict of interest of any author.

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Assessment of Pre and Post dental treatment Anxiety among Saudi Arabian population

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ABSTRACT

AIM: To estimate the prevalence and associations of dental anxiety and to find out the methods to relieve anxiety in a sample of the Saudi Arabian population. **MATERIAL & METHODS:** Four hundred subjects from Saudi Arabia of different age groups were evaluated by a questionnaire. **RESULTS:** A majority of the subjects (80%) were accompanied by a friend or relative to the dental clinic. 70.5% of the subjects were anxious or afraid to go to a dental clinic but 76.7% of the subjects said that they were more relaxed after spending sometime in the clinic. The factors which relaxed them were presence of accompanying person and communication with the dentist whereas communication with other patients in the waiting area and the ambience of the clinic were not major factors. 32% of the subjects found that the treatment procedure during their first appointment was painful. Most of the patients found visit to a dental clinic was better than that to a general hospital. Most of the subjects (89.3%) responded that their overall experience about their first dental visit was good or fair and only 10.7% said that it was poor. 61.3% of the subjects were willing to go back to the same dentist. **CONCLUSION:** The prevalence of dental anxiety was high (70.5%). The apprehension seen towards dental treatment is based on misconceptions and fear of the unknown. My suggestion is to conduct awareness camps in localities and organize school trips to allay the fear of unknown.

الملخص

الأهداف: تحديد معدل انتشار ومسببات القلق الناتج من مشاكل الاسنان , ولمعرفة وسائل تخفيف هذا القلق في عينة من السعوديين. **المنهج:** تم تقييم 400 شخص سعودي من مختلف الاعمار بواسطة استبيان. **النتائج:** أغلب المشاركين (80%) حضروا لعيادة الاسنان برفقة صديق او قريب . 70,5% من المشاركين كانوا قلقين وخائفين للذهاب الي عيادة الاسنان , ولكن 76,7 من المشاركين شعروا بالراحة والاسترخاء بعد الجلوس لبعض الوقت بعيادة الاسنان. من اسباب استرخاء المشاركين وشعورهم بالارتياح وجود المرافق وايضا التواصل مع طبيب الاسنان وليس تواصلهم مع المرضي الاخرين او وجودهم في بيئة العيادة . 32% من المشاركين افادوا بوجود الم خلال المعالجة في اول موعد . زيارة عيادة الاسنان افضل من زيارة المستشفى العام لدي أغلب المشاركين . 89,3% من المشاركين افادوا ان تجربتهم في الزيارة الاولي لعيادة الاسنان كانت جيدة وفقط 10.7% كانت تجربتهم سيئة. 61.3% من المشاركين رغبوا في العودة لمراجعة نفس طبيب الاسنان **الإستنتاج:** معدل انتشار القلق الناتج من مشاكل الاسنان عالي (70.5%) . وهذا القلق والخوف بني علي المفاهيم الخاطئة والخوف من المجهول. نقترح حلقات توعية في المناطق وتنظيم رحلات للمدارس لرفع الخوف من المجهول

INTRODUCTION

Dental treatment is an integral part of health care system. In dental hospital it is noticed that most of the patients are anxious during the appointments. The prevalence of dental anxiety has been shown to range between 4 and 20% in the general population of industrialised countries [1-3]. Fear is the distressing emotion aroused by danger or pain, real or imagined. When fear becomes irrational it becomes a phobia. Dental anxiety may in some cases progress on to become a phobia [4]. Sometimes it is difficult to manage a patient in the dental clinic psychologically. Dental anxiety partially limits, or completely prevents, utilisation of oral health care services [5, 6]. Patients are apprehensive for simple dental procedures. This apprehension prevents them from visiting the dental clinic when the dental disease is at an initial stage and they visit dental clinic only when the disease has reached an advanced stage and more aggressive treatment approach is required for the treatment. This affects the oral health and quality of life .When professional care is provided, it is often given under general anaesthesia without consideration of the aetiological factors behind dental fear. Ideally, the

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management of patients with dental anxiety requires psycho-behavioural and sedation procedures [7, 8] as alternatives to general anaesthesia. Such techniques have been shown to improve patient capacity to cope with dental care over time [9].

Dental fear and anxiety may have a complex and multifactorial psychological aetiology. Most dental providers are well versed in treating pain with pharmacological tools but very few understand and address the underlying fears of the patient [10].The aims of this study were to estimate the prevalence and associations of dental anxiety and to find out the methods to relieve anxiety in a sample of the Saudi Arabian population. Although similar studies have been done previously, most of the studies have focussed on a given set of population for example university students or adolescent females [11, 12].

MATERIALS AND METHODS

A simple random sampling method was chosen for this study. 20 dental students of college of dentistry – Majmaah University, who belonged to different regions of Kingdom of Saudi Arabia were chosen, they were explained about the purpose of the study. Each student was given 20 questionnaires, the students then interviewed the subjects belonging to their respective areas during vacations. This helped us to get a representative sample from different regions of Saudi Arabia. As the questionnaire was administered by trained dental students, all 400 questionnaires were complete and were included in the study.

The study included only those subjects who had undergone dental treatment and the questionnaire was administered after treatment. As the subjects were answering the questionnaire under free will and were free to choose not take part in the study, there were no ethical implications and hence ethical approval was not obtained.

Questionnaire consisted of seventeen questions which were divided in to four sections. The first section had questions which were related to subject's apprehension before visiting a dental clinic. Section 2 consisted of questions that were related to change in attitude during and after treatment. Questions in section 3 were related to their experience during treatment. The last set of questions was designed to evaluate the subjects' views after treatment.

Table 1: Quesstionnaire with results

No	Question	Response of Subjects		
1	At what age did you visit to dental clinic first time?	18- 30 years 63%		30-45 years 37%
2	Who accompanied you to the dental clinic?	Friend or relative 80%		Alone 20%
3	Were you anxious or afraid to go to the clinic?	Yes 70.5%		No 29.5%
4	If yes what was the reason for your fear?	Friends Family member experience 80.5%		Self research 19.5%
5	What made you anxious?	Fear of pain 54.6%	Appearance and sound of the instruments, injection or light 34.4%	Dental personnel (dentist or assistant) 11%
6	After spending some time in the dental clinic, were you less anxious (relaxed) than earlier?	Yes 76.7%		No 23.3%
7	Did the presence of accompanying person help you to relax?	Yes 74.6%		No 25.4%
8	Did the communication with dentist help you to relax?	Yes 80.1%		No 19.9%
9	Were there any audio visual aids to help you relax?	Yes 48.2%		No 51.8%
10	Did the communication with other patients in the waiting area help you to relax?	Yes 40.1%		No 59.4%
11	Did the ambience/ surrounding help you to relax	Yes 54.4%		No 45.6%
12	Did you receive any oral injection during the first dental visit?	Yes 64.7%		No 35.3%
13	Was the oral injection more painful than extra oral injection?	Yes 65%		No 35%
14	Was the treatment procedure during their first appointment painful?	Yes 32%		No 68%
15	How was your dental experience as compared to hospital visit for a non dental procedure?	Better than hospital visit 55%	Hospital visit better than dental visit 21%	Both were same 24%
16	How do you rate the first dental experience?	Fair 55.1%	Good 34.2%	Poor 10.7%
17	Would you like to go back to the same dentist?	Yes 61.3%		No 38.7%

The answers were tabulated and descriptive analysis was done to arrive at the results.

RESULTS

A total of 400 responses were analyzed and tabulated (Table 1), as the questionnaire was interviewer administered 100% response rate was achieved. All the respondents belonged different parts of Saudi Arabia and were between18 to 45 years of age. The questions 1-5 were designed to ascertain the attitude of the subjects towards dental treatment prior to their visit to the dental clinic. It was noticed that irrespective of the age group 80 % of the subjects were accompanied by a friend or relative to the dental clinic. 70.5% of the subjects were anxious or afraid to go to a dental clinic; among these subjects 80.5% were afraid after hearing about dental experiences of friends and family and not because of any firsthand experience. 54.6% of the subjects said that they were anxious because they expected the procedure to be painful, whereas 34.4% were afraid of the dental chair, equipment's and oral injections. Only 11% were afraid of the dental personnel (Figure 1).

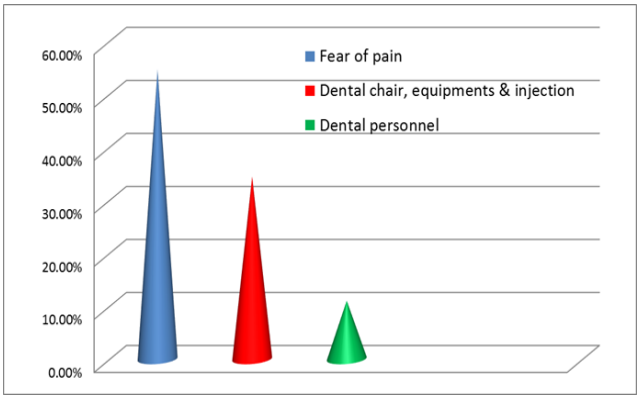

Figure 1: What made you anxious?

The questions 6-11 were designed to assess any change in attitude and the reasons for change, during or after the treatment. 76.7% of the subjects said that they were more relaxed after spending sometime in the clinic. Among these persons, 74.6 % said that the presence of accompanying person was an important factor in helping them to relax. 80.1% said that communication with the dentist helped them to relax. 51.8% of the subjects did not find any audio-visual aids which could help them to familiarize with the treatment procedures, which could help to ally their anxiety. Communication with other patients in the waiting area and the ambience of the clinic were not major factors and only 40.1% and 54.4% respectively found them to be of any help (Figure 2). Questions 12-14 were designed to evaluate the experience of the subjects during treatment. 64.7% of the subjects received oral injection during their first appointment and 65% of them said that oral injection was more painful than any other injection taken elsewhere on the body (extra oral). 32% of the subjects said that the treatment procedure during their first appointment was painful.

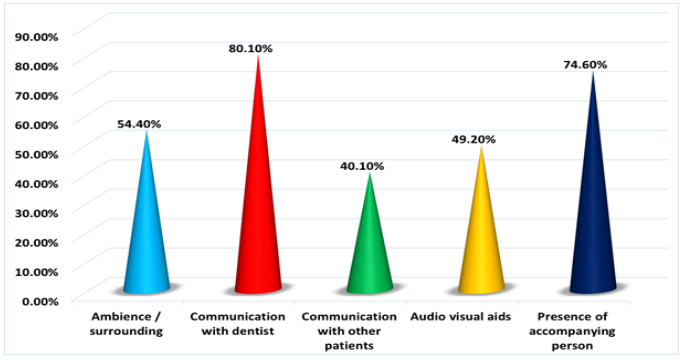

Figure 2: Factors help subjects to relax after some time

Questions 15-17 were designed to check for the patient's views and attitude post treatment. 55% of the patients found that the visit to a dental clinic was better than that to a general hospital, whereas 21% said that the hospital visit was better and 24% found both to be the same (Figure 3). 89.3% of the subjects responded that their overall experience about their first dental visit was good or fair and only 10.7% said that it was poor (Figure 4). 61.3% of the subjects were willing to go back to the same dentist. As this was a descriptive study, no statistical tests were used. Only the percentages of responses were calculated.

DISCUSSION

In dental practice it is seen that the number of people with dental problems and the number of people who seek dental treatment voluntarily is highly disproportional [13], even when the dental treatment is easily available to the general population. The reason for this discrepancy may be psychological barriers which exist due to misconceptions about dental treatment.

The presence of dental anxiety among general population is a known fact; its prevalence varies depending on the sample population [14]. In my study I found that majority of the subjects were afraid/ anxious to go to the dental clinic, the reason for this

fear was the impression created by peers and family members about dental treatment and not based on actual knowledge gained either by research on the internet or by accompanying someone to the dental clinic. Many of the respondents also said that they were afraid because they expected the treatment to be painful and were anxious of sitting alone on the dental chair, and the instruments. Hence we can infer that the pre-visit anxiety is mostly due to fear of the unknown and general population needs to be made aware of the dental procedures.

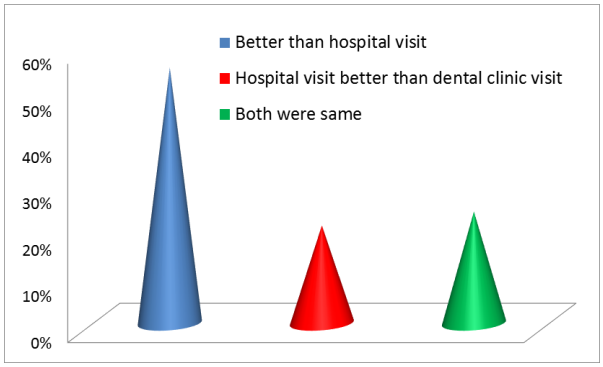

Figure 3: How was your dental experience as compared to hospital visit for a non dental procedure?

When the subject was asked about their anxiety or fear after spending sometime in the dental clinic, surprising a huge proportion of the people said that they were more relaxed. Many factors like communication with the dentist, who explained the procedure or reassurance by the accompanying person also helped them to relax. One important point noted in our study was that more than half of the subjects said that there were no audio-visual aids available in the clinic. In previous studies it is reported that the presence of charts or videos which explain various treatment procedures in the waiting area helps to allay the fear in the patient and makes him more receptive to treatment [15]. Studies have also shown that communication with the dentist helps in behavior management of the patient [16] which was also proved in this study.

Even though majority of the subjects said that they received oral injection during their first dental appointment, they also said that except for the injection, they did not find the treatment procedure to be painful. I suggest that oral injections during first visit should be avoided but if the need for the injection is justified, then minimizing the pain may also make the patient more comfortable.

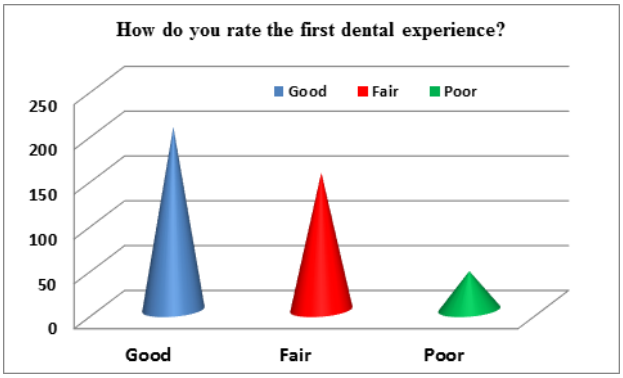

Figure 4: How do you rate the first dental experience?

Majority of the respondents found the visit to a dental clinic to be better than visit to the general hospital and the overall experience was rated to be good or fair by a large number of subjects. This suggests that once the patient is familiar with the treatment procedures and dental clinic, he/she is not scared or afraid of the dental procedure or dentist.

CONCLUSION

Based on the findings of this study I can conclude that the prevalence of dental anxiety was high (70.5%). The apprehension seen towards dental treatment is majorly based on misconceptions and fear of the unknown. Hence in order to reduce dental anxiety among general population my suggestion is to conduct awareness camps in localities and organize school trips to allay the fear of unknown. Additionally the dentist can also make the patients experience better by speaking to the patient in his office and familiarizing with him before taking him to the dental chair.

CONFLICT OF INTEREST

There were no financial and personal relationships with other people or organization that could inappropriately influence this work

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Health Seeking Behaviour of women towards Reproductive Tract Infection: A cross-sectional Study at a Primary Health level in Khashm Al'an, Saudi Arabia

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ABSTRACT

BACKGROUND: Reproductive tract infections (RTIs) are global burden and major public health concern, particularly in developing countries. If untreated, RTI's can lead to serious consequences such as ectopic pregnancy, cervical cancer and infertility and increase vulnerability to STIs. However, detection of disease and clinical diagnosis is largely dependent on patient's awareness of RTI symptoms and attitude. There is limited publication on the subject in Gulf Arab states. **OBJECTIVES:** To describe the beliefs and health seeking behavior regarding RTIs among women of the reproductive age, and factors associated with poor knowledge and care seeking behavior for RTIs among Saudi women. **METHOD:** A cross sectional study was conducted on total of 250 women who attended a primary health care clinic at Khashm Al'an in east of Riyadh, Saudi Arabia. Through a structured interview, participants gave data about demographics, knowledge of RTI's and care seeking behavior. Data were described in terms of frequencies and percentages. Relationships between nominal variables were explored by using Chi-square test. Statistical significance was set to 5% or less. **RESULT:** There were 62.8% of women who had heard of RTIs. Women, who were younger, educated, and employed or studying had higher awareness and knowledge on RTIs and sought medical care more than others. Women who were more aware, had experience, were younger, literate and not homebound were more likely to seek medical care if needed for RTIs. Television and Internet were significant sources of information for RTIs. **CONCLUSION:** Reproductive health awareness programs should focus on homebound, less educated and elder women. Television and internet are reliable resources for delivering reproductive health messages to the target population.

الملخص

مقدمة: تعتبر التهابات الجهاز التناسلي في النساء عبء عالمي ومشكلة صحة عامة, خاصة في الدول النامية. عدم معالجة التهابات الجهاز التناسلي تؤدي الي مضاعفات خطيرة مثل الحمل خارج الرحم , سرطان عنق الرحم , عدم الانجاب وايضا زيادة القابلية للإصابة بالأمراض المنقولة جنسيا. اكتشاف المرض وتشخيصه سريريا يعتمد علي وعي المريضات بأعراض التهابات الجهاز التناسلي وسلوكهن . النشر عن هذا الموضوع محدود جدا في منطقة الخليج. **الاهداف:** وصف المعتقدات والسلوك فيما يخص البحث عن المعالجة لمريضات التهابات الجهاز التناسلي للنساء في سن الانجاب، ولوصف العوامل المؤدية لضعف المعرفة والبحث عن العلاج وسط المريضات السعوديات . **المنهج:** دراسة مقطعية لعدد 250 امرأة من مراجعات عيادة الرعاية الصحية الاولية بخشم العان بشرق الرياض, بالمملكة العربية السعودية . تم جمع البيانات من المشاركين بواسطة مقابلات منتظمة وقد تم جمع بيانات عن ديموغرافيا المشاركين وعن معرفتهم بالتهابات الجهاز التناسلي وسلوك البحث عن العلاج. وصفت البيانات بالتكرارات والنسب واستخدم اختبار مربع كاي لاكتشاف العلاقات بين المتغيرات الاسمية وحددت الدلالات الإحصائية ب 5 % او اقل. **النتائج:** وجد ان 62,8% من النساء سمعن بالتهابات الجهاز التناسلي . النساء المتعلمات والاصغر سنا واللاتي يعملن او يدرسن اكثر وعيا ومعرفة بالتهابات الجهاز التناسلي ويسعين للعلاج اكثر من الاخريات. النساء المتعلمات والاصغر سنا والاكثر وعيا وتجربة يسعين للعلاج من امراض الجهاز التناسلي. من اكثر الوسائل فعالية في التوعية التلفزيون والانترنت. **الإستنتاج:** يجب ان تركز التوعية بصحة الجهاز التناسلي علي ربات البيوت الاقل تعليما والاكبر سنا. التلفزيون والانترنت من الوسائل التي يمكن الاعتماد عليها في توصيل رسائل التوعية لصحة الجهاز التناسلي للنساء .

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INTRODUCTION

RTI in female usually originate in the lower genital tract as vaginitis or cervicitis and may produce symptoms such as abnormal vaginal discharge, genital pain, itching and burning feeling with urination [1, 2]. A patient will be diagnosed to have RTI if she has any of these diseases: infection that result from overgrowth of organisms normally present in the reproductive tract, infection associated with medical procedures, insertion of intra uterine devices, and sexually transmitted infections (STIs) [3, 4].

Majority of patients with RTI are asymptomatic which is a barrier to effective prevention and treatment [5]. RTI is a global burden and a major public health concern, particularly in developing countries.[2] There are very limited studies on RTI in the Arabian Gulf States [6]. In Saudi Arabia there is a special need to conduct more prevalence studies to assess the magnitude of the problem.

Carcinoma of the cervix ranks ninth in the frequency of carcinomas among Saudi females, with a prevalence rate of 3.6%,[7] caused by Human Papillomavirus [8]. KSA has recorded 1768 Saudi nationals testing HIV positive since 1984 when the Kingdom began monitoring the disease. Nearly 46% of adult patients contracted the virus through the sexual route [9-11]. Information about patients belief and health seeking behavior will facilitate to physicians to give them appropriate health education and helping for appropriate allocation of scarce resources and planning of cost effective health care strategies. There is always a need to create awareness programs and improve public knowledge about RTIs, to support other preventive measures[6]. The Aim of this study is to describe health seeking behavior with regard to RTIs, assess level of awareness and knowledge among Saudi women of child bearing age.

METHODS

This is a cross-sectional study conducted on 250 women at a reproductive age attended primary health services at Khashm Al'an, in east of Riyadh, Saudi Arabia, in June 2013. The data has been collected by using a structured and translated questionnaire used in a study by Rabiu et al [2]. The questionnaire consists of three parts, the first part consists of socio-demographic data, including age, marital status, educational level, and occupation. The second part assesses their belief of reproductive tract infection, while third part assesses health seeking behavior. All questions have been constructed in the same style, and either direct (single or multiple choice], or open ended. A pilot study on the questionnaire was carried out on 20 persons face to face by an expert Arabic nurse and questions were modified for clarity and comprehension.

ETHICAL APPROVAL

Verbal consent from the participants was obtained before they were interviewed by the trained nurse. Moreover, the ethical approval was obtained from King Abdullah International Medical Research Center, Riyadh. Participants were interviewed in privacy and Confidentiality of data assured by coding participant.

STATISTICAL ANALYSIS

Data entry and analysis was done using SPSS version 18. Data was analyzed descriptively with mean, frequencies, and percentages. Further analysis carried out using Chi-square and Odds ratio. P-value was considered significant at ≤0.05.

RESULTS

A total of 250 women completed the questionnaire. Age of participants ranged between 18 and 50 with a mean of 35.1. Of all respondents, (76.8%) were married, (43.6%) illiterate, (17.8%] were university education, (83.2%) house wife, and <10% were divorced or widowed (Table 1).

250 patients, (82.8%) believed sexual intercourse is a mode of contracting RTI's, followed by poor hygiene (77.6%), and only (28.4%) were for unsafe delivery. (Figure 1)

Of the STIs, syphilis was named as a commonly known infection by 32.6% of the patients, while 20.2% of the patients named HIV, and 3.4% named hepatitis and herpes equally.

Hundred sixty two (64.8%) of the respondents had heard of RTIs. Fifty six (22.4%) of the respondents had experienced symptoms of RTIs during the last year. When asked about the source of information, (34.8%] of the patients got their information about RTIs from health care workers, and only (7.6%) got

their information from magazine. Internet was a source of information about RTI for 12.8%, and 32% gained their knowledge from friends and relatives. (Figure 2).

Table 1: Participants Demographics

Characteristic	Number	Percentage
Mean Age 35.1	N=245	(%)
Age in years		
18-30	98	40
31-40	68	27.8
41-50	79	32.2
Marital status	N=250	(%]
Single	34	13.6
Married	192	76.8
Divorced	12	4.8
Widowed	12	4.8
Educational level	N=250	(%]
Primary	38	15.2
Intermediate	27	10.8
Secondary	32	12.8
University	44	17.8
Illiterate	109	43.6
Occupation	N=250	(%]
House wife	208	83.2
Student	16	6.4
Employed	26	10.4

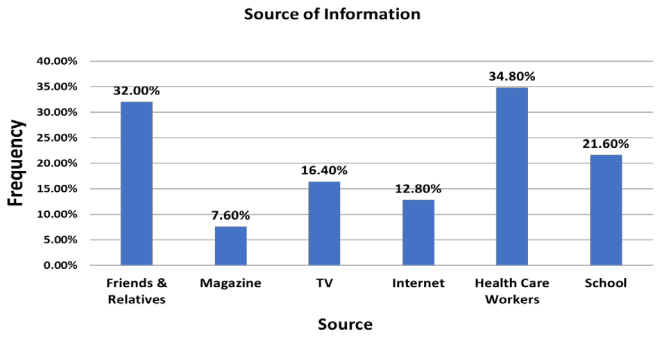

Figure 1: Perceived mode of transmission of STIs among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

The perceived effect of different contraception methods on transmission of RTIs, more than half of the respondents did not know about the effect of contraception methods in contracting RTIs. Where (8.4%] wrongly believe that condom increased the risk of contacting RTIs. And (36%] of the patients believe that IUCD increased the risk of contracting RTIs. (Table 2)

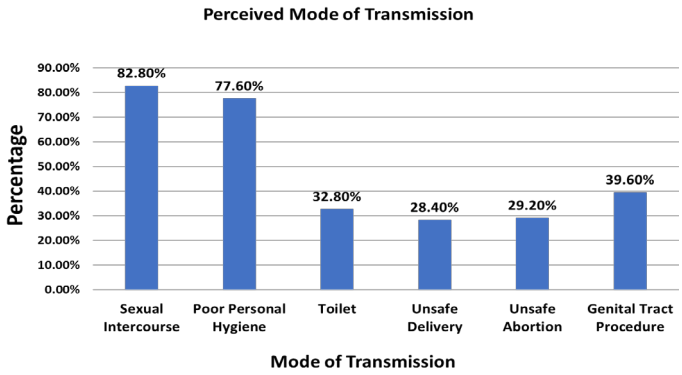

Figure 2: Source of Information about RTIs among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

Symptoms of RTIs, Vaginal discharge and vulval itching were chosen by (92.0%) and (91.2%) respectively of the patients as known symptoms, and (22.4%) checked painful urination. (Table 3)

Table 2: Perceived Effect of Contraception Methods among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013 . (correct answers in Bold)

Type of contraception	N=250	Percentage
Condom		
Increase	21	8.4
Decrease	78	**31.2**
No effect	17	6.8
I don't know	134	53.6
IUCD		
Increase	**90**	**36**
Decrease	15	6
No effect	23	9.2
I don't know	122	48.8
OCP		
Increase	58	23.2
Decrease	17	6.8
No effect	34	**13.6**
I don't know	141	56.4
Implants		
Increase	27	**10.8**
Decrease	3	1.2
No effect	10	4
I don't know	210	84

Regarding complications of RTIs, Cervical cancer had been chosen by (53.6%) of the patients, and chronic pelvic pain by (45.6%) of known complications of RTIs, while (15.2%) thought ectopic pregnancy as known complications of RTIs. (Table 3) 61.8% of the patients sought medical care when they experienced symptoms of RTIs, and (32.6%) of the patients used herbal treatment.

Table 3: Knowledge of symptoms and complications among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

	Number of Cases	Percent of Cases
***Knowledge of symptoms**		
Vaginal discharge	230	92.0
Vulval itching	228	91.2
Lower abdominal pain	113	45.2
Painful urination	56	22.4
Genital sore	60	24.0
Pain during menses	33	13.2
Genital/groin swelling	58	23.2
Painful intercourse	46	18.4
***Knowledge of complications**		
Infertility	64	25.6
Cervical cancer	134	53.6
Heavy menses	51	20.4
Ectopic pregnancy	38	15.2
Chronic pelvic pain	114	45.6
Miscarriage	79	31.6
Still birth	50	20.0
Congenital anomaly	49	16.0

Governmental health centers and general hospital were the most visited health facility by the patients for treatment of RTIs, reported by (44.1%) and (40.6%) of the patients respectively. Shame was reported by (57.1%) of the patients as a reason for not seeking medical care.

Awareness of RTIs varied significantly between age groups, education, occupation, and marital status. Young Population (18-30years] where more aware RTIs (Chi-sq=28.75, p-value<0.001). Literacy had an effect on awareness of RTIs; (83%) of the literate group had heard of RTIs compared to only (41.3%) of those illiterate(Chi-sq=46.85, P<0.001).

Being an outsider (student, employed] helped women in hearing about RTI's, as 90.5% of outsider women had heard of RTI (Chi-sq=14.95, P<0.001) (Table 4).

Health Seeking Behavior :

Subjects between 18-30 years old, Literate and subjects who are not homebound (Students, Working] were the most to seek medical care. Those divorced or widowed were less likely to seek medical care than singles and married (Chi sq=19.7, P <0.001) (Table 5).

Experience of RTIs vs behavior:

Subjects who ever experienced symptoms of RTIs were 2.9 times more likely to seek medical care than those who never experienced RTIs.

Awareness of complications vs behavior:

Patients who were able to recognized Ectopic pregnancy as a complication of RTIs were more to seek medical care than those who failed to recognize it (Chi sq= 5.347, P=0.021). For the rest of complications there was no significant difference in seeking medical care.

Table 4: Awareness of RTIs among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

Have you heard of RTI (% within Socio-demographic data]		
	Yes	No
Socio-demographic data		
Age in groups		
18-30	83.7%	16.3%
31-40	60.3%	39.7%
41-50	45.6%	54.4%
Chi-sq=28.753		
P value<0.001		
Educational group		
Illiterate	41.3%	58.7%
Literate	83.0%	17.0%
Chi-sq=46.853		
P value<0.001		
Occupational group		
House wife	59.6%	40.4%
Outsider	90.5%	9.5%
Chi-sq=14.590		
P value<0.001		

Source of Information:
Subjects who got their information from TV, Internet or School were more likely to seek medical care while no significant difference was notice in behavior among those got their information from other sources (Table 8).

Table 5: Health Seeking Behavior among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

	Health Seeking Behavior (% within Socio-demographic data]	
	Number of Sought medical care	Number of Didn't seek medical care
Socio-demographic data		
Age in groups		
18-30	80(81.6%)	18(18.4%)
31-40	50(73.5%)	18(26.5%)
41-50	45(57.0%)	34(43.0%)
Chi-sq=13.2		
P value<0.001		
Marital group		
Never married	23(67.6%)	11(32.4%)
Married	147(76.6%)	45(23.4%)
Divorced	4(33.3%)	8(66.7%)
Widowed	4(33.3%)	8(66.7%)
Chi-sq=19.7		
P value <0.001		
Educational group		
Illiterate	62(56.9%]	47(43.1%]
Literate	116(82.3%]	25(17.7%]
Chi-sq=19.3		
P value<0.001		
Occupational group		
House wife	142(68.3%]	66(31.7%]
Outsider	36(85.7%]	6(14.3%]
Chi-sq=5.2		
P value<0.023		

DISCUSSION

This study showed that most of the participants 40% are 18-30 years age group, which is part of the active reproductive age group and more prone to RTIs. Nearly 65% of the respondents had heard of RTIs which is low percentage, compared to a study conducted in Nigeria that showed (77.2%) of respondents were aware of RTIs, maybe because RTIs are considered to be endemic in Nigeria [2]. Eighty two point eight percent (82.8%) of the respondents thought that sexual intercourse is the mode of contacting RTIs, (77.6%) of the patients thought that poor hygiene is a mode of contracting RTIs. Mazrou et al. mention that (46%) of adult HIV patients contracted HIV through the sexual route [9-11]. In Lima, Peru, knowledge of sexual intercourse as one way of transmitting RTI was high and in Nigeria, they were attributing more significance to toilet rather than sexual intercourse as a mode of acquiring RTIs [2,12].

When inquiring about naming of RTIs; Syphilis was named by (32.6%), Gonorrhea by (31.5%), HIV by (20.2%), and Candida by (4.5%). While in Nigeria, (23.4%) mentioned Gonorrhea, (16.0%) and (9.9%) mentioned Syphilis and Candida respectively [2]. The most common response of all known symptoms was vaginal discharge (selected by 92.0% of patients) and vaginal itching (91.2%). It is worthy to note that genital sore was poorly perceived as a symptom of RTIs.

Table 7: Effect of experience of RTIs on seeking medical care behavior among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

	Sought medical Care	Did not seek medical care	Total
Experienced RTIs	48 (85.7%]	8 (14.3%]	56
No Experience of RTIs	130 (67.0%]	64 (33%]	194
Total	178	72	250
Odds Ratio= 2.9 (95% C.I. = 1.3, 6.6]			

Because of its association with increased incidence of HIV/AIDS, there is need to raise the community awareness of the importance of the symptom as it relates to HIV transmission. In a study in rural Lebanon, lower abdominal pain (41.1%), painful coitus (40.7%), and vaginal itching (38.5%] were the leading symptoms experienced. Only (24.5%) had experienced vaginal discharge [1]. Although none of the respondents had mentioned Human Papilloma virus in naming RTIs; cervical cancer was the first perceived complication of RTIs followed by chronic pelvic pain and the least perceived by all of the responses was ectopic pregnancy. This is

different from the study in Nigeria, where they found infertility in the top of the list of complication of RTIs, while poor perception for cervical cancer and other complications [2]. There was a significant association between awareness and younger age group (18-30) years age. This is similar to West Bengal study, (56.9%) where knowledge about RTIs was more in (20-29) age group [13].

Regarding prevalence of symptoms, (22.4%) of respondents had experienced at least one symptom in the previous 12 months. This finding is much lower than that reported from a study in West Bengal, where (66.1%) of women of reproductive age reported symptoms related to the reproductive tract [13].

Table 8: Relation between source of information and behavior among women attendees at PHC level at Khashm Al'an, Riyadh, June 2013

Source of Information	Sought Medical Care N(%)	Didn't seek Medical Care N(%)	Chi sq & p value
TV	37 (90.2%)	4 (9.8%)	8.67 p<0.003
Internet	30 (93.8%)	2 (6.3%)	9.10 p<0.003
School	46 (85.2%)	8 (14.8%)	6.57 p<0.01
Friends	59 (73.8%)	21 (26.3%)	0.37 p<0.54
Magazine	16 (84.2%)	3 (15.8%)	1.69 p<0.19
Health workers	68 (78.2%)	19 (21.8%)	3.15 p<0.076

In this study, the majority of the respondents (61.8%) sought medical care if they would perceive to have an RTI. This is may be due to that cervical cancer is known to result from RTIs and to prevent such dreaded complications women tend to seek medical care rather than face the consequences. It also indicates that intervention to reduce morbidity arising from RTIs should be directed mainly towards preventing RTIs rather than cure as most of the respondents will seek treatment when symptomatic. The younger age group and literate sought medical care more than others. Governmental Health Centers were the main facilities where treatment was sought accounting for (40.4%) and (37.2%) for general hospitals are free and accessible. In Nigeria study (31.5%) accounted for Governmental Health Centers [4], and is different from what is reported in West Bengal where most respondents (41.5%) patronized private hospitals [13].

Conclusion

In conclusion, majority (>50%] of women had little or no idea about the role of contraceptives in preventing RTIs. Most of the women (>90%] rated discharge and itching as common symptoms of RTI however >50% of women have little or poor knowledge of complications of RTI. Relatively younger (18-30 years], literate and those with more access to outside world were more aware about RTIs than otherwise. Those who had awareness, experienced, were younger (18-30 years), educated, or were not housewives, were more likely to seek medical care when needed. Television, Internet and education institutes were more effective source of information about RTI, for seeking medical care if needed.

This study recommends community based awareness programs on RTIs that target the less educated, middle aged (31-50) and housewives. More effort should be made by the Health care providers especially physicians and health educators in counseling patients to prevent RTIs. More focus should be made on complications of RTIs and modes of prevention in the content of awareness. Finally, more use should be made of mass media, i.e. television, radio, and internet, for raising awareness about RTIs. This study was done in one center only due to limited sources so results could not be generalized for all Saudi women. Further study at multi level health centre or community based will be needed.

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Thyroid Disease and Indicator of Autoimmune Illness Among Vitiligo Patients

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ABSTRACT

BACKGROUND: The precise pathogenesis remains not fully understood. However, it is thought to involve an autoimmune process directed against melanocytes. Thyroid disorders and autoimmune thyroid diseases have been shown in some reports to be associated with vitiligo. Additionally, there is limited and conflicting data examining the association of antinuclear antibodies (ANA) with vitiligo. **METHODS:** We conducted a cross-sectional study among 50 vitiligo patients diagnosed according to established clinical criteria at King Khalid University Hospital, Riyadh from January 2008 through December 2010. Blood samples were obtained from the study participants and were tested for antinuclear antibodies (ANA), thyroid stimulating hormone (TSH) and free thyroxin hormone (T4). **RESULTS:** Males represented 62% and the mean age was 28.2±11.0 years. A total of 20 (41.7%) had positive ANA values (titer ≥1:40). The mean TSH level was 2.69±2.17 µIU/mL. The mean free T4 level was 16.40±2.74 pmol/L and all patients were in the normal range (10.3-25.8 pmol/L). There were no statistically significant associations between ANA and age, gender, extent of skin lesion, face inclusion, and free thyroxin levels. On the other hand, positive ANA level had significant association with TSH level (p=0.041). With exception of ANA, TSH level had no statistically significant associations with the above variables. **CONCLUSIONS:** The results of the current study showed higher prevelance of ANA in vitiligo patients, probably suggesting autoimmune process. The current finding may need to be confirmed in a larger prospective multicentric study before recommending periodic screening of patients with vitiligo for thyroid function and antithyroid antibodies.

Key words: Vitiligo; autoimmune ; thyroid disease; antinuclear antibodies

الملخص

مقدمة: بالنسبة لمرض البهاق لم تعرف بدقة الية المرض ولكن يظن أنها خلل ذاتي في جهاز المناعة ضد الخلايا الصبغية. افادت بعض التقارير وجود علاقة بين البهاق وأمراض و اضطرابات الغدة الدرقية الناتجة من الخلل الذاتي في جهاز المناعة, بالإضافة لوجود بيانات محدودة ومتضاربة فيما يخص العلاقة بين البهاق والاجسام المضادة للنواة. **المنهج:** دراسة مقطعية لعدد 50 مريض بالبهاق تم تشخيصهم سريريا في مستشفى الملك خالد الجامعي بالرياض في الفترة من يناير 2008 الي ديسمبر 2010. تم سحب عينات دم للمشاركين بالدراسة لتحليل الاجسام المضادة للنواة و الهرمون المحفز للغدة الدرقية وهرمون الثيروكسين الحر. **النتائج:** كانت نسبة الذكور بالدراسة 62% ومتوسط اعمارهم 28,2 ± 11سنة . وجد ان الاجسام المضادة للنواة ايجابية في 20 من المشاركين (41.7%) (العيار: ≤1:40. متوسط الهرمون المحفز للغدة الدرقية 2,69 ± 2,17 ميكرو وحدة دولية لكل ملم , وهرمون الثيروكسين الحر 16,40 ± 2,74 pmol/L وقد كان كل المرضي في المستوي الطبيعي (10,3 الي 25,8 pmol/L). لا توجد علاقة ذات دلالة احصائية بين مضادات النواة والعمر او الجنس او الاصابات الخارجية في الجلد او الوجه او مستوي هرمون الثيروكسين الحر. من ناحية اخري توجد علاقة ذات دلالة احصائية بين ايجابية الاجسام المضادة للنواة ومستوي الهرمون المحفز للغدة الدرقية (p=0.041). بإستثناء الأجسام المضادة للخلية فان مستوي هرمون الثيروكسين الحر لا توجد لديه علاقة ذات دلالة احصائية مع المتغيرات اعلاه. **الإستنتاج:** نتائج الدراسة أوضحت وجود معدل انتشار عالي للأجسام المضادة للنواة لمرضي البهاق , مما يؤشر لإحتمال الخلل الذاتي لجهاز المناعة . هذه النتائج تحتاج لدراسات مستقبلية متعددة قبل التوصية بعمل مسوحات لمرضي البهاق لمعرفة وظائف الغدة الدرقية ومستوي الاجسام المضادة بها

INTRODUCTION

What's known?

The causes of vitiligo are unknown. However, it has been reported that vitiligo is associated with other autoimmune diseases, with thyroid disease being the commonest.

Vitiligo is an acquired, non-contagious disorder of the skin and mucous membranes that is characterized by progressive, well circumscribed, depigmented macules and patches that occurs secondary to selective destruction of melanocytes, apparently

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on an autoimmune basis [1]. Vitiligo is the most common pigmentation-related disorder that varies based on region [2] with a prevalence of 0.06 to 2.28% among adults whereas this was 0.0 – 2.16% in children/adolescents populations [3] Gujarat, India is considered to have the highest prevalence in the world, at about 8.8% [4]. Men and women are equally affected, but young Females (< 30 years of age) usually acquire the disease earlier than males [5,6]. The average age of onset of vitiligo among patients with a positive family history (seen in up to 20% of them) is younger than sporadic ones (18.7 ±12.5 vs. 20.4 ±13.9 years) [7,8].

The pathogenesis of this disease is still not clear and is thought to be multifactorial and polygenic; however, several theories have been proposed to explain the loss of epidermal melanocytes in this disorder [1,9]. There is substantial evidence for the immune mediated destruction of melanocytes [10]. Although the triggers and specific nature of the autoimmune response remain unknown, circulating autoantibodies to melanocytes and various melanocytic proteins components, detectable in many patients, have been considered to be secondary humoral responses to melanocyte destruction rather than primary cause [11].

It is also suggested by the concomitant occurrence of other autoimmune diseases in patients with vitiligo. Some of them is well established for example thyroid disorders, particularly Hashimoto's thyroiditis and Graves' disease, are commonly associated with vitiligo, as are other endocrinopathies, such as Addison's disease and diabetes mellitus. Alopecia areata, pernicious anemia, systemic lupus erythematous, inflammatory bowel disease, rheumatoid arthritis, psoriasis, and autoimmune polyglandular syndrome also are associated, though the significance of some of these associations is debated [12-14].

The association of vitiligo with these disorders suggests the presence of shared genetic factors that contribute to the development of these diseases. In a genome wide association study, several genes with known associations with other autoimmune disorders were identified as potential susceptibility loci for generalized vitiligo, including PTPN22, LPP, IL2RA, UBASH3A, C1QTNF6, and genes encoding MHC I and MHC II molecules[15, 16]. These genes provide potential therapeutic targets for treatment as well as for approaches to presymptomatic diagnosis and disease prevention in individuals with inherited susceptibility to autoimmune diseases [17].

Autoimmune thyroid disorders have been associated with vitiligo, and the incidence of clinical or subclinical involvement of the thyroid is more common in patients with vitiligo as compared to healthy subjects [18]. Autoimmune thyroid disease is seen in approximately 21% of children and adult patients with vitiligo compared to 3% in non-vitiligo patients [19,20]. It was found predominantly among older women and in subjects with a positive family history of thyroid disease [21]. Thus, screening of patients with vitiligo for thyroid function and antithyroid antibodies to diagnose early changes in the function of this gland becomes relevant and necessary [22].

Given the frequency with which concomitant autoimmune disorders occur in patients with vitiligo, it was suggested to screen for selected diseases (eg, thyroid function, CBC, and fasting blood glucose level), especially in children and young women [23]. There is limited data examining the association of antinuclear antibodies (ANA) and vitiligo. In a recent retrospective study performed among 135 patients with vitiligo, ANA were found in 33% of patients and the highest proportion of thyroid abnormalities was found among patients aged 21-30 years [23]. However, other studies could not detect association of ANA with vitiligo neither in children nor in adults [24].

We conducted this study among a group of vitiligo patients with the following objectives:

1. To estimate the prevalence of positive antinuclear antibodies (ANAs), as indicator of autoimmune illness
2. To estimate the level of thyroid stimulating hormone (TSH) and free thyroxin hormone (T4), as possible indicators of thyroid disease
3. To evaluate the association of ANA, TSH, and T4 with demographic (age and gender) and clinical (body site affected and percentage of affected body surface area) characteristics of vitiligo patients, and finally
4. To study the association between ANA level and levels of TSH and T4.

METHODS

Design and population:

We conducted a cross-sectional study among a group of patients diagnosed with vitiligo. We examined 50 consecutive patients with vitiligo diagnosed according to established clinical criteria [1], of both sex, irrespective of age, and attending the dermatology outpatient clinic of the King Khalid University Hospital, Riyadh from January 2008 through December 2010. Data collected in the current study included demographic, clinical, and laboratory data. Demographic and clinical data included age, sex, site of skin lesion and the percentage of body surface area affected.

Laboratory methods:

Blood samples were obtained from the study participants to separate serum and were tested for ANA, TSH, and T4. ANA tests were performed by indirect immunofluorescence (IIF) technique for the detection of autoantibodies using the following dilutions 1:40, 1:80, 1:160, and 1:320 (INOVA Diagnostic Inc., San Diego, CA). According to manufacturer, detectable ANA (fluorescence observed) at titer ≥1:40 was considered positive results. Positive levels were further categorized as mildly elevated (titers 1:40 and 1:80) and elevated (titers 1:160 and 1:320).(25, 26) Serum TSH and T4 were estimated using electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). According to manufacturer references, normal values for TSH were between 0.25 and 5.0 µIU/mL (milli-international units per liter) while normal values for free T4 were values between 10.3 and 25.8 pmol/L (picomole per liter). Therefore, low TSH values were considered at <0.25 µIU/mL while higher values were considered at >5.0 µIU/mL. Likewise, low free T4 values were considered at <10.3 pmol/L while high free T4 values were considered at >25.8 pmol/L.

STATISTICAL ANALYSIS

Patient's demographic, clinical, and laboratory data were described as mean and standard deviation or median and inter-quartile range for continuous data or proportion for categorical data. The differences of age, affected body surface area, TSH, and T4 levels by two-level groups (such as gender and ANA groups) were examined using student t-test for parametric data and Mann-Whitney U test for non-parametric data, as appropriate. The differences of age, affected body surface area, TSH, and T4 levels by three-level groups (such as affected body surface area groups) were examined using one-way analysis of variance (ANOVA) for parametric data and Kruskal-Wallis ANOVA for non-parametric data, as appropriate. The associations between categorical data were examined using Chi-square or Fisher exact, as appropriate. All tests were two-tailed and p-value of <0.05 was considered significant. SPSS software (release 18.0, SPSS Inc., Chicago, U.S.) was used for all statistical analyses.

ETHICAL STATEMENT

This study was conducted on hospital patients. Appropriate ethical committee approval and patient consent were obtained before actual data collection. All the data were securely maintained by separating participants' identification and associated clinical and laboratory data. All the data were anonymously analyzed so as to maintain individual privacy. All records with results and progress both electronic and written were maintained by the researchers in a secured place with a lock. At the end of the study, summary of the result was sent to advisor committee. Upon completion of the study the final report was submitted to the Saudi Commission for Health Specialization.

RESULTS

Table 1 showed demographic and clinical characteristics of vitiligo patients seen at King Khalid University Hospital, Riyadh from January 2008 through December 2010. A total 50 patients were included in the study. Males represented 62% while females represented 38%. The mean age was 28.2±11.0 years. The age range was between 12 and 68 years. The majority of patients (82%) were less than or equal to 35 years. The majority (83.3%) of vililigo patients had whole body skin lesions including the face while the minority (16.7%) had whole body skin lesions sparing the face. The average percentage of affected body surface area was 31.7±10.3.

A total of 20 (41.7%) out of 48 vitiligo patients had ANA elevated values (titer ≥1:40). Three-fourth (15/20, 75%) of these patients had mildly elevated values (titers 1:40 and 1:80) while one-fourth (5/20,

25%) of these patients had moderately elevated values (titers 1:160 and 1:320) (Table 1).

Table 1: Demographic and clinical characteristics of vitiligo patients seen at King Khalid University Hospital, Riyadh from January 2008 through December 2010

Characteristics	N (%) or mean±SD
Age (mean±SD, years)	28.2±11.0
Age (median & IQR, years)	27.0 (21.0-33.3)
Age groups (%)	
≤25 years	21 (42%)
26-35 years	20 (40%)
>35 years	9 (18%)
Gender	
Male	31 (62%)
Female	19 (38%)
Body site affected	
Whole Body	40 (83.3%)
Whole Body without face	8 (16.7%)
Body Surface Area affected (mean±SD)	31.7±10.3
Body Surface Area affected	
<30%	17 (34%)
30%	18 (36%)
>30%	15 (30%)
Antinuclear Antibody (ANA)	
Negative (<1:40)	28 (58.3%)
Elevated (>1:40)	20 (41.7%)
Antinuclear Antibody (ANA)	
Negative (<1:40)	28 (58.3%)
Mildly elevated (titers 1:40 and 1:80)	15 (31.2%)
Elevated (titers 1:160 and 1:320)	5 (10.4%)
TSH (mean±SD, µIU/mL)	2.69±2.17
TSH (median & IQR, µIU/mL)	2.44 (1.25-3.03)
TSH groups	
Low (<0.25 µIU/mL)	2 (4.2%)
Normal (0.25-5.0 µIU/mL)	41 (85.4%)
High (>5.0 µIU/mL)	5 (10.4%)
T4-Free thyroxine (mean±SD, pmol/L)	16.40±2.74
T4-Free thyroxine groups	
Normal (10.3-25.8 pmol/L)	48 (100.0%)
High (>25.8 pmol/L	0 (0.0%)

The mean TSH level was 2.69±2.17 µIU/mL. Since the distribution of TSH level was skewed to the right with a lot of value toward the zero side, we calculated the median and inter-quartile range at 2.44 (1.25-3.03) µIU/mL. A total of 5 (10.4%) out of the 48 vitiligo patients had high TSH values (>5.0 µIU/mL) while only 2 patients (4.2%) had low TSH value (<0.25 µIU/mL).

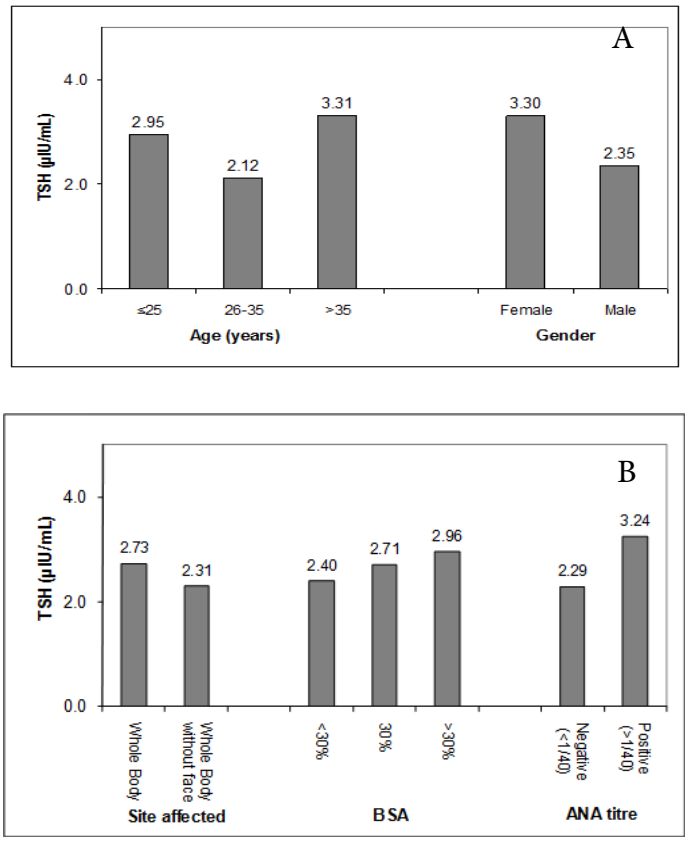

Figure 1: Thyroid stimulating hormone (TSH, µIU/mL) by demographic (A), clinical and laboratory characteristics (B) among vitiligo patients seen at King Khalid University Hospital, Riyadh from January 2008 through December 2010

ANA levels were categorized as negative and positive. Tables 2 showed the differences in demographic, clinical, and laboratory characteristics of vitiligo patients by ANA groups. Those who had positive ANA were slightly older (29.2±12.4 year) than those who had negative (27.8±10.4 year) ANA. However, there were no statistically significant associations between ANA groups and age or sex (p=0.670 and p=0.959, respectively). The frequency of those who escaped the skin lesions in face were more common among those who had negative results of ANA (22%) compared to those who had positive ANA levels (10%).

Table 2: Demographic and clinical characteristics by ANA groups among vitiligo patients seen at King Khalid University Hospital, Riyadh from January 2008 through December 2010

Characteristics	Antinuclear Antibody (ANA)		P value
	Negative (<1/40)	Positive (>1/40)	
Age (mean±SD, years)	27.8±10.4	29.2±12.4	0.670
Age groups (%)			
≤25 years	12 (42.9%)	8 (40.0%)	0.974
26-35 years	11 (39.3%)	8 (40.0%)	
>35 years	5 (17.9%)	4 (20.0%)	
Gender			
Male	18 (64.3%)	13 (65.0%)	0.959
Female	10 (35.7%)	7 (35.0%)	
Body site affected			
Whole Body	21 (77.8%)	18 (90.0%)	0.437*
Whole Body without face	6 (22.2%)	2 (10.0%)	
Body Surface Area affected (mean±SD)	30.1±8.2	34.5±12.6	0.149
Body Surface Area affected			
<30%	11 (39.3%)	5 (25.0%)	0.585
30%	9 (32.1%)	8 (40.0%)	
>30%	8 (28.6%)	7 (35.0%)	
TSH (mean±SD, µIU/mL)	2.29±1.93	3.24±2.40	0.135
TSH (median & IQR, µIU/mL)	1.68 (0.96-3.00)	2.73 (2.12-3.47)	0.041
TSH groups			
Low (<0.25 µIU/mL)	2 (7.1%)	0 (0.0%)	0.447
Normal (0.25-5.0 µIU/mL)	24 (85.7%)	17 (85.0%)	
High (>5.0 µIU/mL)	2 (7.1%)	3 (15.0%)	
T4-Free thyroxine (mean±SD, pmol/L)	16.54±2.78	16.21±2.75	0.682

However, this finding did not reach statistical significance (p=0.437). The median and inter-quartile range of TSH level was statistically significantly (p=0.041) higher among those who had positive ANA level (2.73, 2.12-3.47 µIU/mL) compared to those who had negative ANA levels (1.68, 0.96-3.00 µIU/mL). The levels of free thyroxin were similar among both who had positive and negative ANA levels (16.21±2.75 and 16.54±2.78 pmol/L, p=0.682, respectively).

We examined the differences in demographic, clinical, and laboratory characteristics of vitiligo patients by affected body surface area groups (data are not shown). There were no statistically significant associations among ANA, TSH, and free thyroxin levels and the degree of affected body surface area (p=0.585, 0.846, 0.940, respectively). Gender was the only significant characteristics observed in relation to affected body surface area. Those who had 30% affected body surface area were more likely to be females than males (61% versus 39%, p=0.041). Figure 1 showed the TSH levels by demographic, clinical, and laboratory characteristics. TSH levels were lower among those aged 26-35 years compared to other ages and in males compared to females (Figure 1A).

TSH levels were higher among patients with whole body vitiligo including the face compared to patients with whole body vitiligo sparing face and in patients with more affected body surface area compared to those with less affected body surface area (Figure 1B).

However, all the above differences were not statistically significant using non-parametric tests (Mann-Whitney U test and Kruskal-Wallis ANOVA). On the other hand, TSH levels were significantly higher in those who were ANA positive compared to those who were ANA negative (p=0.041). There were no statistically significant differences in free thyroxin levels by age groups, gender, body site affected, percentage of affected body surface area, or ANA levels using parametric tests (student t-test and ANOVA). On the other hand, free thyroxin levels was significant higher among patients with normal and higher TSH compared to those with lower TSH (p<0.001).

Discussion

In a cross-sectional study done among Saudi patients with vitiligo, 41.7% had mild to moderate ANA

elevation defined as titer ≥1:40. The prevalence of ANA in our study was generally higher than reported in many other studies suggesting an autoimmune origin of vitiligo. For example, ANA were found in 33% of patients in a recent retrospective study performed among 135 patients with vitiligo (80 women and 55 men with mean age of presentation of 36.8 years). [23] In another study among 55 Iranian patients with vitiligo, ANA was found in only 7.3% of the patients [27].

ANA are frequently found in healthy populations. Although a positive ANA test, by itself, does not establish a diagnosis of a certain autoimmune disease, it can be helpful when used with other clinical features. Moreover, some studies reported similar levels of ANA among vitiligo patients compared to the general population [14,20,24]. Additionally, it was suggested that positive ANA finding in the absence of physical signs and symptoms has limited diagnostic utility [25]. However, the higher prevalence of ANA in our study may suggest that autoimmunity might plays an important role in the pathogenesis of vitiligo. Supporting this finding, it was shown that vitiligo appears to be an autoimmune disease involving T-cell mediated melanocytes destruction [25,28].

The current study showed a weak non-significant association between ANA and older age. This finding was in accordance with that reported by previous studies [29]. It was reported that ANA was more common in adults with vitiligo compared to children with vitiligo[29]. Interestingly, it was shown that the frequency ANA positivity do not differ significantly across the age range of healthy individuals aged 20 to 60 years [26].

The current study showed non-significant associations of both TSH and ANA with the extent of skin lesions. The non-significance is probably due to the small sample size. Similar to our findings, it was also observed that vitiligo patients with autoimmune thyroid disease were more likely to have more than 25% of their body affected by skin lesions compared vitiligo patients without autoimmune thyroid disease but did not show statistical significance (67% vs. 21%, p=0.083) [30]. Unfortunately there is lack of data evaluating the association between the ANA level and extent of lesion in vitiligo patients.

A recent study examined 186 adult vitiligo patients for the differences between familial and sporadic cases of vitiligo [31]. In this study, patients in familial group showed more widespread depigmentation compared with sporadic cases. Additionally, the widespread depigmentation was associated with the presence of autoantibodies (p = 0.03) including ANA (p<0.001) in sporadic cases of vitiligo.

The current study showed non-significant associations of both TSH and ANA with the extent of skin lesions. The non-significance is probably due to the small sample size. Similar to our findings, it was also observed that vitiligo patients with autoimmune thyroid disease were more likely to have more than 25% of their body affected by skin lesions compared vitiligo patients without autoimmune thyroid disease but did not show statistical significance (67% vs. 21%, p=0.083) [30]. Unfortunately there is lack of data evaluating the association between the ANA level and extent of lesion in vitiligo patients. A recent study examined 186 adult vitiligo patients for the differences between familial and sporadic cases of vitiligo [31]. In this study, patients in familial group showed more widespread depigmentation compared with sporadic cases. Additionally, the widespread depigmentation was associated with the presence of autoantibodies (p = 0.03) including ANA (p<0.001) in sporadic cases of vitiligo.

Approximately 15% of our vitiligo patients had abnormal TSH level. This was higher than subclinical hypothyroidism or hyperthyroidism observed in many studies examining adult vitilgo patients where these rates ranged between 2.1 and 5.6% [32,33]. However, our TSH rate was lower than reported before among vitiligo patients where 20-22% had abnormal TSH level [30]. The difference between this study and other studies may be partially explained by the different age, gender, and nationality of studied patients in these studies. Thyroid disorders and autoimmune thyroid diseases have been associated with vitiligo, and the incidence of clinical or subclinical involvement of the thyroid is more common in patients with vitiligo as compared to healthy Subjects [34]. A recent systematic review estimated the prevalence of thyroid disease, autoimmune thyroid disease and presence of thyroid-specific autoantibodies in patients with vitiligo at 15.1%, 14.3% and 20.8%

(respectively) with significant relative risks of 1.9, 2.5 and 5.2 (respectively) [35].

The usefulness of periodic screening (at least every 3 years) to evaluate the coexistence of different auto-antibodies in patients affected by vitiligo has been suggested, to better follow-up the complexity of autoimmune diseases associated with vitiligo [14]. Since the interval between the onset of vitiligo and the occurrence of thyroid disease can be as long as 20 years, this periodic screening may useful for long period after the onset of vitiligo [36].

What's new?

The results of the current study showed higher prevelance of ANA in vitiligo patients, probably suggesting autoimmune process. The current finding may need to be confirmed in a larger prospective multicentric study before recommending periodic screening of patients with vitiligo for thyroid function and antithyroid antibodies.

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Relativity of Platelet Count with Automated Cell Counter & Manual Haemocytometer using Peripheral Blood Smear

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ABSTRACT

BACKGROUND: Obtaining accurate, precise, and reliable platelet counts as a reference for the calibration of hematology analyzers has been a continuing problem due to varying results. We proposed the relativity of platelet count by Automated Cell Counter and Manual Haemocytometer with Peripheral smear for focusing the accuracy and & cost effectiveness, without compromising the accuracy of test results. **METHODS:** Seventy-Five blood samples in EDTA-anticoagulant vacutainer tubes were obtained and randomly divided into 3 group; Group I-normal control (n = 25), Group II-mild thrombocytopenia (n = 25) and Group III-severe thrombocytopenia (n = 25). Each blood sample had two peripheral blood smears made and stained on an automatic slide stainer. Peripheral blood smears were analyzed for platelet count by manual microscopy, an automated hematology analyzer Beckman Counter Diff 2 in all groups. The agreement between the two methodologies were assessed using the paired t-test and correlation coefficient analyses. **RESULTS:** The coefficient of variation values significantly differed in Group C and were high for Automated cell counter. High postitive correlation was also observed between Automated cell counter values and manual method ($r=0.82$, $p<0.001$) in group A and B. However, values in group C were not significantly correlated. **CONCLUSION:** Based on the results observed in severe thrombocytopenia, the automated cell counter was more reliable and significant than the manual method. However, manual platelet count using by haemocytometry and morphological study of the blood smear may be promising if platelets are very low and giant platelets.

KEY WORDS: Thrombocytopenia peripheral blood smear, platelet count, automated Cell Counter and haemocytometer

الملخص

مقدمة: تحديد قيمة عدد الصفائح الدموية بصورة دقيقة ويعتمد عليها كمرجع لمعايرة اجهزة تحليل الدم يعتبر من المعضلات المستمرة وذلك لتفاوت قيم النتائج. نقترح نسبية عد الصفائح الدموية بعداد الخلايا الاتوماتيكي والعداد اليدوي مع عمل مسحة دم طرفي وذلك للتركيز علي الدقة والتكلفة والفعالية مع عدم الاخلال بدقة نتائج الاختبار. **المنهج:** تم استخدام 57 عينة دم في أنابيب EDTA المضادة للتخثر ووزعت عشوائيا الي 3 مجموعات , المجموعة الاولي طبيعية وهي للمقارنة وعددها 25 والمجموعة الثانية تعاني من انخفاض بسيط في عدد الصفائح الدموية وعددها 25 اما المجموعة الثالثة فعددها 25 وتعاني من انخفاض شديد في عدد الصفائح الدموية. كل عينة دم صاحبها مسحتان من الدم الطرفي تم تحضيرهما وصبغتهما علي شرائح صبغية اتوماتيكية. تم تحليل المسحات في كل المجموعات بعد الصفائح الدموية يدويا بالميكروسكوب , و اوتوماتيكيا بجهاز بيكمان لتحليل الدم . تم اختبار التوافق بين الطريقتين احصائيا باستخدام اختبار t ذو الطرفين و اختبار معيار العلاقة . **النتائج:** معيار قيم المتغيرات اختلفت اختلافا ذو دلالة في المجموعة الثالثة وكانت قيمها كبيرة في النتائج المستخلصة من جهاز العد الاتوماتيكي, لوحظ ايضا علاقة ايجابية كبيرة بين النتائج المستخلصة من جهاز العد الاتوماتيكي والنتائج اليدوية ($r = 0.82$) ($p<0.001$) للمجموعتين الاولي والثانية. اما قيم المجموعة الثالثة فلم يكن لها علاقة ذات دلالة . **الإستنتاج:** اعتمادا علي النتائج التي لوحظت من العينات التي تعاني من انخفاض شديد في عدد الصفائح الدموية فان جهاز العد الأوتوماتيكي اكثر اعتمادية وموثوقية من الطريقة اليدوية . ولكن ايضا الطريقة اليدوية باستخدام عداد الخلايا الدموية مع دراسة شكل الخلايا في مسحات الدم يمكن ان تكون واعدة في حالة كانت الصفائح الدموية قليلة جدا وعملاقة.

INTRODUCTION

In many disorders, Platelet count estimation is an important element of the diagnostic and treatment process. In patients with abnormal thrombocytes where platelet transfusion is required, the reliability of the platelet count is highly desired and necessary to provide appropriate treatment. Hematology analyzers are intended to count (patient) blood

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samples. Many blood centers use these analyzers to perform quality control on the blood components that they produce. The platelets are counted with hematology analyzers, but varying results among different hematology analyzers are observed, making comparisons very difficult.

Measurement of platelet counts using automated hematology analyzers is usually quite precise and accurate. However, the accuracy of automated platelet counts can be compromised when measuring very low platelet counts or in the presence of interference from non-platelet particles or platelet abnormalities.

Recent studies, mainly focusing on the counts of low levels of platelets, demonstrated that automated counts were not as accurate in severely thrombocytopenic samples. Traditional microscopic methods are very intense with variable results and are highly dependent on the individual training. Recent developments in automated peripheral blood differentials using a computerized system have shown many advantages as a viable alternative. But some cases like morphology abnormality and very low platelet count in not give accurate results.

The aim of this study was to determine the reliability and accuracy of the Beckman Counter Diff2 automated system with manual microscopic estimates from the peripheral Blood smear

METHODS

Blood Samples

Blood samples were obtained from 75 patients who were randomly divided into three groups. Group I (n = 25) had the patients with normal count, Group II (n = 25) had the patients with mild thrombocytopenia and Group III (n = 25) had the patients with severe thrombocytopenia. Venous blood specimens were collected and within 20 seconds from blood sampling, blood was transferred to a tube containing ethylenediaminetetraacetic acid (EDTA), and within 4 hours a whole-blood count was performed using an automated cell counter and manual method. Notation was made, if clots were seen in the blood sample or if the amount of blood in the tube was grossly inadequate such that a disproportionately high concentration of EDTA would be present; these samples were excluded from the study.

Automated Analyzer Platelet Counts

After thorough mixing of each blood sample on an automated mixer for 10 min, a complete automated blood count was performed using Beckman coulter Diff 2 (Beckman, USA), which was maintained and calibrated as recommended by the manufacturer.

Manual Platelet Estimation

This study done by using improved Neubaure chamber (Paul Marienfeld GmbH & Co). Thin air-dried blood smears made after thorough mixing of each sample were stained manually with a May-Grünwald-Giemsa stain and examined under light microscopy with a X100 oil-immersion lens.

The slides were entirely scanned for platelet aggregates and/or macrothrombocytes and, if any, the samples were excluded from the study. If neither aggregates nor macrothrombocytes were found, the red cell: platelet ratio was calculated in the monolayer zone of the smear as follows: The number of erythrocytes observed in a quarter of the oil-immersion field was multiplied by four instead of counting all the erythrocytes in the field, which is a laborious and time consuming method. Then all the platelets in the same field were counted. Other fields were examined in the same way until we reached a minimum number of 1000 erythrocytes. The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count (RBC) ($x10^6$cells/µl) to give an approximate manual count ($x10^3$cells/µl). A morphological study done by peripheral blood smear stained by Leishman's stain. After processing the samples, the cell counter values and manual method values were compared in all the groups.

STATISTICAL ANALYSIS

The data was entered into Excel spreadsheet (Microsoft, Redmond, Washington) but was exported to SPSS 20.0 for data analysis. Averages were reported for quantitative variables. Two-independent sample t-test was applied to compare quantitative values. Pearson-correlation was also applied to observe correlations. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Seventy-five patients randomly divided into three

groups were compared for platelet count by automatic methods and manual cell count using hemacytometer. Group-A: Consisted of twenty-five patients with normal platelet count values ranging from 1, 50,000 to 4, 00,000/mm^3 Group-B: - consisted of twenty-five patients ranging from 30,000 to 1,50,000/mm^3 and Group-C: consisted of twenty-five patients with low platelet count ranging from 5,000 to 30,000/mm^3

In the present study, all group samples were analyzed and coefficient of variation was calculated by statistical method. Group A samples were estimated by Automated cell counter and manual method by number chamber, No significant difference was observed in the coefficient of variation between automated cell counter and manual method (33.44 vs 24.97, p=0.189). In Peripheral smear, the platelets appeared to be adequate with normal morphology. In group B, again no significant difference was observed in the coefficient of variation between cell counter result and manual method (41.86 vs 36.93 p=0.239). However, in group C, coefficient of variation values were significantly high for automated cell counter as compared to manual method values (24.50 vs 22.06, p=0.009) Figure 1. When correlations were applied, high postitive correlation was observed between Automated cell counter values and manual method (r=0.82, p<0.001) in group A and B. However, values in group C were not significantly correlated.

Table1: Distribution of Coefficient of Variation bewteen Different Groups

Groups	Methods	CV
Group I	Automated Cell Counter	33.44
	Manual	24.97
Group II	Automated Cell Counter	41.86
	Manual	36.93
Group III	Automated Cell Counter	24.5
	Manual	22.06

DISCUSSION

The comparison was done irrespective of age, sex and specific disease. The patient's sample were taken randomly and processed for study. The aim of the study was correlation of various methods of platelet counting so that we can get reliable assessment of platelet count to avoid unnecessary platelet transfusion therapy and the main objectives are the reliable platelet counting for prophylactic platelet transfusion in severe thrombocytopenic patients and the second most important object was to find out the factors responsible for unreliable platelet counting. Stibbe W et al were measured and the platelet counts compared using the various blood cell counters of the central laboratory, University Clinic, Göttingen. Using the H 6000, Hemalog 8 (both Technicon) and ELT-8 (Ortho Instruments), product-moment correlation coefficients (r) ranging from 0.88 to 0.92 were obtained between the different counters. They found the correlation coefficients of the thrombocounter (Coulter) to the other instruments were only 0.84 and 0.85. Discrepant platelet counts (difference greater than 40x10^9/L) were reinvestigated using the Neubauer chamber. From a total of 354 platelet measurements, falsely high concentrations were observed only in three cases and falsely low concentrations in seven cases [3, 4]. Our study shows group I and group II not significant

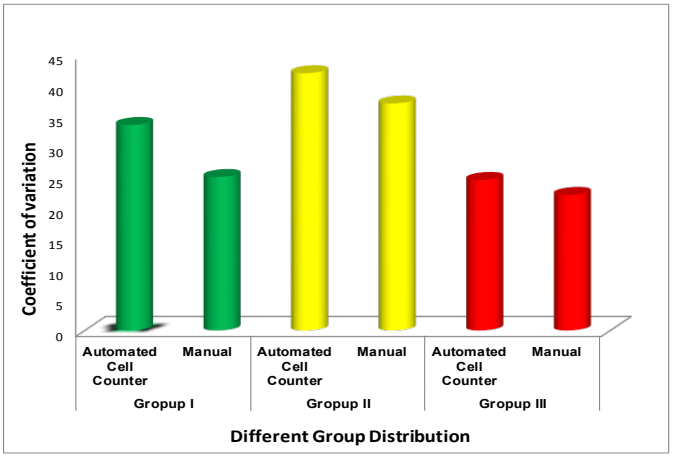

Figure 1: Comparison of platelet count reproducibility (coefficient of variation) in 3 different groups using manual and automated methods

Lawrence JB et al studied on very low platelet count about 20 × 10^9/L (20,000/µl) threshold for prophylactic platelet transfusion may be unnecessarily high. The widespread use of this threshold may reflect lack of confidence in the reliability of low platelet count [5]. They evaluated the performance of automated platelet counts and their relation to clinical bleeding. First, they prepared serial blood dilutions with "target" platelet counts from 2 to 40 × 109/L for the 48 measurements on 2 × 10^9/L "target" dilutions, values of 1 or 2 × 10^9/L were obtained with the Sysmex NE-8000 analyzer (mean 1.44 × 109/L; SD 0.31 × 109/L). Similarly, for 5 × 10^9/L "target" counts, automated

counts were 3-6 × 10^9/L (mean 4.42 × 109/L; SD 0.18 × 10^9/L) [6]. Similar results were observed with all other "target" levels, with coefficients of variation (CV) from 6.39% to 7.71% with 10-40 × 10^9/L "target" values. Secondly, they compared triplicate automated and manual platelet counts in thrombocytopenic patients with platelet counts from 4–30 × 10^9/L. The triplicate automated platelet counts differed by no more than 5 × 10^9/L among themselves, whereas the manual counts varied by as much as 30 × 10^9/L. Mean platelet counts: automated, 14.40 × 10^9/L (CV 10.12%); manual, 16.48 × 10^9/L (CV 30.39%) (P = 0.038 for counts; P < 0.001 for CV). Finally, they observed highly significant correlations between the automated platelet count and major and minor bleeding manifestations. Thus, automated platelet counts are highly reliable and accurately predict clinical bleeding.

The use of automated analyzers should facilitate improved prophylactic platelet transfusion protocols. In this condition our result also support if platelet count is very low than automated cell count is given accurate and reliable results [7,8]. Malok M estimated that the traditional method of estimating platelet counts from peripheral blood smears to evaluate automated results appears to provide adequate quality assurance. Accurate platelet counts in severe thrombocytopenia are very difficult to take decisions at prophylactic platelet-transfusion thresholds.

Until recent years, the accuracy of platelet counts has been limited by the reliance of hematology analyzers on calibration material values derived from the manual platelet counting method. The calibration of automated cell counter in thrombocytopenia and the reduction of variation between instruments have been hindered by a lack of adequate quality control materials, making the accuracy of automated methodologies in routine practice difficult to assess. This situation could now be highly improved by the use of the International Reference Method (IRM) to assign calibration materials and by further knowledge of the accuracy and limitations of the particular types of automated platelet count available to the clinician These changes will improve clinical confidence in the accuracy of a platelet count and thus inform clinical decisions at the current level of prophylactic platelet transfusions.

The clinical decision to proceed with prophylactic platelet transfusions is widely based on trigger points for platelet counts are equal to 20, 10, or even 5 x 10 [(9)] /L. But an increasing number of publications show evidence that the conventional automated platelet counting methods are unable to provide consistently accurate results in this lower thrombocytopenic range.

These measurement errors are mainly associated with the most commonly used impedance principle; optical methods seem to be more precise. The problems of counting imprecision in the low thrombocytopenic range can be avoided with direct or indirect immunological counting methods using monoclonal antibodies or by time-consuming manual procedures. But how should new counting procedures be evaluated? Which method should be used as the "gold standard" for platelet counting? A way out of this apparent dilemma is the application of a statistical procedure as proposed by Gautschi et al. This mathematical model allows a reference method independent evaluation of new methods by calculation of the limits of detection (LD) and limits of quantification (LQ) based on the imprecision profile of the investigated method. Using this evaluation procedure, it can be shown that immunological automated counting methods can provide reliable, sufficient, and prompt platelet counts, especially in the thrombocytopenic range [9,10].

Briggs C was observed the platelet count four main procedures, manual phase contrast microscopy, impedance, optical light scatter/fluorescence and flow cytometry. Earlier methods to enumerate platelets were inaccurate and irreproducible. The manual count is still recognized as the gold standard or reference method, and until very recently the calibration of platelet counts by the manufacturers of automated cell counters and quality control material was performed by this method [11]. However, it is time-consuming and results in high levels of imprecision. The introduction of automated full blood counters using impedance technology resulted in a dramatic improvement in precision. However, impedance counts still have limitations as cell size analysis cannot discriminate platelets from other similar-sized particles. More recently, light scatter or fluorescence methods have been introduced for automated platelet counting, but there are still occasional cases where an accurate platelet count

remains a challenge. Thus, there has been interest in the development of an improved reference procedure to enable optimization of automated platelet counting.

This method utilizes monoclonal antibodies to platelet cell surface antigens conjugated to a suitable fluorophore. This permits the possible implementation of a new reference method to calibrate cell counters, assign values to Calibrators, and to obtain a direct platelet count on a variety of pathological samples. In future, analysers may introduce additional platelet parameters; a reliable method to quantify immature or reticulated platelets would be useful [11,12].

There were different methods of platelet count in hematology laboratory and these methods were manual counting, automated cell counting, platelet count by peripheral blood smear, immunoplatelet counting and radioisotope labeling technique for platelet counting. Many authors had given their thoughts on the methods of platelet counting. Reliability of platelet count by comparison with manual method was studied by Lawrence J.B (1995). Koh T (1996) also concluded the discrepancy of platelet numbers between automated blood cell analysis and manual counting in patients with thrombocytopenia. In 21st century (2004), Web DI, suggested the platelet count assessment from peripheral blood smear (PBS) [13,14,15].

Mainly automated systems were two types, semi-automated and fully automated. Semi-automated required sample preparation (for example dilution) by a technician and fully automated equipment performed all steps subsequent to obtaining the sample itself. The principle of electronic counting based on Electrical Impedance or Light Scattering [16]. The methods were used for comparison of platelet counts are automated cell counter Act Diff 2 and manual platelet counting by hemacytometer with diluting fluid 1% ammonium oxalate which lyses the red cells and preserves the platelet. In automatic analyzer the processing was done automatically within few minutes. In automated counting the principle is based on Impedance and Impedance counting first described by Wallace Coulter (1956). Lindsay RM (1975) evaluated the counting of platelets by electronic methods, had become widely accepted by laboratories and method was considered to give better reproducibility than the conventional microscopic count. Mayer K (1980) gave a comparative evaluation of the latest instrumentation by automated platelet counters [17,18,19,20].

Conclusion

In this study, on the basis of results, it is recommend that a manual platelet count using haemocytometer and morphology study of the blood smear should be performed if platelets is very low and giant platelets.

Conflict of Interest

This research project was self-sponsored without any financial aid from any institution/ company. There was no financial interest of/in any company or institution that might benefit from their publication. The authors have no competing/conflict of interests associated with this publication.

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Antibiotics - Is there a Need for Antibiogram?

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ABSTRACT

Antibiotic resistance is a global problem, the solutions are entirely local issue. The anti-microbiological data revealed an alarming increase in the incidence of antibiotic resistant, Methicillin-resistant Staph.aureus and other resistant bacteria in many upgraded and Speciality Hospital in many developing countries. The disturbing fact has been a major driver for framing antibiotic policy to improve the hospital infection control. Education about the specific use of antibiotics both in hospitals and in community is vital if we are to tackle resistance to antibiotics effectively. Increase in monitoring, surveillance and imparting knowledge of infection control principles and practices will be able to bring down the rate of antibiotics resistance. Microbial isolates and antimicrobial susceptibility testing pattern were studied in cumulative samples from ICU (n=1773), Surgery ward (n=1446), Medicine ward (n=1092), Cardiac centre (n=719), Collection centre (n=1522) from Super speciality hospital in developing country. Pus samples (n=5544), Urine samples (n=3204) Respiratory samples (n=1297) and Blood samples (n=903) were analysed and results of antimicrobial susceptibility testing pattern were worked out. The antibiogram should be monitored by a control unit under strict supervision of Experts.

KEY WORDS: Antibiogram, Antibiotics

الملخص

المقاومة للمضادات الحيوية مشكلة عالمية, ويكون الحل محليا . بيانات مضادات الميكروبات في المستشفيات التخصصية في بعض البلدان النامية توضح زيادة مقلقة في معدل حدوث المقاومة للمضادات الحيوية, مثل بكتريا المكورات العقدية المقاومة لعقار الميثيسيلين. هذه الحقائق ادت الي تكوين سياسات لاستخدام المضادات الحيوية للتحكم في العدوي. التوعية بالاستخدام الامثل والدقيق للمضاد الحيوي في المستشفيات وفي المجتمعات من الوسائل الحيوية للحد من المقاومة للمضادات الحيوية بصورة فاعلة. ايضا المراقبة والتقصي والمعرفة لأساسيات وممارسات السيطرة علي العدوي يخفض من مستوي المقاومة للمضادات الحيوية.**المنهج:** تم عمل دراسة وذلك بعزل البكتريا وعمل اختبار الحساسية للمضادات الحيوية لعينة تراكمية عددها 1773 مريض بالعناية المركزة وعدد 1446 من قسم الجراحة و1092 من قسم الباطنية و719 من مركز القلب و1522 من مركز التجميع بإحدى المستشفيات التخصصية بدولة نامية . كانت عينات الصديد 5544 وعينات البول 3204 وعينات الجهاز التنفسي 1297 وعينات الدم 903 وقد تم تحليل العينات واستخراج النتائج لاختبار حساسية المضادات الحيوية . يجب عمل التخطيط والرصد والمراقبة للمضادات الحيوية من قبل وحدة تحكم و بإشراف دقيق وصارم من الخبراء.

INTRODUCTION

The rapid increase in the incidence of antimicrobial resistance in recent years has deemed it a major public health concern. Improper treatment of infections due to resistance to conventionally prescribed antibiotics has led to increased morbidity and mortality. The Antibiotic resistance is a global problem, the solution are entirely local issue. Briefly, an antibiogram as defined by the Clinical and Laboratory Standards Institute is an overall profile of the antibiotic susceptibility of an organism to a collection of antimicrobial agents routinely tested and used. Most hospitals issue once a year an “Antibiogram chart”, which is a summary of the most important antibiotic resistance pattern of that hospital for the year [1].

The anti-microbiological data revealed an alarming increase in the incidence of antibiotic resistant, Methicillin resistant Staph aureus, and other resistant bacteria in many Hospitals. The antibiogram gives useful information for the selection of an empiric antibiotic treatment with specific bacteria [2,3]. Measuring resistance to antibiotics enables epidemiologists and healthcare providers to monitor trends, develop guidelines for optimal empiric therapy, and provide impetus for and ascertain the success of educational efforts promoting the judicious use of antibiotics as antimicrobial resistance is not uniform [4, 5].

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Alternatively, consistently accurate data can not only result in the successful remedy of an infection, but also provide clinicians and health professionals with confidence in their therapeutic prescribing and their ability to treat [6]. Lack of standardization in the construction of antibiogram can result in misinterpretation of data and inappropriate prescribing of empiric antimicrobial therapy. Guidelines for antimicrobial susceptibility testing and the presentation of cumulative test data antibiogram are available for many hospitals [7,8,9,10,11].

Properly prepared antibiogram are important in the monitoring and control of antimicrobial resistance. The disturbing fact has been a major driver for framing antibiotic policy and improvement of hospital infection control. The present collaborative study aims to determine if existing hospital antibiogram could be used to estimate the percent of specific, drug-resistant in multiple sites in one Medical College with Super specialty Hospital in developing countries in order to see the solution to the arising problems and its implications for the other applicable places

METHODS

Study Design: A 2 years collaborative follow up study was done with Department of Microbiology, Dayanand Medical College Hospital, Ludhiana, India which is one type of Super speciality Hospital in developing country.

Data collection: A request for antibiogram from 2008-2010 were sent to all laboratory facilities performing microbiological culture and antimicrobial susceptibility testing in the above study place in order to examine and report the status of antimicrobial resistance to prepare cumulative antibiogram.

Sample size: Microbial isolates and antimicrobial susceptibility testing pattern were studied in the following sample size from ICU (n=1773), Surgery ward (n=1446), Medicine ward (n=1092), Cardiac centre (n=719) and Collection centre (n=1522). Also subgroup of various samples namely: - Pus samples (n=5544), Urine samples (n=3204) Respiratory samples (n=1297) and Blood samples (n=903) were also studied.

Analysis: We obtained aggregated data within antimicrobial susceptibility and the status of antimicrobial susceptibility in the individual antibiogram was evaluated using criteria from the Clinical Laboratory Standard Institute [1,2].

Careful monitoring was done regarding the production and distribution of antibiotic culture plates. Antibiograms were evaluated on the basis of antimicrobial susceptibility testing pattern of Gram +ve and -ve isolates.

Limitation: In this study, we were unable to examine the role of laboratory error or differences in susceptibility testing methods.

RESULTS

The percentage of antimicrobial susceptibility testing of gram –ve isolates were high in Amikacin, Netlimicin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam, Imipenem and Meropenem. Also, the percentage of antimicrobial susceptibility testing of gram +ve isolates were high in Erythomycin, Gentamycin, Amikacin, Clindamycin, Lincomycin, Vancomycin and Teicoplanin (Table 1 & 2).

Table1: Percentage of Antimicrobial Susceptibility Testing of Gram-ve Isolates to different Antibiotics

Antibiotic	E coli	Pseudo	Acino	Klebsiella
Genta	35	30	15	30
Amika	78	41	22	68
Netl	68	34	48	53
Cipro	14	37	10	18
Cephlex	16	4	3	12
Ceftz	22	31	10	16
ceftx	20	30	8	14
Cefpz	19	31	7	8
Pipera	13	38	7	11
Piper/tazo	80	66	77	24
Cepz+sulb	72	46	47	61
Imip	99	75	68	96
Mero	97	68	58	90
Cotri	15	16	19	19

In Surgical wards the antimicrobial susceptibility testing pattern for gram–ve isolates were Amikacin, Netilmicin, Piperacillin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam, and Imipenem whereas Erythromycin, Gentamycin, Amikacin, Netilmicin and

Vancomycin were for for gram + isolates (Fig 3, 4).

Table 2: Percentage of Antimicrobial susceptibility testing of gram+ve isolates to different antibiotics

Antibiotic	Staph. aureus	Enterococcus
Pen	27	13
Eryth	50	24
Oxa	41	-
Genta	52	20
Amika	51	28
Netl	27	28
Cipro	41	22
Clinda	74	-
Linco	100	100
Vanco	100	96
Teico	98	97

In ICU the antimicrobial susceptibility testing pattern for gram–ve isolates were Amikacin, Piperacillin+Tazobactum and Imipenem whereas Amikacin, Netilmicin and Vancomycin were for gram + isolates (Table 3, 4 and Fig 1, 2).

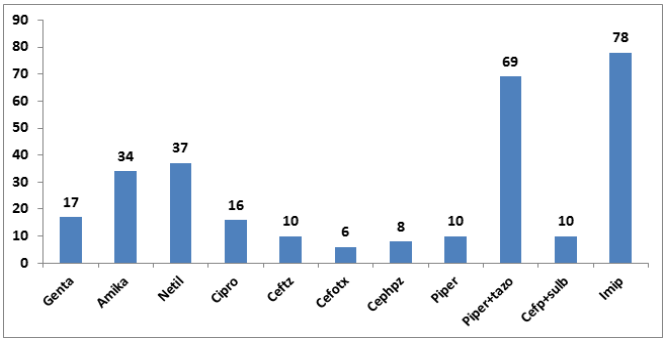

Figure 1: Sensitivity Pattern of G-ve Isolates from ICU (n=1542)

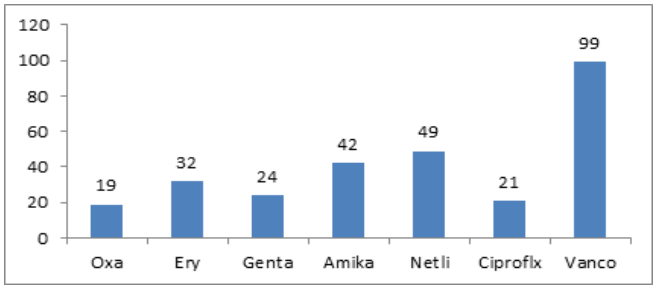

Figure 2: Sensitivity Pattern of G+ve Isolates from ICU (n=231)

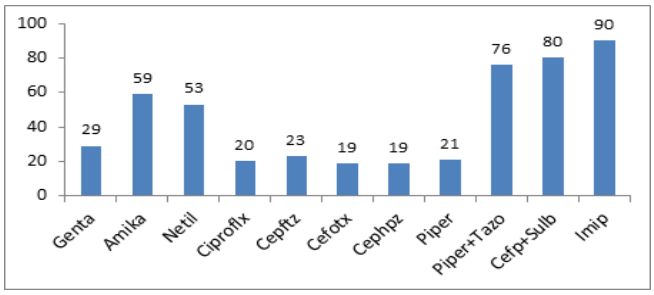

Figure 3: Sensitivity Pattern of G-ve Isolates from Surgery wards (n=1098)

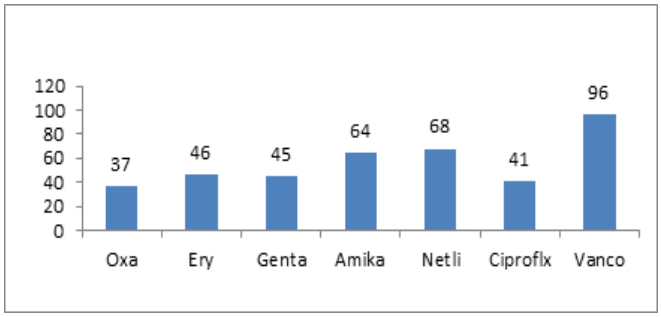

Figure 4: Sensitivity Pattern of G+ve Isolates from Surgery wards (n=348)

Table 3: Culture Reading from ICU

Org./ Antibio	Genta	Amk	Netil	Cipro	Ceptz	Ceftx	Cefpz	Pipera	Piper+ Tazo	Cefp+ Sulb	Imip
Acineto	10	13	40	4	1	2	1	2	76	8	65
Pseudo	23	33	26	32	24	22	24	28	66	44	72
E. coli	21	58	45	10	7	6	5	3	63	14	97

Table 4: Culture reading from ICU

Org./Antibio	Oxa	Ery	Genta	Amik	Netil	Cipro	Vanco
S.aureus	25	38	33	55	79	22	100
Enterococcus	--	18	14	6	10	12	96

In Medical wards the antimicrobial susceptibility testing pattern for gram–ve isolates were Amikacin, Netilmicin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam and Imipenem whereas Oxacillin, Gentamycin, Amikacin, Netilmicin and Vancomycin were for gram + isolates (Fig 5,6).

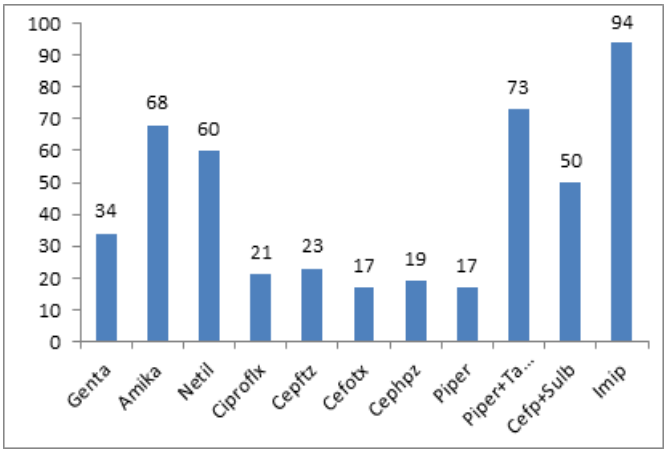

Figure 5: Sensitivity Pattern of G-ve Isolates from Medical wards (n=852)

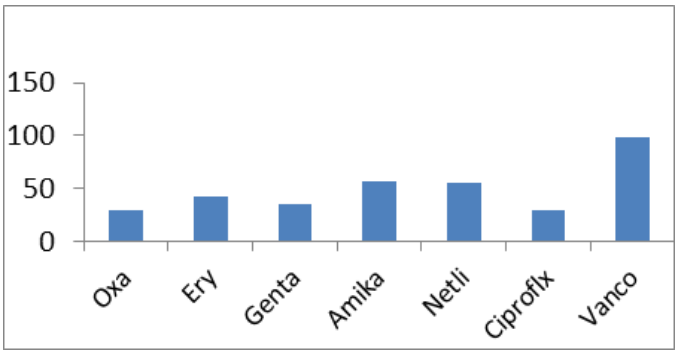

Figure 6: Sensitivity Pattern of G+ve Isolates from Medical wards (n=240)

In Cardiac centre the antimicrobial susceptibility testing pattern for gram –ve isolates were Gentamycin, Amikacin, Netilmicin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam, and Imipenem whereas Amikacin, Netilmicin and Vancomycin were for gram+ isolates (Fig 7,8).

In Collection centre the antimicrobial susceptibility testing pattern for gram –ve isolates were Amikacin, Netilmicin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam, and Imipenem whereas Amikacin, Netilmicin and Vancomycin were for gram+ isolates (Fig 9,10).

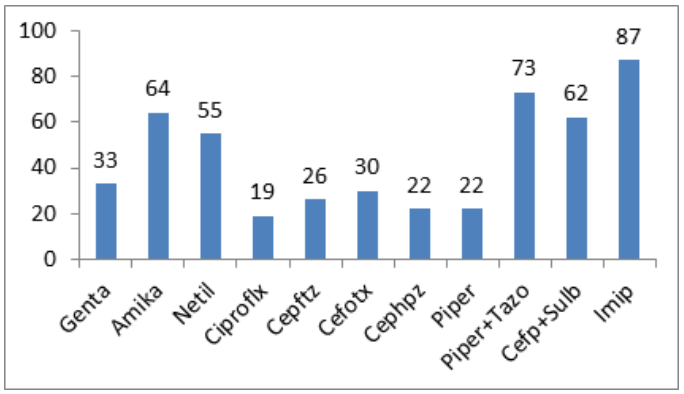

Figure 7: Sensitivity Pattern of G-ve Isolates from Cardiac Centre (n=592)

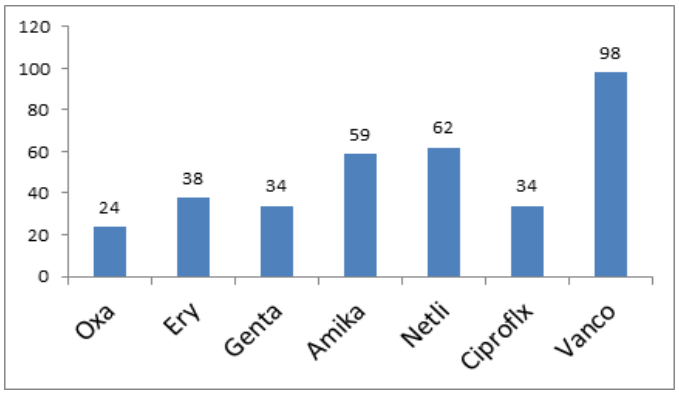

Figure 8: Sensitivity Pattern of G+ve Isolates from Heart Centre (n=127)

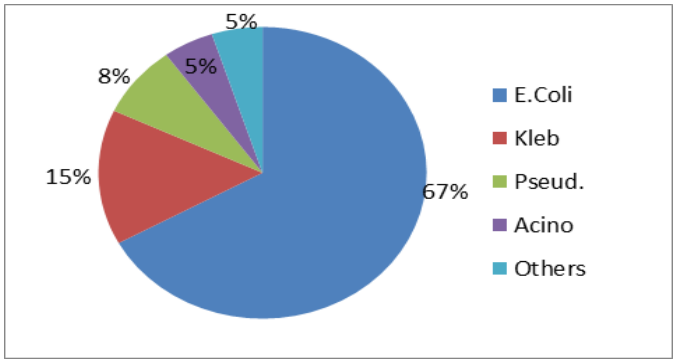

Figure 9: G-ve Isolates from Collection Centre

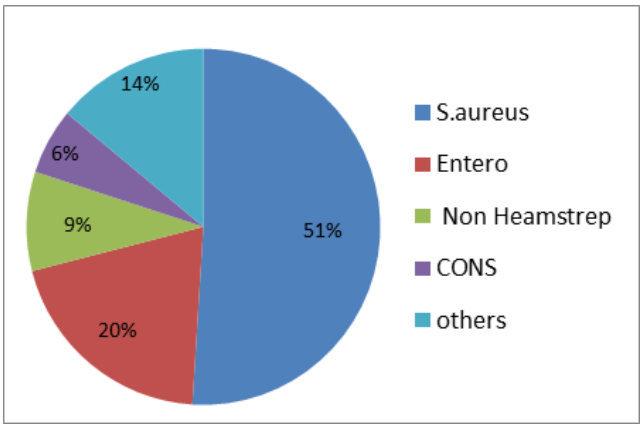

Figure 10: G+ve Isolates from Collection Centre

In pus samples the antimicrobial susceptibility testing pattern for gram–ve isolates were Piperacillin+Tazobactum, and Imipenem whereas Amikacin, Netilmicin, Clindamycin and Vancomycin were for gram + isolates (Fig 11, 12).

In urine samples the antimicrobial susceptibility testing pattern for gram–ve isolates were Amikacin, Netilmicin, Norfl, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam and Imipenem whereas Nitrofurantoin are for gram + isolates (Fig 13, 14).

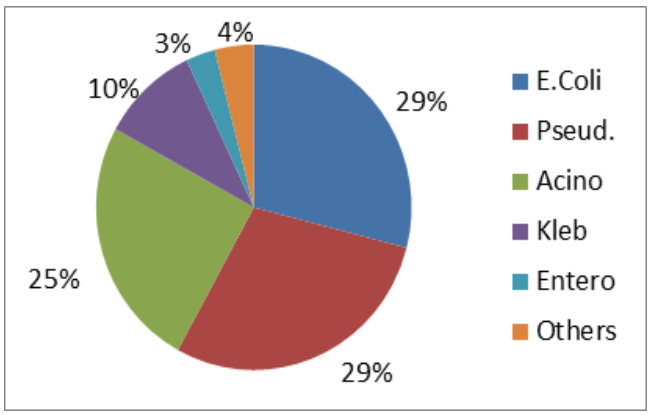

Figure 11: G-ve Isolates in Pus samples

In Respiratory samples the antimicrobial susceptibility testing pattern for gram–ve isolates were Piperacillin+Tazobactum, and Imipenem whereas Erythromycin, Amikacin, Netilmicin and Vancomycin were for gram + isolates (Fig 15, 16).

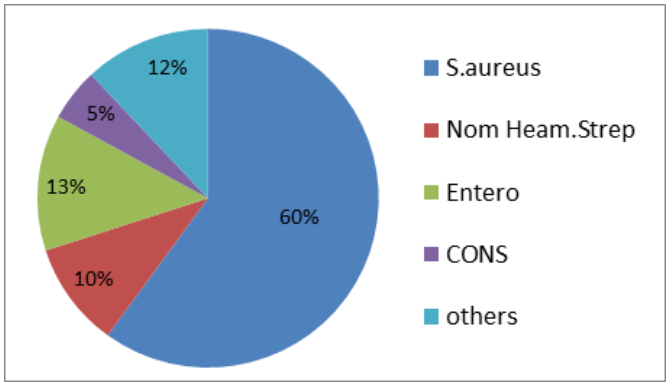

Figure 12: G+ve Isolates in Pus samples

In Blood samples the antimicrobial susceptibility testing pattern for gram–ve isolates were Chloromycin, Amikacin, Piperacillin+Tazobactum, Cefoperazone+ Sulbactam and Imipenem whereas Oxazolidinones, Erythromycin, Chloramfenicol, Amikacin, Netilmicin, Ciprofloxacin and Vancomycin were for gram + isolates (Fig 17, 18).

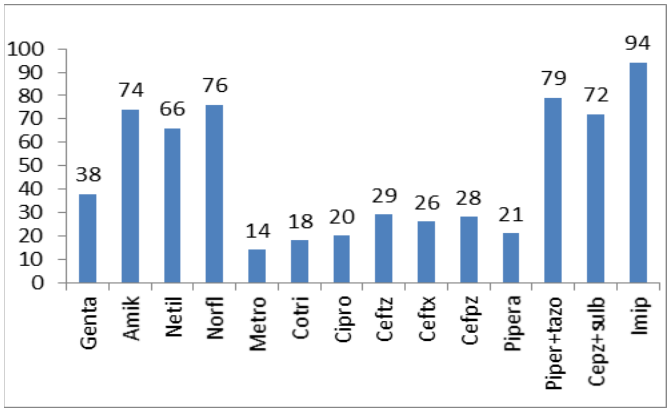

Figure 13: Sensitivity Pattern of G-ve Isolates in Urine samples (n=2565)

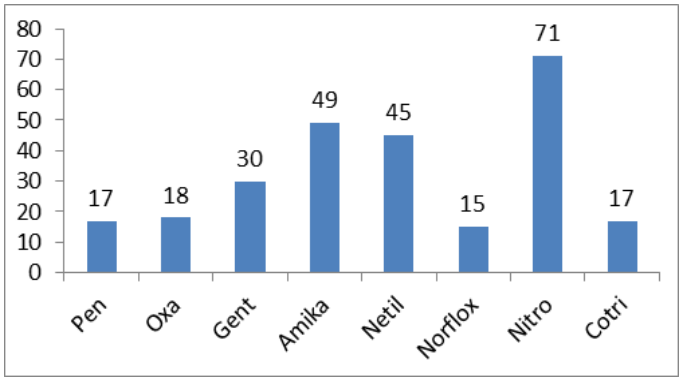

Figure 14: Sensitivity Pattern of G+ve Isolates in Urine samples (n=639)

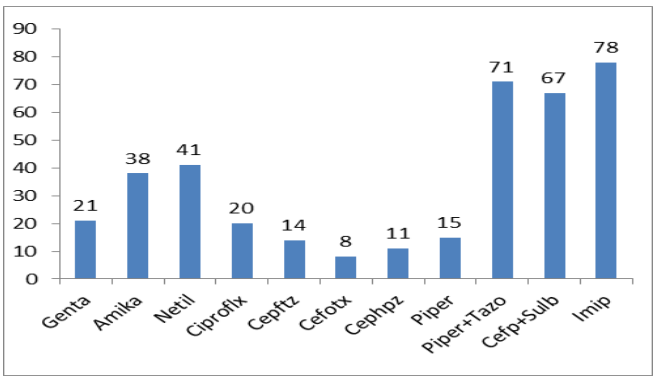

Figure 15: Sensitivity Pattern of G -ve Isolates in Respiratory samples (Sputum,Tracheal Secr. & BAL) (n=1212)

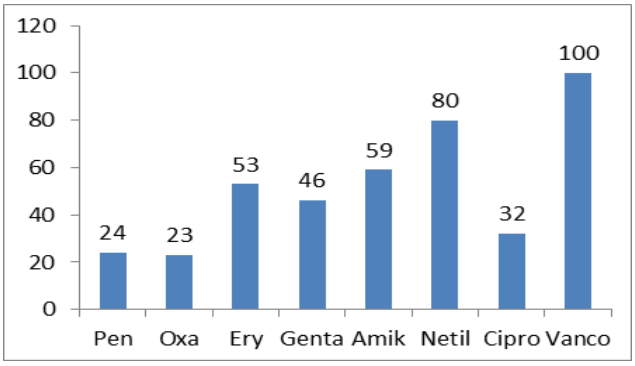

Figure-16, Sensitivity Pattern of G+ve Isolates in Sputum, Tracheal Secr. & BAL samples (n=85)

DISCUSSION

Most hospitals and laboratories routinely generate antibiogram; therefore, obtaining this information is relatively easy. The present study suggests that antibiogram may be an adequate method for conducting drug-resistant surveillance, illustrating the comparability of aggregated antibiogram and provided site specific point estimates of antibiotic resistance similar to the studies of antimicrobial susceptibility testing study conducted in various sites to detect resistance trends over time [8,9]

Antibiogram may be able to follow trends in antimicrobial resistance [10]. At the present juncture, we could not document the ability of antibiogram to detect trends. Drawbacks include the inability to evaluate resistance to multiple drugs. Relatively few drugs can be evaluated because of laboratory variations in antibiotics selected for susceptibility testing.

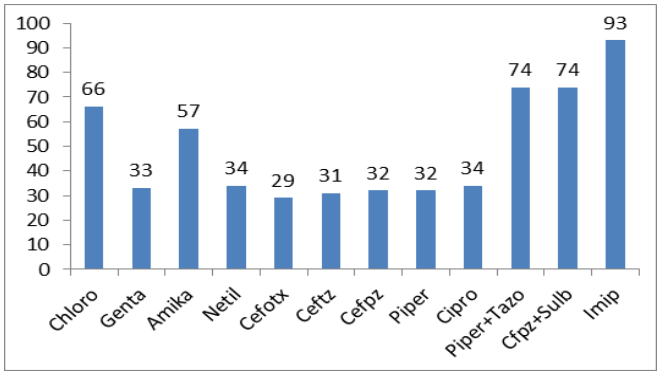

Figure 17: Sensitivity Pattern of G-ve Isolates in Blood samples (n=432)

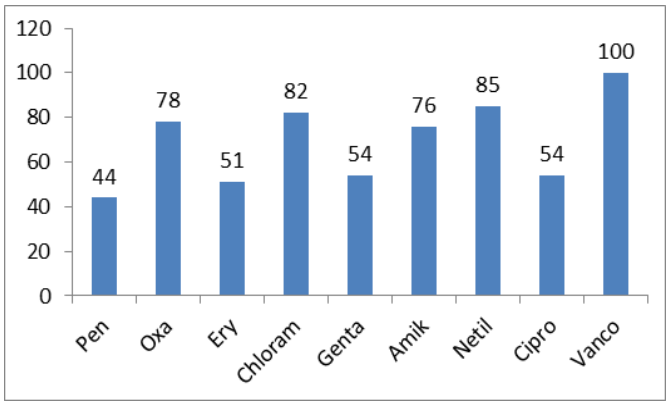

Figure 18: Sensitivity Pattern of G+ve Isolates in Blood samples (n=471)

They might encourage hospital laboratories to standardize their susceptibility panels to facilitate aggregation of results. These findings are also supported by various studies [12, 13, 14]. It is important that the recommended reports generated should be informed to clinicians while choosing antibiotic treatment regimen. Expert from Microbiologist and Pharmacist should think seriously for the addition of extra antibiotics plate culture for wide antimicrobial susceptibility testing.

CONCLUSION AND RECOMMENDATIONS

Aggregating antibiogram is useful for infections in hospital-acquired infections. Evaluation of antibiogram collected from different hospitals and clinics need to improve communication.

Online and update Medical Education is required to educate and making aware to the treating doctors in order to reduce resistance pattern and reduction of infections. Clinicians and researchers are now acknowledging the importance of preventing resistant infections through appropriate use of antibiotics.

Microbiologist and Pharmacist should think seriously in hospital laboratories to standardize their susceptibility panels with addition of extra antibiotics plate culture for wide antimicrobial susceptibility testing report to make easier for clinician in choosing the best antibiotics and will avoid misused and will prevent antibiotics resistance. Education about the specific use of antibiotics both in hospitals and in community is vital if we want to tackle resistance to antibiotics effectively. Increase in monitoring, surveillance and imparting knowledge of hospital infection control principles and practices will be able to bring down the rate of nosocomial infections. *"We learn now". "Now is the time to fight".*

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Spontaneous congenital intrauterine depressed skull fracture in Newborn

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ABSTRACT

Spontaneous congenital intrauterine depressions of skull in newborn has an incidence of 1/10,000 birth. These skull depression have two pathogenic types; deformed skull depression with and without fracture. The cause of skull depression being the pressure exerted by the fist of the fetus on the skull or skull being pressed against the sacral promontory. The treatment of choice for selected cases is surgical elevation.

Key words: neonatal, congenital, depressed fracture, spontaneous, sacral promontory.

الملخص

معدل حدوث انخفاض الجمجمة التلقائي الخلقي داخل الرحم يساوي 1 لكل 10,000 ولادة. هذا الانخفاض يتكون من نوعين , انخفاض مصحوب بكسر في الجمجمة والاخر بدون كسر , من اسباب هذا الانخفاض ضغط قبضة الجنين علي الجمجمة او الضغط علي الجمجمة من عظم عجز الام, يتم العلاج جراحيا في بعض الحالات المختارة

INTRODUCTION

A depressed skull fracture is an inward buckling of the calvarial bones and where no fracture line can be found, is referred to as Ping- Pong or Pond fracture or Greenstick fracture [1]. Sponteneous congenital depressions of skull have an incidence of 1/10000 birth in western countries [1,2]. Congenital depression of fetal skull not associated with trauma is a rare event. Infrequently noted that spontaneous intrauterine skull depressions are seen in neonates after easy and uneventful normal delivery or even after cesarean section. Etiology and pathogenesis of these congenital skull depressions are open to conjecture and the optimal method of management remains controversial. The actual cause is not known, but it is suggested that because of the cartilaginous nature of the fetal skull, compression by fetal limbs or pressure of the fetal head against the maternal bony structure, mainly the sacral promontory causes the most of the so-called "spontaneous" congenital depressed skull fractures. They are not true fractures, rather these are focal congenital molding depressions [2]. On the other hand, trauma to the mother's abdomen and traumatic delivery are accepted pathological mechanisms for such lesions [3].

One third of the depressed fractures are simple, one third are associated with dural laceration and one fourth have cortical laceration. Depressed skull fractures typically requires surgical elevation if the depth of the depression is thicker than the calvarium [4]. Associated intracranial injuries are rare. Theoretically a depression of more than 5 mm may impinge on the cerebral cortex, resulting in localized compression of the brain with resultant cerebral edema and decreased blood flow [5].

This condition can be diagnosed clinically. Plain x-ray of skull may show the degree of deformation. No fracture line is noted because of the resilient cartilaginous nature of the neonatal skull, and there are no secondary findings of an acute injury, such as soft tissue swelling, intracranial hemorrhage or adjacent brain edema seen on the CT scan [6].

The management of depressed skull fracture of neonates is discussed controversially. Small lessions are likely to resolve sponteneously without any surgical intervention [7]. Large lessions (>3cm) with a mass effect leading to midline shift, require corrective surgery. In addition, it is advisable to elevate severe depressions to prevent cortical injury from sustained pressure [8,9].

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Treatments advocated have included surgical elevation[10,11] usually by Burr-hole operation for large depression, elevation by digital pressure on the edges of the depression [12] by serial manipulation for several days for minor depression, elevation by vacuum extractor or a breast pump [13] for minimum to moderate size depression and watchful waiting [14] for minimal depression. In general larger and deeper depressions are treated more aggressively either by neurosurgical correction or suction elevation [10,11]. We report this case because of infrequent occurrence but with awareness of neurological consequence which may need good clinical assessment, evaluation and timely intervention.

Case Report

It was the fifth delivery of the mother. The actual delivery was at term, cephalic presentation and amniotic fluid was clear. Delivery was done by cesarean section. It was a female baby, body weight was 3100 gm, length was 54 cm. Apgar score 9 after 01 minute and 10 after 5 minute. During her stay in the hospital the child had normal neurological status. After delivery by cesarean section the left fronto- parietal bone depression was evident. The depression measured 2x3cm with a deapth of 1.5cm. The anterior frontonel measured 1x1cm and was normotensive. There was no maternal history of abdominal trauma during pregnancy and there was no complication or difficulty with cesarean section.

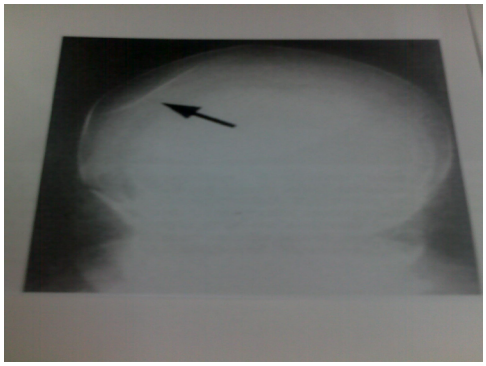

Figure 1: Frontal skull radiography showing "Ping-pong ball" fracture of the left parietal bone.

At craniogram in antero-posterior projection, at the place of depression of the left fronto-parietal bone any fracture line was not visible. Cerebral ultra sonogram was normal without any sign of intracranial bleeding or contusion. CT scan of brain showed depression and angulations of bone on the left Fronto-parietal region. There was no intracranial hemorrhage or cerebral edema. Ventricular system was normal and there was no mid-line shift. The case was evaluated by Neurosurgeon and decided for surgical correction as the depressed segment was of large size.

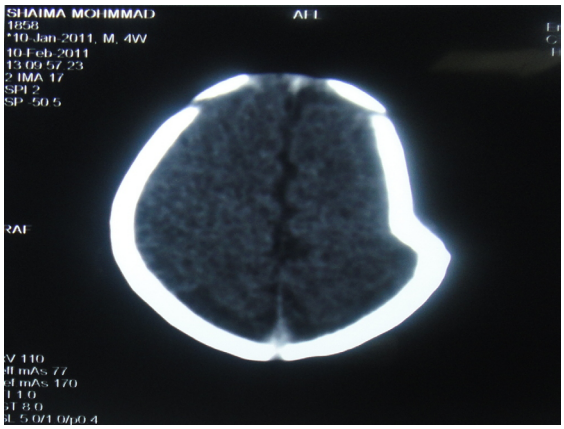

Figure 2: Axial computed tomography brain scan showing a depression in the left parietal region with no associated fracture.

The rationale for this policy is (a) to relieve local and general compression of the brain; (b)to prevent irritation of the brain and possibly the future development of seizures; and (c) cosmetic [15]. Accordingly on the 6th day of life, under General anesthesia the depression was elevated by Burr-hole operation. The dura matter was stripped and a periostial elevator was used to elevate the depressed fracture. Postoperative recovery was uneventful and was discharged from the hospital on 3rd post operative day at good shape and neurological status.

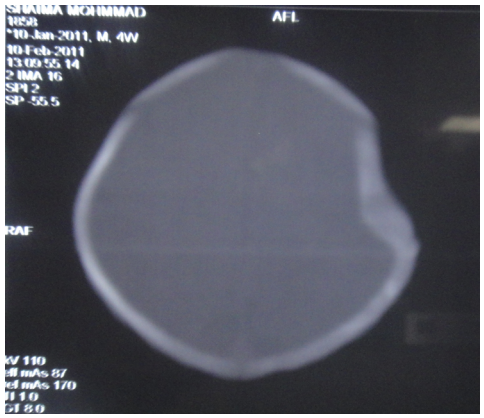

Figure 3: Axial computed tomography brain scan(Bony domain) showing a depression in the left parietal region with no associated fracture.

The patient was followed-up at 2 weeks, 2months, 6 months and ten months of age was found at normal neurological status. Milestones of development were normal.

Discussion

Congenital depression of the neonatal skull is a rare finding in our newborn population. We have encountered only one patient among 6528 births in the past 5 years in our hospital, from July 2006 to June 2011.

Regarding the pathogenesis we distinguished two types of congenital depressions of neonatal skull. (a) Deformed skull depression, which is deformation without fracture (b) Fractured skull deformation, which is depression with fracture [5]. It is impossible to differentiate between these two entities clinically. In our case the diagnosis was made by the use of X-ray and CT- scan of brain which ruled out the presence of any fracture, cerebral compression, hemorrhage or cerebral edema. Spontaneous intrauterine fracture of skull in neonates among African women may be more common and a large series by Axon and Levy [16] suggests that the most frequent mechanism may be occult trauma from pressure of the fetal head on the sacral promontory, resulting in molding.

In some reported cases, uncomplicated sponteneous vaginal or cesarean section delivaries, surprisingly yielded infants with depressed skull fractures [10]. The congenital depressed fracture, unlike the linear fracture, is caused by inward bending of the skull surface, leading to so called Pin-Pong ball or Pond fracture. In the neonate with a depressed fracture, usually there is no break in the surface continuity of the skull and on an X-ray the fracture appears as a line of increased density [17].

Normally the neonatal skull is very soft, and bones are separated by membraneous sutures, which makes the skull very pliable. There are some controversy as to whether depressed skull fracture in the newborn should be treated or not and if treatment is given whether it should be surgical or not. Some researcher states that in his experience all depressed skull fractures in neonates have corrected themselves sponteneously [18]. To our openion, this may be so, but there is no long term follow up studies to know the outcome in terms of neurological defeciency.

Sequelae due to acute head injury are well recognized in older children. These include, cranial nerve palsies, focal cerebral deficits, cerebral atrophy and post traumatic epilepsy [19].

A similar case report was published in Dove press journal: Research and Reports in Neonatology [20], which like our case was not a true fracture but rather a depression, which also was treated by surgical correction.

It is therefore, in conclusion more acceptable that congenital depressed skull fracture in newborn should be elevated as soon as possible after birth, although not necessararily as an emergency. Rational for this policy is (i) to relieve local and general compression of brain, (ii) to prevent irritation of brain and possibly the future development of seizures; and (iii) cosmetic [15].

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Custom Ocular Prosthesis as a Palliative Aid for a Pediatric Patient with a Surgical Enucleation following Retinoblastoma

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ABSTRACT

BACKGROUND & AIMS: Congenital absence or loss of the ocular globe during childhood not only compromises the normal development of the orbital region, it causes esthetic and psycho-social disturbances as well. Literature demonstrates the necessity of prevention and early detection in order to minimize the sequelae and disturbances in orbital growth.The purpose of this article is to highlight customized scleral shell prosthesis as a simple yet effective treatment modality for anopthalmic pediatric patients **METHODS:** For this anophthalmic pediatric patient who reported to us, we decided to fabricate a customized ocular prosthesis by modifying the stock eye prosthesis. Clinical relevance: Fabrication and installation of eye prosthesis is essential to the rehabilitation process especially in a child, so as to produce satisfactory development of the region and for their emotional wellbeing.

Key words: Ocular prosthesis, scleral shell, anophthalmos, retinoblastoma

الملخص

مقدمة : عدم وجود مقلة العين الخلقي او فقدان مقلة العين اثناء الطفولة لا يؤثر فقط علي التطور الطبيعي لمحجر العين ، بل و يسبب اضطرابات جمالية و نفسية واجتماعية . توضح المراجع اهمية وضرورة الوقاية من و الكشف المبكر لهذه الحالات من أجل التقليل من مضاعفات عدم نمو محجر العين . **الهدف:** يهدف هذا المقال لتسليط الضوء علي استخدام بديل اصطناعي مخصص لصلبة العين باعتبارها طريقة بسيطة للعلاج و لكنها فعالة لحالات فقدان مقلة العين لدي الاطفال . **المنهج :** تم صناعة بديل مخصص لصلبة العين وتركيبه لطفل يعاني من فقدان العين . **الاستنتاج :** تصنيع وتركيب بديل صناعي للعين أمر ضروري في عملية إعادة التأهيل وخاصة في الأطفال ، وذلك للحصول علي نتائج جيدة في تطور ونمو المنطقة و في سلامة الصحة النفسية و العاطفية.

INTRODUCTION

The loss of the ocular globe during childhood, be it due to congenital, traumatic or pathological etiologies, affects the growth and development of the orbital area, which in turn may result in hypoplasia and facial asymmetry, and represents an additional factor of aesthetic and psychological imbalance [1]. According to Heher et al [2], ocular tumors such as retinoblastoma are the major causes of ocular globe enucleation during first childhood. In a study with childhood cancer series, the retinoblastoma has been ranked second in occurrence, corresponding to 17.1% of all types of cancer occurring in infants [3]. Considered the most common primary intraocular malignancy of childhood, this neuroblastic tumor may be unilateral or bilateral [4].

Clinical implications: Unlike adults, a child can neither express nor realize the situation of loss of an eye, but non acceptance in the peer group and society in general will undoubtedly have a lasting psychological impact on the child. Hence it is important to provide prosthesis to not only preserve the socket but also to ensure the psychological wellbeing of the child. This article highlights the management of a pediatric patient with the loss of ocular globe following retinoblastoma using a simple yet effective clinical technique.

CASE REPORT

The subject was a four year old female child of poor socioeconomic status, who reported to the clinic for prosthetic rehabilitation of a left ocular defect. On recording the history and obtaining the previous medical records, it was found that the patient had retinoblastoma of the left eye as a result of which the eye had to be enucleated. On examination, it was observed that the mucosa surrounding the eye was healthy and post-surgical healing was uneventful.

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It was decided to give the ocular prosthesis by modifying the stock eye prosthesis. The procedure was explained to the patient/ parent and consent was taken for making photographic records. Since this technique was a combination of custom eye prosthesis and prefabricated scleral shell prosthesis, clearance from the institutional ethical clearance committee was obtained.

Clinical Procedure

Lignocaine hydrochloride (2%) topical anesthetic (Neon Laboratories Limited, Mumbai, India) was applied on the tissues of the left eye before making the impression to prevent discomfort to the patient during the procedure. An impression of the affected side of the eye was recorded using custom ocular tray fabricated using self-cure resin (DPI, Mumbai, India) made from a moulage of the contralateral eye . The advantage of using a custom ocular tray is that it ensures the emergence profile of the ocular bulb closely matches the unaffected eye. The handle of the ocular tray was made hollow so that the alginate could be syringed into the defect. The periorbital tissues were coated with a layer of petrolatum jelly so that the impression material could be easily separated from the tissues. Impression was recorded using alginate (Plastalgin, Septodont, India) and was poured in Type 2 Gypsum (Kalstone, Kalabhai, Mumbai, India). The intaglio surface on the cast was coated with separating medium and waxed up using type 2 modeling wax (Hindustan Dental Products, Hyderabad, India). A suitable scleral shell was selected in accordance with the patient's contralateral eye and trimmed to the recorded dimensions. The scleral shell color was then customized according to the patient's contralateral iris size and color; and dewaxing and packing was done with acrylic resin on the intaglio surface of the shell. The prosthesis was finally retrieved to be trimmed, finished and polished. It was then stored in distilled water for 48 hours to let residual monomer leach out to prevent irritation to the sensitive palpebral mucosal tissue.

During the prosthesis insertion phase, the palpebral area was examined and the parents of the child were shown the correct procedure to insert and remove the prosthesis for regular cleaning and maintenance procedures. They were asked to report back on a biannual basis for follow up visits which was duly followed and has so far been uneventful.

Discussion

Loss of the ocular globe may affect normal growth in the periorbital region and may contribute to asymmetry of the facial skeletal complex. Another challenge that needs to be countered is the constant growth of the facial skeletal complex that may necessitate periodic alterations to the existing prosthesis or may need to be changed. Therefore, the prosthesis should be made slightly larger to simulate normal development of the surrounding tissues. It has also been advised to remake the prosthesis at periodic intervals to keep pace with growth till the eye socket is fully developed at around 12 years of age [5].

Two options are available for artificial eye prosthesis; one is a pre-fabricated ocular prosthesis and the other being custom-made ocular prosthesis. literature has also described customizing a scleral shell prosthesis into a custom ocular prosthesis to improve the fit and seating of the prosthesis [7,8] which was used in this case to ensure that the best of both the techniques was achieved. Though there are articles in literature reporting the same, there is a lack of evidence base data regarding the use of this technique with pediatric patients.

Limitations of this technique are that the clinician is dependent on the availability of a pre-fabricated eye with properly matching iris and pupillary part. Also, the long-term color stability of the heat-cured acrylic and the strength of its union with the stock eye will have to be closely evaluated [9]. The option of an ocular implant was also considered but since it necessitated preservation of tenons capsule, which had been already excised when the patient reported, hence this treatment modality was ruled out

Keeping in mind that we deal with pediatric patients, unforeseen complications may also arise. Care must be taken to prevent voids that can accumulate mucus or debris, which may be a potential source of infection in children. Moshfegi et al reported cases of a 30-month- and 32-month-old boy, each of whose eyes had been enucleated for retinoblastoma and swallowed their respective ocular prosthesis on different occasions. But they also mentioned

that each of the children was having life stresses in addition to their medical treatment that may have accounted for their behavior [10].

Conclusion

Irrespective of which technique is followed, the use of a customized ocular prosthesis during childhood entails periodic changes with successive increase in order to accompany the expansion of the anophthalmic cavity, and it is in fact the only way to aesthetically rebuild the anophthalmic socket. It is also deemed necessary to determine periods of ocular prosthesis change during craniofacial growth in order to minimize the possible discrepancy between the compromised and the healthy sides, thus ensuring a balance and harmony of the facial development. Most importantly, there is also a need of providing psychological support to the pediatric patient.

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MEDICAL QUIZ

A 50-year-old lady presented to the outpatient clinic with history of dry cough for the last 2 months she also mentioned 1 month history of low grade fever, joints pain and cutaneous nodules on both pretibial area and around the elbow joints. Physical examination revealed a temperature of 38.6°C, bilateral periarthritis affecting the ankle joints and prominent tender erythematous lesions over the shin and the extensor surfaces of both elbow joints and forearms (Figure 1). A set of relevant investigations were carried out and a summary of the results are shown (Table 1). Chest x-ray (Figure 2) and computed tomography showed bilateral hilar lymphadenopathy.

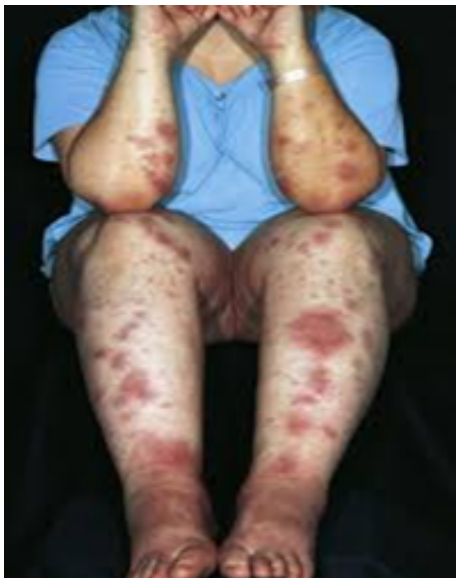

Figure 1

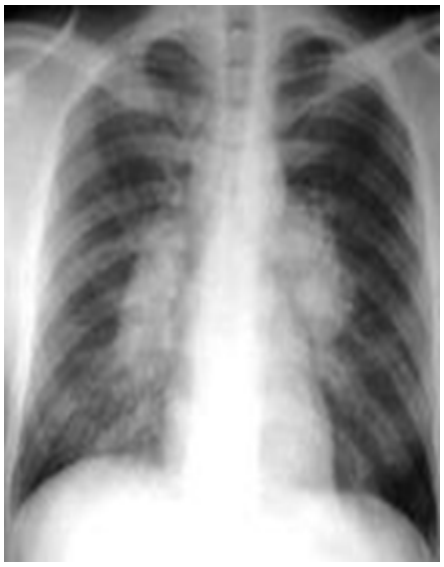

Figure 2

Table 1: Summary of investigations

Investigation	Results
Complete blood count, urinalysis, renal function test, rheumatoid factor	Within normal limits
Serum calcium and angiotensin-converting enzyme levels	Both are high
Erythrocyte sedimentation rate (ESR)	High at 50 mm/h
Chest x-ray and computed tomography	Bilateral adenopathy
Lung function test	Normal
Skin test for purified protein derivative antigen	Negative

1. What is the nature of the erythematous rash seen in this patient?
   a. Erythema multiforme.
   b. Rheumatoid nodules.
   c. Erythema marginatum.
   d. Erythema nodosum.
   e. Eyrthema chronica migrans.

2. What is the diagnosis?
   a. Systemic lupus erythematosus
   b. Rheumatoid arthritis.
   c. Sarcoidosis
   d. Steven- Johnson syndrome
   e. Lyme disease

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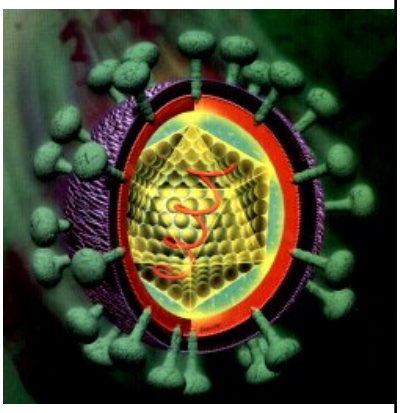
The answers are (d) erythema nodosum (c) sarcoidosis

FDA approves Sovaldi for Chronic Hepatitis C



The U.S. Food and Drug Administration approved Sovaldi (sofosbuvir) to treat chronic hepatitis C virus (HCV) infection. Sovaldi is the first drug that has demonstrated safety and efficacy to treat certain types of HCV infection without the need for co-administration of interferon. Sovaldi is the second drug approved by the FDA recently to treat chronic HCV infection. On November 22, the FDA approved Olysio (simeprevir).

Hepatitis C is a viral disease that causes inflammation of the liver that can lead to diminished liver function or liver failure. Most people infected with
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Sovaldi is a nucleotide analog inhibitor that blocks a specific protein needed by the hepatitis C virus to replicate. Sovaldi is to be used as a component of a combination antiviral treatment regimen for chronic HCV infection. There are several different types of HCV infection. Depending on the type of HCV infection a patient has, the treatment regimen could include Sovaldi and ribavirin or Sovaldi, ribavirin and peginterferon-alfa. Ribavirin and peginterferon-alfa are two drugs also used to treat HCV infection. Sovaldi's effectiveness was evaluated in six clinical trials consisting of 1,947 participants who had not previously received treatment for their disease (treatment-naive) or had not responded to previous treatment (treatment-experienced), including participants co-infected with HCV and HIV. The trials were designed to measure whether the hepatitis C virus was no longer detected in the blood at least 12 weeks after finishing treatment (sustained virologic response), suggesting a participant's HCV infection has been cured.

Results from all clinical trials showed a treatment regimen containing Sovaldi was effective in treating multiple types of the hepatitis C virus. Additionally, Sovaldi demonstrated efficacy in participants who could not tolerate or take an interferon-based treatment regimen and in participants with liver cancer awaiting liver transplantation, addressing unmet medical needs in these populations. The most common side effects reported in clinical study participants treated with Sovaldi and ribavirin were fatigue and headache. In participants treated with Sovaldi, ribavirin and peginterferon-alfa, the most common side effects reported were fatigue, headache, nausea, insomnia and anemia.

Sovaldi is the third drug with breakthrough therapy designation to receive FDA approval. The FDA can designate a drug as a breakthrough therapy at the request of the sponsor if preliminary clinical evidence indicates the drug may demonstrate a substantial improvement over available therapies for patients with serious or life-threatening diseases. Sovaldi was reviewed under the FDA's priority review program, which provides for an expedited review of drugs that treat serious conditions and, if approved, would provide significant improvement in safety or effectiveness.

NEW JNC 8 HYPERTENSION GUIDELINES: WHAT DOES THE PANEL RECOMMEND NOW?

The Eighth Joint National Committee (JNC 8) has released new guidelines on the management of adult hypertension.

RELATED: Cardiovascular Disease Resource Center

The authors formed nine recommendations which are discussed in detail along with the supporting evidence. Evidence was taken from randomized controlled trials, the gold standard for establishing efficacy and effectiveness. Some of the new major recommendations include:

1. In patients aged ≥60 years, initiate pharmacologic treatment in systolic BP ≥150mmHg or diastolic BP ≥90mmHg and treat to a goal systolic BP <150mmHg and goal diastolic BP <90mmHg. (Strong Recommendation–Grade A)
2. In patients aged <60 years, initiate pharmacologic treatment at diastolic BP ≥90mmHg and treat to a goal <90mmHg. (For ages 30–59 years, Strong Recommendation–Grade A; For ages 18–29 years, Expert Opinion–Grade E)
3. In patients aged <60 years, initiate pharmacologic treatment at systolic BP ≥140mmHg and treat to a goal <140mmHg. (Expert Opinion–Grade E)
4. In patients aged ≥18 years with chronic kidney disease, initiate pharmacologic treatment at systolic BP ≥140mmHg or diastolic BP ≥90mmHg and treat to goal systolic BP <140mmHg and goal diastolic BP <90mmHg. (Expert Opinion–Grade E)
5. In patients aged ≥18 years with diabetes, initiate pharmacologic treatment at systolic BP ≥140mmHg or diastolic BP ≥90mmHg and treat to a goal systolic BP <140mmHg and goal diastolic BP <90mmHg. (Expert Opinion–Grade E)
6. In the general nonblack population, including those with diabetes, initial antihypertensive treatment should include a thiazide-type diuretic, CCB, ACE inhibitor, or ARB. (Moderate Recommendation–Grade B) This recommendation is different from the JNC 7 in which the panel recommended thiazide-type diuretics as initial therapy for most patients.
7. In the general black population, including those with diabetes, initial antihypertensive treatment should include a thiazide-type diuretic or CCB. (For general black population: Moderate Recommendation - Grade B; for black patients with diabetes: Weak Recommendation–Grade C)
8. In the population aged ≥18 years with chronic kidney disease, initial (or add-on) antihypertensive treatment should include an ACE inhibitor or ARB to improve kidney outcomes. (Moderate Recommendation–Grade B)
9. If goal BP is not reached within a month of treatment, increase the dose of the initial drug or add a second drug from one of the classes in Recommendation 6. If goal BP cannot be reached with two drugs, add and titrate a third drug from the list provided. Do not use an ACEI and an ARB together in the same patient. If goal BP cannot be reached using only the drugs in Recommendation 6 because of a contraindication or the need to use more than 3 drugs to reach goal BP, antihypertensive drugs from other classes can be used. (Expert Opinion–Grade E)

The full guidelines have been published online at JAMA.

STANDARDS OF MEDICAL CARE IN DIABETES 2014

American Diabetes Association, Diabetes Care Volume 37, Supplement 1, January 2014

Diabetes mellitus is a complex, chronic illness requiring continuous medical care with multifactorial risk reduction strategies beyond glycemic control. Ongoing patient self-management education and support are critical to preventing acute complications and reducing the risk of long-term complications. Significant evidence exists that supports a range of interventions to improve diabetes outcomes.

The American Diabetes Association's (ADA's) Standards of Care are intended to provide clinicians, patients, researchers, payers, and other interested individuals with the components of diabetes care, general treatment goals, and tools to evaluate the quality of care. The Standards of Care recommendations are not intended to preclude clinical judgment and must be applied in the context of excellent clinical care and with adjustments for individual preferences, comorbidities, and other patient factors.

The recommendations include screening, diagnostic, and therapeutic actions that are known or believed to favorably affect health outcomes of patients with diabetes. Many of these interventions have also been shown to be cost-effective. The Standards of Care conclude with evidence and recommendations for strategies to improve the process of diabetes care. It must be emphasized that clinical evidence and expert recommendations alone cannot improve patients' lives, but must be effectively translated into clinical management.

PROMOTION OF BREASTFEEDING

The promotion of breastfeeding in developing nations struggling with hunger directly addresses the problem of infant undernutrition. Thirty-five percent of all childhood deaths are attributed to undernourishment, which is the largest single contributor to childhood mortality worldwide (Horton, et al. 2010). Childhood undernutrition refers to the inadequate nutrition of children under the age of five. After the first 24 months of an infant's life, the effects of childhood undernutrition are largely irreversible, such as stunting, which refers to low height for one's age, wasting, which is low weight for one's height, and the impairment of cognitive ability. It is therefore critical that infants obtain proper nutrition within the first 1000 days of their life (IFPRI, 2010).

Breast milk can provide infants with all the nutrients they need; however, many mothers are unaware of this fact, while others are unable to breastfeed due to undernutrition and micronutrient deficiencies, particularly in vitamin A, iron, and folic acid (IFPRI, 2010). Breastfeeding also can serve as an uncontroversial means of birth control: fertility reduction due to breastfeeding is similar to the effect of other methods of contraception (Pérez-Escamilla, 2007). Fertility reduction decrease population growth, which is interlinked with food insecurity as presented in the Cost of Inaction.

Breastfeeding will be promoted in areas affected by hunger, especially in areas with high rates of child undernutrition. Promotion will occur throughout the community, in pre-existing hospital clinics, as well as any new facilities that are developed by the innovation villages. The government will be responsible for the oversight of all programs, while each hospital's or heath care facility's program would be under the care of the hospital managers. The managers would be held accountable for the training of their staff by government institutions.

At hospitals, after the birth of a child, the following protocol, adapted from the World Health Organization's (WHO) and the United Nations Childhood Fund's (UNICEF) "Baby Friendly Hospital Initiative" would be implemented (UNICEF, 2010).

1. A nationwide breastfeeding policy will be implemented at each hospital. Under the oversight of the manager of the health care facility or the hospital, the heath care staff will be properly trained on the policy and its implementation methods.
2. The health care staff will inform all pregnant women about the benefits of breastfeeding, providing them with pamphlets and any additional resources available. All pregnant women will be given this information throughout their gestation and reminded of the importance after the birth of their child.
3. Health care staff will aid mothers in initiating breastfeeding within one-half hour to hour of birth to ensure establishment and sustainment of breastfeeding through infancy (UNICEF and WHO, 2009).
4. Show mothers how to breastfeed and how to maintain lactation. Provide them pamphlets and additional information concerning these subjects. Health care staff will also teach them how to use the hospital's electric breastfeeding pump, if applicable at that particular hospital, along with proper storage containers. As one of the key problems women faced with breastfeeding is their inability to breastfeed while at work (“Infant Feeding in Emergencies,” 2010). An available electric pump would allow them to store breast milk that could be given to their infants while they are away. The cost of electric breastfeeding pumps range from 50 to 900 US dollars, and therefore, may not be able to be available in every hospital and health care clinic (Comit Stores, 2009).
5. Health care staff will promote exclusive breastfeeding for the first six months, informing the women that breast milk has all the key nutrients an infant needs. Exclusive breastfeeding refers to only breast milk feeding with no other foods or fluids, with the exception fo drops or syrups as micronutrient supplements and/or medicines. After six months, infants should receive complementary foods with continued

Promotion of breastfeeding

Medical supply

breastfeeding for up to two years and beyond. Woman should be informed that breastfeeding is also a more efficient way of distributing calories. Infants between zero to six months need, on average, 627 calories/day,which can all be acquired through breast milk, while a breastfeeding mother only needs 500 extra calories per day (Atlas of World Hunger, 2010). This is approximately a 20% increase in caloric efficiency. Mothers, therefore, only need to be worried about acquiring food for themselves, instead of needing to be concerned with purchasing formula, which can put further strain on economic resources for impoverished families struggling with hunger.

6. They should also encourage breastfeeding on demand and discourage the use of pacifiers, as pacifiers can provide mothers who have anxieties concerning breastfeeding with a means of weaning their children off of breast milk. Instead, health care staff should consult women about their anxieties when educating them about the benefits of breastfeeding (UNICEF and WHO, 2009) ("Exclusive Breastfeeding," 2010).
7. Health care facilities should foster the establishment of breastfeeding support groups and provide mothers with information upon their discharge from the hospital or clinic.
8. As many pregnant women suffer from micronutrient deficiencies, mothers should be given supplements in vitamin A, iron, and folic acid. Also, they should be informed about the importance of procuring an extra 500 kcal per day (UNICEF, 2010).

The above image is an example of an instruction card for the promotion of "Best Practices in Agriculture, Nutrition, and Health" with a special focus on breast feeding (IFPRI, 2010).
Initiatives in the community are also important, especially in areas where women do not tend to go to the hospital to give child birth. The promotion in the community would follow a similar set of guidelines as the hospital protocol, as it would:

- Educate about the importance of breastfeeding/ Encourage exclusive breastfeeding/ Provide information on breastfeeding and lactation
- Inform women about nearby available electric pumps and how they work
- Provide women with micronutrients, especially vitamin A, iron, and folic acid.

The World Bank estimates that the cost of breastfeeding promotion initiatives would be approximately $2.893 billion worldwide, while it would cost about $85.2 million worldwide to provide pregnant women

who have low levels of micronutrients with iron and folic acid supplements (Horton, et. al, 2010). Vitamin A supplements cost approximately 2-4 cents per dosage per person (Sommer, 2001).

One problem with breastfeeding is the passing of AIDS from mother to her child through breast milk. About 42% of the children affected with AIDS each year, acquire the disease through breastfeeding. UNICEF has been working to provide voluntary confidential counseling testing, antiretroviral drugs, and counseling regarding infant feeding options and how to minimize risk of transmission ("Mother-to-Child Transmission of HIV/AID," 2010). This type of information should be available at the local health facilities, as well as included in the promotion programs of the community.

Breastfeeding promotion could have an immediate impact on child undernutrition rates, and therefore can be considered a short term solution. Mothers who are taught about the benefits of breastfeeding and how to properly breastfeed can immediately begin providing their children with the adequate nutrition they need. Promotion of breastfeeding with continue in the communities strongly afflicted by hunger and undernutrition rates until it becomes an integral part of the community. The actual time this will take will vary from place to place, and therefore does not have an exact period of implementation, but will continue throughout the 100 year period until it is unnecessary.

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GUIDELINES FOR MANUSCRIPT PREPARATION

A. TYPES OF MANUSCRIPTS

I. ORIGINAL MANUSCRIPTS

Manuscripts submitted in this category are expected to be concise, well organized, and clearly written. The maximum length is 5000 words, including the abstract, references, tables, and figure legends. The maximum length is 5000 words, including the abstract, references, tables, and figure legends.

- The structured abstract must not exceed 250 words.
- The title must not exceed 130 characters.
- A maximum of 4 tables and 4 figures is allowed.
- References should not exceed a maximum of 100.
- The abstract must be organized as follows:
- Background & Aims
- Methods
- Results
- Conclusions
- Do not use abbreviations, footnotes or references in the abstract.
- An electronic word count of the abstract must be included.
- Three to ten key words at the end of the abstract must be provided.

The manuscript must be arranged as follows:

- Title page
- Abstract
- Introduction
- Materials and methods (or Patients and methods)
- Results
- Discussion
- Acknowledgements
- References
- Tables
- Figure legends
- Figures

Acceptance of original manuscripts will be based upon originality and importance of the investigation. These manuscripts are reviewed by the Editors and, in the majority of cases, by two experts in the field. Manuscripts requiring extensive revision will be at a disadvantage for publication and will be rejected. Authors shall be responsible for the quality of language and style and are strongly advised against submitting a manuscript which is not written in grammatically correct English. The Editors reserve the right to reject poorly written manuscripts even if their scientific content is qualitatively suitable for publication. Manuscripts are submitted with the understanding that they are original contributions and do not contain data that have been published elsewhere or are under consideration by another journal.

II. REVIEW ARTICLES

Review articles on selected clinical and basic topics of interest for the readers of the Majmaah Journal of Health Science will be solicited by the Editors. Review articles are expected to be clear, concise and updated.

- The maximum length is 5000 words, excluding the summary, references, tables, and figures.
- References should not exceed a maximum of 150.
- The inclusion of a maximum of 4 high-quality tables and 4 colored figures to summarize critical points is highly desirable.
- Review articles must be accompanied by a title page and a summary.

- Reviews should include at least one Key Point Box, with a maximum of 5 bullet points, that briefly summarizes the content of the review.

Review articles are reviewed by the Editors and may be sent to outside expert reviewers before a final decision for publication is made. Revisions may be required.

III. EDITORIALS

This section consists of invited brief editorial comments on articles published in the Majmaah Journal of Health Science

The length of an editorial should not exceed 1500 words, excluding references.

- A maximum of 1 table or 1 figure is allowed.
- References should not exceed a maximum of 20.
- A title page must be provided.

IV. CASE REPORTS

Case reports would be only accepted if they represent an outstanding contribution to the Etiology, pathogenesis or treatment of a specific condition.

- The maximum length is 3000 words, including the summary and references.
- A maximum of 2 tables and 2 figures is allowed.
- References should not exceed a maximum of 15.
- A title page must be provided.

V. LETTERS TO THE EDITOR

Letters to the Editor will be considered for publication if they are related to articles published in recent issues of Majmaah Journal of Health Science. Occasionally, Letters to the Editor that refer to articles not published in Majmaah Journal of Health Science will be considered.

The length of a Letter to the Editor should not exceed 800 words.

- A maximum of 1 table or 1 figure is allowed.
- References should not exceed a maximum of 10.
- No more than 4 Authors may appear in the author list.

VI. COMMENTARIES

International commentaries will be solicited by the Editors only.

- Commentary articles should not exceed a maximum of 800 words, excluding tables or figures.
- A maximum of 1 table or 1 figure is allowed.
- References should not exceed a maximum of 10.
- A title page must be provided.

B. MANUSCRIPT SUBMISSION

ORGANIZATION OF THE MANUSCRIPT

- The submitted manuscript must be typed double-spaced throughout and numbered (including references, tables and figure legends). Preferably using a "standard" font (we prefer Times/Arial 12).
- For mathematical symbols, Greek letters, and other special characters, use normal text. The references must be in accordance with the Vancouver reference style (see References).
- Approved nomenclature for gene and protein names and symbols should be used, including appropriate use of italics (all gene symbols and loci, should be in italics) and capitalization as it applies for each organism's standard nomenclature format, in text, tables, and figures.
- Full gene names are generally not in italics and Greek symbols are not used. Proteins should not be italicized.
- Improperly prepared manuscripts will not be entered into the peer review process and will be sent back to the author for correction.

TITLE PAGE MUST CONTAIN:

- A title of no more than 130 characters.

- Running title (not to exceed 60 characters)
- Names of the Authors as it should be published (first name, middle initial, last name)
- Affiliations of all authors and their institutions, departments, or organizations (use the following symbols in this order to designate authors' affiliations: *, †, ‡, §, ¶, ||, #, **, ††, ‡‡, §§, ¶¶, || ||, ##).
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- Number of figures and tables.
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- Conflict of interest.
- Financial support.

Animal trials: Manuscripts reporting experiments using animals must include a statement giving assurance that all animals received human care and that study protocols comply with the institution's guidelines. Statistical methods used should be outlined.

Human trials: Manuscripts reporting data from research conducted on humans must include a statement of assurance in the methods section of the manuscript reading that:

1. Informed consent was obtained from each patient included in the study and
2. The study protocol conforms to the ethical guidelines of the 1975 declaration of helsinki as reflected in a priori approval by the institution's human research committee.

Randomized controlled trials: Any paper that is a randomized control trial should adhere to the guidelines that can be found at the following web-site: www.consort-statement.org. The checklist should be printed out and faxed to the Editorial office at the time of submission. The trial registration number must be included on the title page of the manuscript reporting a registered clinical trial. Failure to do so will prevent entry to the peer review process.

Drugs and chemicals: Drugs and chemicals should be used by generic name. If trademarks are mentioned, the manufacturer's name and city should be given. All funding sources supporting the work, either public or private, especially those from pharmaceutical companies, must be provided.

Genetic Sequence data: In papers reporting a novel DNA or amino sequence, verification that the data have been or will be submitted either to Gen-Bank or EMBL is required. Please provide this verification and the accession number in the covering letter.

REFERENCES

References must be in accordance with the Journal of Hepatology reference style. References are ordered as they appear in the text and citation numbers for references are placed between "brackets" ("[ ]") in the text as well as in the reference list.

Authors should be listed surname first, followed by the initials of given names (e.g. Bolognesi M). If there are more than six authors, the names of the first six authors followed by et al. should appear.
Titles of all cited articles are required. Titles of articles cited in reference list should be in upright, not italic text; the first word of the title is capitalized, the title written exactly as it appears in the work cited, ending with a full stop. Journal titles are abbreviated according to common usage, followed by Journal years, semicolon (;) before volume and colon (:) before full page range (see examples below).

All articles in the list of references should be cited in the text and, conversely, all references cited in the text must be included in the list.

Personal communications and unpublished data should be cited directly in the text by the first Author, without being numbered. Please make sure you have the latest, updated version of your reference management software to make sure you have the correct reference format for Majmaah Journal of Health Science.

An example of how references should look within the text:

"HVPG was measured by hepatic vein catheterization using a balloon catheter according to a procedure described elsewhere [14, 15] and used as an index of portal hypertension [16]."

An example of how the reference list should look:

[14] Merkel C, Bolognesi M, Bellon S, Zuin R, Noventa F, Finucci G, et al. Prognostic usefulness of hepatic vein catheterization in patients with cirrhosis and esophageal varices. Gastroenterology 1992;102:973-979.
[15] Groszmann RJ, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. Hepatology 2004;39:280-282.

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A maximum of 4 figures is allowed
(This can be modified if needed by Editorial board).

- Figures will be often, but not always, re-designed by graphic designers. By signing and transferring the Copyright Agreement to MJHS, the author gives permission to the graphic designers to alter the visual aspect of any figures, tables, or graphs. The scientific content of figures will not be altered. Please provide this information with your covering letter.
- All graphics submitted to Majmaah Journal of Health Science should be sent at their actual size, which is 100% of their print dimension and in portrait orientation.
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- Figures should be supplied in the following preferred file formats: PDF (*.pdf), Power Point (*.ppt), Adobe Illustrator (*.ai, *.eps), Photoshop (*.psd) files in grayscales or in RGB color mode. It is highly recommended that figures not be sent in JPG (*.jpg) format.
- Photographs (scans, immunofluorescences, EM, and histology images) should be submitted as: 1. TIFF (*.tif) with a resolution of at least 300 pixels per inch, or
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2. 600 dpi for black and white figures
3. 1200 dpi for line-art figures

- For all photomicrographs, where possible, a scale should appear on the photograph. Photographs of identifiable patients should be accompanied by written permission to publish from patient(s).
- • Furthermore, panel lettering should be in Arial bold 14 pt, capitalized and no full stop (A, B) while lettering in figures (axes, conditions), should be in Arial 8 pt, lower case type with the first letter capitalized and no full stop. No type should be smaller than 6 pt.

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- Tables should be provided as Word files (*.doc) or Illustrator/InDesign (*.ai, *.eps, *.indd) compatible files. No TIFF and JPG files are acceptable for table submission.
- When submitting tables in Microsoft Word table function, no tab, space or colors should be used. Tables should contain a maximum of 10 columns.
- Tables submitted in landscape orientation will not be accepted. Tables should include a title, table legend, and if necessary footnotes.
- Include tables in the submitted manuscript as a separate section.

FIGURE LEGENDS

- Figure legends should be listed one after the other, as part of the text document, separate from the figure files.
- Please do not write a legend below each figure. Each figure legend should have a brief title that describes the entire figure without citing specific panels, followed by a description of each panel, and the symbols used.
- Enough information should be provided in the figure legend text to permit interpretation of figures without reference to the text; but should not contain any details of methods, or exceed 100 words.
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(i.e. "Fig. 1."). Every figure legend should have a Title written in bold.
- If a figure contains multiple sections (i.e. A, B, C, D) the letter for these subsections should be in capital letters. Within the figure legend text the capital letters should be surrounded by parenthesis [i.e. (A)(B)(C)(D)].
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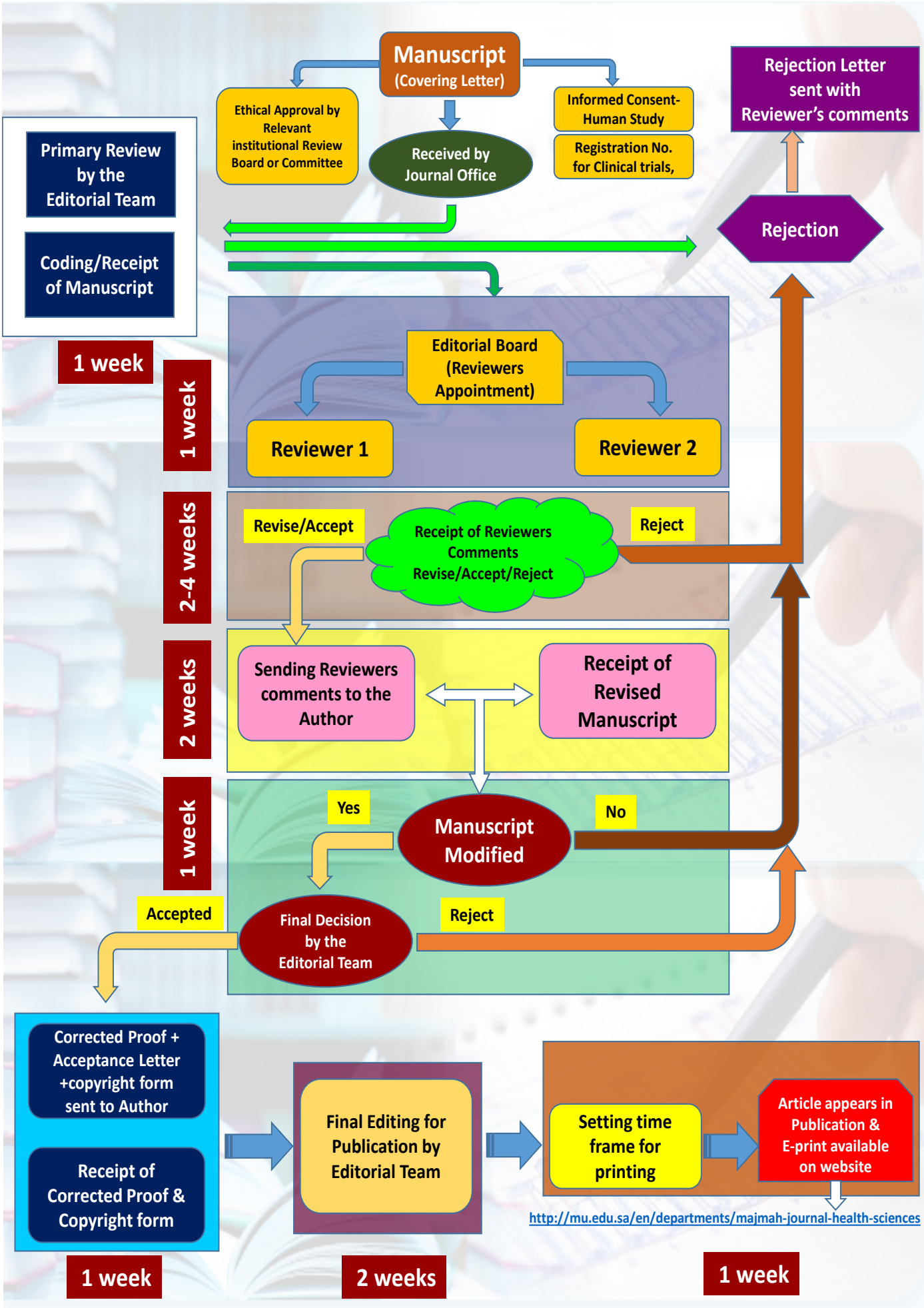
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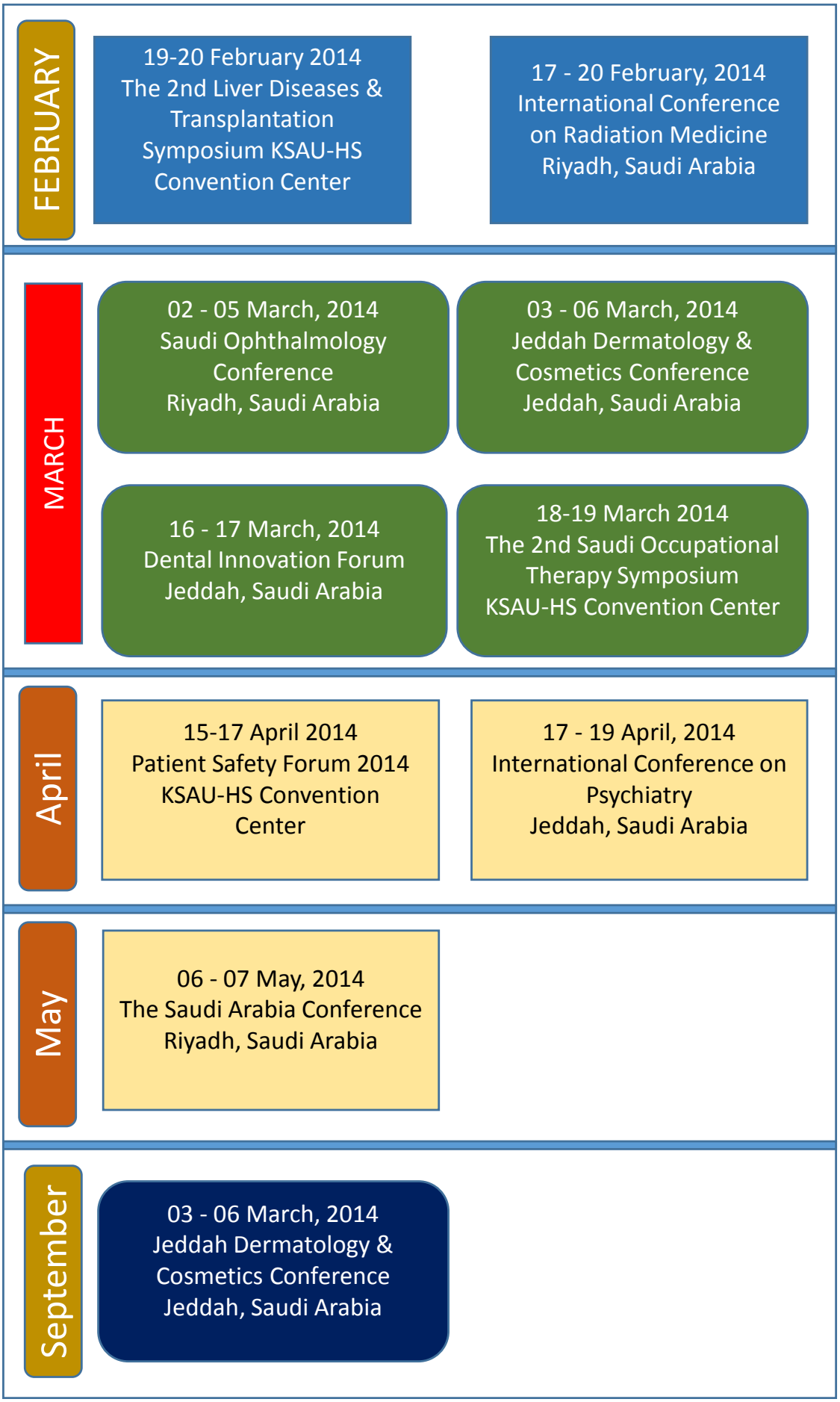
Methodological & Statistical instructions for Authors submitting manuscripts to the Majmaah Journal of Health Science

The manuscripts should include a complete and detailed description of what was done. This includes a description of the design, measurement and collection of data, the study objective and major hypotheses, type and source of subjects, inclusion and exclusion criteria and measures of outcome, number of subjects studied and why this number was chosen. Any deviation from the study protocol should be stated. The baseline characteristics of any compared groups should be described in detail and -if necessary -adjusted for in the analysis of the outcome.

For randomized clinical trials the following should also be clearly documented: treatments, sample size estimation, method of random allocation and measures taken for maintaining its concealment including blinding, numbers treated, followed-up, being withdrawn, dropping out, and having side effects (numbers and type). The statistical methods used should be relevant and clearly stated. Special or complex statistical methods should be explained and referenced.

Complex analyses should be performed with the assistance of a qualified statistician. Unqualified use of such analyses is strongly discouraged. The underlying assumptions of the statistical methods used should be tested to ensure that the assumptions are fulfilled.

For small data sets and if variable distributions are non-normal, distribution free (non-parametric) statistical methods should be used. The actual p values - whether significant or not - should always be presented (not NS). Confidence intervals convey more information than p values and should be presented whenever possible. Continuous variables can always be summarized using the median and range which are therefore preferred. Only in the infrequent case of a Normal distribution are the mean and standard deviation (SD) useful. Complex analyses (including Cox and logistic regression analysis) should be presented in sufficient detail: i.e. variable scoring, regression coefficients, standard errors and any constants. Odds-ratios or relative risks are not sufficient documentation of such analyses. The handling of any missing values in the data should be clearly specified. The number of statistical tests performed should be kept at a minimum to reduce spurious positive results. Explorative (hypothesis generating) analyses without confirmation using independent data are discouraged. Figures showing individual observations e.g. scatter plots are encouraged. Histograms may also be useful. Tables should indicate the number of observations on which each result is being based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