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Ref: Postgrad Med. 2014 May;126(3):239-45. doi: 10.3810/pgm.2014.05.2772.

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Role of JCPSP in Advancement of Medical Journals in Pakistan: Raising the Standards through Quality Publication

Zafar Ullah Chaudhry

The prime vision of bringing out a medical journal is to encourage and disseminate quality research. College of Physicians & Surgeons Pakistan (JCPSP) is a world recognized Institute of postgraduate medical education and its sole objective is to promote specialist medical practice through coupled facilitation of postgraduate medical training and research. Journal of CPSP is an Impact Factor peer-reviewed research journal. It always strives for quality publication. It is a non-profitable journal, which is beyond thinking, in the current era of commercial publication. Authors do not have to pay for publication at JCPSP. The articles are available on the website by the first week of publication, being an Impact Factor journal, large number of bytes are reported. This portal, therefore, encourages authors from all over the world to send their quality research for publication to JCPSP. In 2017 (till 20th December), 321 out of 1,028 (32.1%), manuscripts submitted to JCPSP were from outside Pakistan. In order to facilitate future researchers that is the trainee Fellow, CPSP conducts workshops on research methodology, basic biostatistics, epidemiology, and medical writing, publication ethics, proposal / synopsis writing, and reporting research as per standard guidelines of medical journals.

Health related issues are quite unique for different regions of the world. Burden of disease and its associated morbidity and mortality is huge in developing countries. However, medical research currently dominated by affluent countries, hardly address these issues. This highlights the importance of Impact Factor journals from developing world, in which quality research papers can be published. JCPSP is one such journal. It receives a number of articles from developed and developing countries on various subjects, both basic and clinical sciences. With strict criteria at initial assessment, laid down according to the scope of the Journal and communicated through the guidelines to prospective authors, many articles are returned for addressing the deficiencies or considered unsuitable for processing in JCPSP. Authors are, therefore, encouraged to read 'instructions to the authors' carefully before submitting their manuscript to JCPSP. Our website provides a checklist for all the essential components of manuscript that must be followed before submission, specially "authors' certification form", and authors' contribution as per ICJME criteria, IRB / ERC formal approval/ exemption letter, declaration of conflict of interest and adherence to guidelines of reporting various types of studies. For facilitation, authors are advised to go through CONSORT statement for standardized reporting of randomized trials (RCT), registration of RCTs, STROBE checklists for reporting of cohort, case-control, and cross-sectional observational studies. It also includes STARD flow diagram and checklist, MOOSE, QUOROM and PRISMA guidelines.

JCPSP has recently indigenously developed its own Online Journal Management system. In future, we plan to receive articles through this system. We will continue to follow hybrid system, a mix of current pattern of submission and new OJS of CPSP, till authors get used to it. It will help in quick processing of articles and will decrease waiting time from submission till publication. The authors can personally track the stage of processing of their submissions. JCPSP has also acquired Digital Object Identifier (DOI); it will help the visibility and citation of the articles published in JCPSP.

JCPSP, as per policy of the parent institute CPSP, always collaborated with editors from Pakistan and abroad. The sole purpose of this collaboration is to improve quality of publication. CPSP recognizes quality journals, published from elsewhere in Pakistan for its residents, seeking fellowship, to publish research-based articles in lieu of dissertations as part of requirement of residency training. This increases the number of submission to national journals. CPSP criteria of recognizing national journals are far more stringent than both the Higher Education Commission (HEC) of Pakistan and Pakistan Medical & Dental Council (PM&DC). It is apparent from latest list of HEC recognized journals, where a journal regretted recognition by CPSP, is given X-category. CPSP has also conducted conferences for medical journal editors and collaborated with the Pakistan Association of Medical Editors (PAME). The members of editorial board of JCPSP have participated and made several presentations in various conferences, and conducted workshops for editors, peer reviewers and authors.

Editors of the medical journals have responsibilities towards investigators, peer reviewers, readers, and the society as well. Accuracy of research findings is of

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utmost importance as published articles contribute to guide and modify the medical practice, and may even impact the health related policies of statutory bodies. To ensure timely publication, a vigilant editorial board is needed. At JCPSP, a team of competent editors and staff work hard to meet this task. Here, I would like to pay tribute to Late Muhammad Zafaruddin, Executive Editor JCPSP, who passed away after short illness few months back. He played an important role in running the day-to-day affairs of the journal. We will always remember his contributions towards medical journalism. I end here by saying that at JCPSP editors enjoy editorial freedom as they have complete authority to run the affairs of the journal without external influence. This is the reason why JCPSP has unique standing in the field of medical journalism. I wish all of our contributors a happy new year and eager to receive any suggestion for further improvement.

EDITORIAL

Evolving and Expanding Role of Pathologists in Multidisciplinary Team Cancer Care

Shaheera Shakeel and Muhammed Mubarak

Multidisciplinary tumour (MDT) board meetings play a pivotal role in the era of personalised oncology; and ultimately providing evidence-based approach towards management plan in cancer care.[1-5] Although, these meetings are accomplished with a combined effort of dedicated team of surgeons, medical oncologists, radiologists, pathologists and other ancillary healthcare professionals, the role of pathologists has always been significant and is currently expanding beyond the traditional domains.[6-10] MDT meetings provide an opportunity where every domain of oncology, pathology, and radiology can share the expertise; discuss the recent advancements, and formulate the best possible management plan for an individual patient under discussion. MDT meetings are increasingly becoming the essential component of large healthcare systems not only to provide the optimal benefit to the individual patient, but also to improve the academic approach and combined effort of assuring quality control measures for the overall benefit of the cancer patients and healthcare providers.[11-13]

The utility of MDT meetings has been observed and reported from various parts of the world, but their establishment in developing countries is still at a primitive stage. Several studies have reported better ultimate outcomes of patients in whom decisions were made by a team of experts from all specialties.[14-16] In fact, pathologists are on the leading front to guide clinicians regarding prognostication and future outcomes of the disease. Pathologists are traditionally providing services for the evaluation of prognostic and predictive pathological markers, but they are still facing significant challenges in predicting the outcome in individual patients. It is crucial to understand the molecular underpinnings of cancer to guide the advancements in molecular targeted approach, which is the true paradigm shift in the current era of diagnosing and treating cancer.

MDT meetings represent a tremendous opportunity to pathologists to explore the natural history of disease process and, hence, the prediction of disease course and outcome; because they are the main driving force behind the targeted approach for optimal treatment strategies. In addition to this academic benefit, MDT meetings provide an ideal opportunity to foster professional relationships among surgeons, oncologists, radiologists and pathologists.[1-10]

Traditionally, the pathologists along with radiologists have been the core members of the MDT cancer care team. Historically, the role of pathologists were crucial in providing the tissue diagnosis, grading and pathologic staging of the cancer, sometimes accompanied by some prognostic information, for an individual patient with suspected cancer. But in this era of precision medicine, their role is evolving and expanding beyond the traditional frontiers involving individual cases to wider aspects of cancer care.[1,3,5,8] With the availability of more precise diagnostic and prognostic markers, the responsibility of pathologists is expanding beyond the traditional responsibilities to cater to the wider perspective of cancer prevention and cancer care. It includes participation of the pathologists in preclinical drug discovery and testing, determination of clinical trial eligibility of patients, assessment of novel prognostic markers, triage of tissue for molecular testing and consultation on the feasibility of such testing, companion biomarkers and diagnostics, biobanking of samples for future clinical testing and research; and last but not the least, the evaluation of quality assessment of cancer programs.[10-19] Although pathologists in developed countries are well trained and prepared to participate in these additional roles, most of the pathologists in developing countries lag behind in these aspects from their counterparts in developed countries. With these additional responsibilities, it is not surprising that a large part of the pathologists' time and effort is spent on oncology-related activities. Increasing demands on pathologists' time and responsibilities in oncology-related activities are compounded by a potential shortage of pathologists, not only in developing countries, but also in developed countries.[1] This is partly due to retirement of a greying workforce and an insufficient number of new doctors entering the system. Efforts are needed to attract more young doctors to the field of anatomic pathology. In addition, there is a need to educate the pathologists, particularly in developing countries, on their expanding roles in multidimensional cancer care programmes.

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In conclusion, the role of pathologists is expanding in many aspects of oncology beyond their traditional roles in diagnosing cancer, pathological staging, and participation in local MDT meetings; and now, impacts most phases of care of the cancer patient. Close working relationships among pathologists, radiologists, oncologists and other members of the MDT team can improve the care of individual cancer patient and are, particularly, pertinent in this era of personalised medicine.

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ORIGINAL ARTICLE

Urinary Retention in Unilateral Total Knee Arthroplasty: Comparison between Continuous Epidural Analgesia and Single-Shot Femoral Nerve Block

Ausaf Ahmed Khan and Robyna Irshad Khan

ABSTRACT

Objective: To compare the frequency of urinary retention and requirement of bladder catheterization in patients undergoing total knee arthroplasty while receiving either continuous epidural analgesia or single-shot femoral nerve block.
Study Design: Randomized controlled study.
Place and Duration of Study: Operating Rooms of Aga Khan University Hospital, Karachi, from January 2014 to January 2015.
Methodology: Patients were randomized in two groups of 30 each, i.e. epidural group (group E) or femoral nerve block group (group F). Baseline parameters were recorded. Postoperatively, patients were followed for upto 24 hours to collect the data regarding urinary retention. Final outcome was taken at 24 hours postoperatively. Data was analyzed to compare the frequency of urinary retention between the two groups.
Results: The average age of the patients was 59.58 ±5.85 years. There were 28 (46.7%) male and 32 (53.3%) female patients. Frequency of urinary retention was significantly high in Group E than Group F (46.7% vs. 6.7%; p=0.0005).
Conclusion: Single-shot femoral nerve block offers a more favorable profile in terms of postoperative urinary retention when compared to continuous epidural analgesia.

Key Words: *Total knee arthroplasty. Femoral nerve block. Epidural analgesia. Urinary retention. Lumbar epidural anesthesia.*

INTRODUCTION

Acute urinary retention is a common complication following lower limb arthroplasty with the incidence ranging from 10.7% to 84%.[1] Postoperative urinary retention can lead to significant morbidity, such as prosthetic infection and sepsis.[2] The spread of infection from the urinary tract to cause deep joint sepsis has been well documented and is more common following knee arthroplasty with a quoted incidence of 2.5% as compared with 0.64% post-hip arthroplasty.[1] Studies have shown increased incidence of urinary retention in patients using epidural when compared with non-epidural techniques.[3-5] The incidence of postoperative urinary retention is significantly lower in patients receiving continuous peripheral nerve blocks compared with epidural anesthesia.[6-8] However, in any of these studies urinary retention has not been looked as the primary outcome.

Pain after total knee arthroplasty (TKA) is severe,[9,10] and effective postoperative pain control allows for earlier ambulation and initiation of physiotherapy, which hastens recovery, reduces the length of stay in the hospital, and lowers the risk of postoperative complications.

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This study is aimed to compare the frequency of urinary retention and requirement of bladder catheterization in patients undergoing TKA while receiving either continuous epidural analgesia or single-shot femoral nerve block; and also compared the frequency of postoperative nausea, vomiting and sedation, as well as surgical outcomes and patient satisfaction between the two groups.

METHODOLOGY

This randomized controlled study was conducted in the operating rooms of the Aga Khan University Hospital after approval from University's Ethics Review Committee (reference number: 2768). Primary investigator obtained written and informed consent from all ASA I, II & III patients of either gender aged between 30 and 70 years, scheduled for unilateral total knee arthroplasty. Exclusion criteria were patient refusal, allergy to local anesthetics, paracetamol or other study drugs, history of opioid dependence, contraindications to spinal/epidural anesthesia, femoral nerve block (coagulation defects, infection at puncture site etc.), inability to use patient-controlled analgesia (as assessed at the time of informed consent), history of urinary retention, neurogenic bladder, or any urologic problem. Patients were randomly allocated by draw method to either of the two treatment groups, i.e. epidural group (group E) or femoral nerve block group (group F). After application of routine monitoring, i.e. electrocardiography (ECG), pulse oximetry (SpO_2), non-invasive blood pressure (NIBP)

and end-tidal carbon dioxide ($ETCO_2$), patients were induced using propofol 2mg/kg and atracurium 0.5 mg/kg; and endotracheal intubation was done. Anesthesia was maintained using isoflorane with oxygen and nitrous oxide, to maintain a MAC of 1.

After induction of general anesthesia, patients were treated according to their group allocation. Patients in epidural group (group E) were turned to lateral position for epidural catheter placement in lumbar region. Epidural catheter was loaded with 0.25% bupivacaine and infusion of bupivacaine 0.1% + fentanyl 2 mcg/ml was started at 8 - 12 ml per hour.

For patients in the femoral nerve block group (group F), ultrasound-guided femoral nerve block was performed using 20 ml 0.375% bupivacaine.

The surgeons were then allowed to proceed with the surgical procedure. After the completion of the procedure, patients were shifted to the Post-Anesthesia Care Unit (PACU). Time of arrival in the PACU was taken as 0-hour. Patient controlled intravenous analgesia (PCIA) was started for the patients in group F; using morphine with a bolus of 1 to 1.5 mg, no background infusion, and a lock-out time interval of 10 minutes. Patients in both groups were prescribed inj. paracetamol 1-gm every 8 hourly till the time proper oral intake is started, when paracetamol was given 1 gm every 8 hours, orally. If there was failure to perform epidural or nerve block, or the epidural was not working, then the patient was excluded from the study. Data regarding urinary retention was collected on the questionnaire at intervals 0 to 24 hours and final outcome for urinary retention was taken at 24 hours.

Sample size calculation was based on the study by Singelyn *et al*., where urinary retention was significantly higher in continuous epidural analgesia group than single shot femoral group (40 vs. 0%). Therefore, P1 was considered as 40% and P=0%. A sample size of 16 patients per group would have a 95% power of detecting a 40% difference in frequency of urinary retention at the 0.05 level of significance. It was decided to include 60 patients (30 in each group) to account for possible drop-outs.

Data was analyzed with SPSS (statistical package for social sciences) version 19. Frequencies and percentages were computed for qualitative variables like gender, urinary retention, postoperative nausea and vomiting (PONV) and sedation. Mean and standard deviation were computed for age, height and weight, maximal knee flexion, hospital stay, time taken to mobilize out of bed, and time of first solid meal intake. Independent sample t-test was applied to compare mean difference between groups and Chi-square test was used to compare frequency of urinary retention between groups. $p \leq 0.05$ was considered level of significance.

RESULTS

A total of 60 patients with unilateral total knee arthroplasty were included in the study. Patients were randomly allocated into two groups. Thirty patients were treated with epidural (Group E) and 30 were treated with femoral nerve block (Group F). The average age of the patients was 59.58±5.85 years. Mean age, weight and height were not significantly different between the groups (Table I). There were 28 (46.7%) male and 32 (53.3%) female patients. Proportion of gender was also not significant between the two groups (p=0.12) as presented in Table I.

Frequency of urinary retention in patients was not significant between groups at 0 hour and at 1-hour but it was observed that at 12 hours urinary retention was significantly high in Group E as compared to Group F (56.7% vs. 26.7%; p=0.018). Final outcome regarding the urinary retention of the patients between groups were assessed at 24 hours as also displayed in Table II. Frequency of urinary retention was significantly higher at 24 hours in Group E than Group F (46.7% vs. 6.7%; p=0.0005).

PONV scores were significantly low (p<0.05) in Group F vs. Group E as shown in Table III. Sedation score and maximal knee flexion at 24 and 48 hours were not significantly different between groups. Mean hospital stay was significantly lower in group F as compared to group E [157 ±9.75 hours vs. 162.7 ±11.77 hours; p=0.046]. Mean time taken to mobilize out of bed, was

Table I: Comparison of characteristics of patients between groups.

Variables	Group E n=30	Group F n=30	P-value
Age (years)	58.97 ±6.01	60.20 ±5.52	0.42
Height (cm)	163.13 ±8.59	164.83 ±8.97	0.45
Weight (kg)	77.57 ±8.09	80.03 ±8.29	0.24
Gender, n (%)			
Male	11 (36.7%)	17 (56.7%)	0.12
Female	19 (63.3%)	13 (43.2%)	
Procedure, n (%)			
Left TKA	11 (36.7%)	15 (50%)	0.29
Right TKA	19 (63.3%)	15 (50%)	

Data are presented as mean ± SD and n (%).
T-test for mean difference; Chi-Square test for proportion difference.

Table II: Compare the frequency of urinary retention in patients undergoing unilateral Total Knee Arthroplasty receiving continuous epidural analgesia vs. single-shot femoral nerve block.

Time (hours)	Urinary retention and need of bladder catheterization		P-value
	Group E	Group F	
	n=30	n=30	
0 hour	28 (93.3%)	26 (86.7%)	0.38
1 hour	18 (60%)	17 (56.7%)	0.79
12 hours	17 (56.7%)	8 (26.7%)	0.018*
24 Hours (Final outcome)	14 (46.7%)	2 (6.7%)	0.0005*

Chi-square test for proportion difference at each point time.

Table III: Comparison of PONV between groups.

Time	Nausea and vomiting scale	Group E n=30	Group F n=30	P-values
Baseline	Zero - none	18 (60%)	30 (100%)	
	1 - mild	7 (23.3%)	0 (0%)	
	2- moderate	5 (16.7%)	0 (0%)	0.001
1 hour	Zero - none	21 (70%)	18 (60%)	
	1 - mild	9 (30%)	7 (23.3%)	0.065
	2- moderate	0 (0%)	5 (16.7%)	
2 hours	Zero - none	22 (73.3%)	18 (60%)	
	1 - mild	6 (20%)	10 (33.3%)	0.49
	2- moderate	2 (6.7%)	2 (6.7%)	
24 hours	Zero - none	18 (60%)	30 (100%)	0.001
	1 - mild	10 (33.3)	0 (0%)	
	2- moderate	2 (6.7%)	0 (0%)	
48 hours	Zero - none	24 (80%)	30 (100%)	
	1 - mild	6 (20%)	0 (0%)	0.024
	2- moderate	0 (0%)	0 (0%)	

Chi-Square and Fisher exact test applied.

significantly low in group F than group E [25.73 ±4.18 hours vs. 38.17 ±6.53 hours; p=0.0005] while mean time of the first solid intake was not significant between groups [Group E vs. F: 9.23 ±2.92 hours vs. 8.57 ±1.94; p=0.302]. Regarding the satisfaction and recommendation of methods of pain relief, 83.3% (25/30) and 86.7% (26/30) patients were satisfied and 83.3% (25/30) and 93.3% (28/30) responded to recommend the same method of pain relief to their family and friends in group E and F, respectively. There were no significant differences between groups in rate of satisfaction and recommendation (p=0.718 and p=0.228 respectively).

DISCUSSION

Postoperative urinary retention remains a challenge and a topic of interest for arthroplasty, contributing to significant morbidity and can be the cause of prosthetic infection and sepsis as a consequence of hematogenic spread of infection secondary to bladder catheterization. The sequelae of postoperative urinary retention hinder mobilization and thus can increase the length of hospital stay. Considerable work has been done to estimate the incidence of postoperative urinary retention in joint arthroplasties. However, the available data regarding the incidence of postoperative urinary retention varies because of differences in the study designs and different criteria used to detect postoperative urinary retention as shown by Bjerregaard *et al*.[11] The findings of this study are consistent with a higher frequency of postoperative urinary retention at 24 hours in patients receiving epidural analgesia (group E) when compared with femoral nerve block (group F). These results are consistent with the findings of a meta-analysis by Fowler *et al*.[12] It showed a reduction in postoperative urinary retention with the use of peripheral nerve blocks. Similar results were shown in systemic reviews by Choi *et al*.[13] and Cook *et al*.[14] Balderi and Carli also reported comparable incidence of postoperative urinary retention in patients receiving peripheral nerve block in lower limb arthroplasty.[15] In their retrospective review of 125 patients, Lingaraj *et al*. showed that use of postoperative epidural analgesia is associated with significant post-operative urinary retention.[3] The study by McQueen *et al*. revealed a significantly increased incidence of urinary retention when comparing epidural with non-epidural anaesthesia.[16] Singelyn *et al*. demonstrated that continuous femoral nerve sheath block has favorable side effect profile in comparison to epidural analgesia and intravenous patient controlled analgesia with morphine.[17]

As stated earlier, literature reports a variable incidence of postoperative urinary retention owing to the difference in the definitions of outcome, i.e. postoperative urinary retention and also there is variance in study designs. Cochrane review by Chan *et al*. showed that there is no significant difference in the risk of urinary retention when comparing femoral nerve block with epidural, while looking at final outcome at 72 hours.[18] Chu *et al*. and Izard *et al*. did not show any difference between regional and general anaesthesia when comparing urinary retention.[19,20] In a retrospective review, Kumar *et al*. showed increased postoperative urinary retention associated with postoperative morphine consumption.[1] Chan *et al*. reported no significant difference in sedation scores, knee flexion at 24 hours, and time to first mobilize out of bed.[18] They showed a reduced risk of nausea/vomiting and a higher patient satisfaction with femoral nerve block when compared to epidural analgesia. However, this Cochrane review included only 10 papers out of 45, comparing epidural with femoral nerve block. It also concluded that continuous femoral nerve block is better than single-shot method.

The present study results are comparable to many of the previous studies; however, there are certain limitations to the generalization of results as the present study mainly focused on unilateral TKA. This study excluded pre-existing bladder outflow obstruction in patients that can confound the results and can be addressed using the International Prostate Symptom Score (IPSS) including patients with pre-existing bladder outflow obstruction in future carefully designed studies. The format of this study did not make it feasible to blinding, which confers a significant risk of bias. A study design that eliminates the risk of bias needs to be considered before the results can be considered generally applicable. Studies can be designed in the future to encompass a greater population of patients with varied procedures feasible for consideration of epidural analgesia vs. femoral nerve block that will better establish the findings of this study.

CONCLUSION

Single-shot femoral nerve block offers a more favorable profile in terms of postoperative urinary retention

avoiding potential complications of urinary sepsis and prosthetic infection, thereby facilitating earlier mobilization and shorter in-hospital stay when compared to epidural analgesia. A recommendation for the change in analgesic modality can be considered after addressing the potential confounders and designing studies that establish these findings more strongly.

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ORIGINAL ARTICLE

Comparison of Retrograde, Primary and Secondary Bonding Materials with Tooth Substance

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ABSTRACT

Objective: To compare the microleakage of MTA (mineral trioxide aggregate) and resin-modified GIC (glass ionomer vitremer) as retrograde endodontic material.

Study Design: Experimental study.

Place and Duration of Study: Operative Department of DIIKIOHS (DUHS) and NED University, Karachi, from February to June 2014.

Methodology: Forty human anterior teeth were divided into four groups. Each tooth was endodontically treated. Apical cavity preparations were performed on all teeth. The retropreparations were filled either with MTA (Group 1), Vitremer (Group 2), or only covered with nail polish (Group 3). The root surfaces of the first three groups were coated with nail polish. In Group 4 (positive control), neither retrograde filling was placed nor the nail polish was applied. The teeth were then suspended in 2% methylene blue dye solution for 10 days at 37°C. Sections were made along the long axis of teeth to determine the depth of linear dye penetration. Using Mann-Whitney test the comparison, p-value $\leq$0.05 was considered as statistically significant.

Results: MTA had no significant difference in apical dye leakage (p = 0.122) than did the vitremer.

Conclusion: MTA is equivalent to vitremer in preventing microleakage when used as retrograde filling material.

Key Words: *Leakage. Mineral trioxide aggregate (MTA). Vitremer. Retrograde filling.*

INTRODUCTION

Surgical endodontic treatment comprises of several steps including apical curettage, apicoectomy and retrograde filling.[1] A successful apicoectomy is the one which achieves complete sealing of the apical area through retrograde filling.[2] A perfect seal is needed in order to avoid bacterial spread and contamination from root canal to periapical area and vice versa.[3]

Many root-end fillings have promised to give an appropriate sealing to apical leakage.[4] The most common are glass ionomer cement, amalgam, gutta percha, zinc oxide eugenol cement, and composite resins.[5] Recent studies have revealed that mineral trioxide aggregate (MTA) are more compatible substance for retrograde fillings.[6,7]

Testing has been done to asses leakage, marginal adaptation and cytotoxicity in many retrograde filling materials.[1] Leakage assessment can be performed by using protein leakage, dye, bacterial leakage methods, fluid filtration, or by scanning electron microscopy (SEM).[8]

A number of studies have been performed on different materials when used as a root-end filling materials using dye leakage model.[9-11] There are a number of dyes that can be used to assess sealing ability of retro filling materials, including, Fuchsin, Methylene blue, Silver nitrate, Rhodamine B, Pelikan ink and India ink.[12]

In this context, this study was conducted to compare the sealing ability of MTA, and resin-modified glass ionomer (Vitremer) as retrograde filling materials using dye penetration technique. The null hypothesis tested was that there would be no difference between the sealing ability of the two materials.

METHODOLOGY

Forty human non-carious single rooted anterior teeth were extracted for periodontal reasons. The samples were randomly selected for *in vitro*, experimental design study conducted at the Operative Department of DIIKIOHS (DUHS) and NED University from February to June 2014. Teeth with enamel cracks or fractures, malformations, developmental defects, restorations or erosions, attritions, incomplete apices and having more than one apical foramen were excluded.

Teeth were cleaned with a dental prophylactic cup having water/pumice slurry and then were kept in distilled water at 4°C in a refrigerator. General steps of chamber opening were performed with small round bur

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(Mani, Japan) stainless steel file (Mani, Japan) of 15 number k-type with 25 mm length was placed into each canal in such a way that file tip could be visualized at the foramen. Teeth were prepared and shaped by using step down technique having MAF of a size 35. Thorough irrigation was performed with sodium hypochloride 1% along with 5ml of sterile distilled water on the completion of instrumentation.

After final irrigation, all canals were dried with absorbent paper point and then obturated with gutta percha and sealapex using finger spreader and lateral compaction technique. The access cavities were closed with Cavit. The teeth were stored in a humid environment by using moist cotton in artificial saliva, during all procedures.

An apical resection was made at 90°C to the long axis of tooth with a 701 taper fissure bur at 3-mm from the apical end.

Retrograde preparations were made using small round bur (Mani, Japan) to a depth of 3mm.

The prepared teeth were then randomly divided into two experimental groups having 15 teeth each, and two control groups having 5 teeth each.

For group 1 (MTA), retrograde filling with ProRoot MTA was mixed with sterile distilled water according to manufacturer's instructions, poured in syringe of vitremer system, and injected into the retrograde preparation. Excess materials were removed and stored in 100% humidity at 37°C.

In group 2, resin-modified GIC, root end filling vitremer, was used according to the manufacturer's directions. It was poured in the syringe of vitremer system, injected in to the retrograde preparation with removal excess materials, light cured, and stored in 100% humidity at 37°C.

Teeth were radiographed to evaluate root and filling of both experimental groups.

Group 3 was the negative control. The retrograde preparations and exposed gutta percha were left untreated but were covered with nail polish to prevent dye penetration. Group 4 was the positive controls. The apical cavity preparations neither had a retrograde filling placed nor a nail polish applied in order to verify the efficacy of the methylene blue dye penetration.

All the preparations and the retrograde filling were performed by the same operator. In the experimental group, double coats of nail polish were applied to all aspects of the roots except the apical end. This was done to seal the roots and avoid the penetration of dye through surfaces other than apex. All the groups' specimens were marked according to their specific group in the container of methylene blue for differentiation purpose. The half of all the specimens was vertically suspended in 2% methylene blue solution. Teeth were place in incubator at 37°C for 10 days. Following that time period, teeth were removed from the dye and rinsed for 15 minutes under running water.

Self-curing resin was used to embed the teeth. Diamond cutting saw (EQ MT 4, MTI Cooperation, USA) (blade of 0.5mm thickness) was used to sectioned teeth in a labio-lingual direction. In this way, two longitudinal halves were produced, but only the half with the better-cut surface was examined. The sample slides were polished and were then inspected under a stereomicroscope (Motic, Honkong) having magnification of 15X. Leakage was then evaluated to determine the maximum linear depth of the dye penetration, dye penetration in each section using a standard score (ISO/TS 11405-2003 Rev 2015).[13] Dye penetration was scored as score 0-no dye penetration; score 1-dye penetration; score 2 - dye penetration from 0.5 to1 mm; score 3 - dye penetration from 1 to 2 mm; score 4 - dye penetration $\leq$2 mm and score 5 - total dye penetration or through-and-through.

Descriptive statistics that involved median and inter-quartile range were reported for the scores of dye penetration. Mann-Whitney test was used to compare the results of two groups. A p-value $\leq$0.05 was considered as statistically significant. Statistical Package for Social Sciences (SPSS) software version 21 was used to analyze the data.

RESULTS

The negative controls, group 3, with nail polish and no root-end filling material, demonstrated no dye penetration. The positive controls, group 4, with no nail polish and no root-end filling, showed leakage of the methylene blue throughout the entire length of all retro-perparations extending into the canal obturated with gutta-percha and sealer (Table I).

Using Mann-Whitney test, there was no significant difference in the median depth of dye penetration between the MTA and the vitremer specimens. The MTA specimens had no significant difference in apical dye leakage (p-value = 0.122) with the vitremer group.

Table I: .

Group	Retrograde filling	T. No.	No. of teeth with leakage score						Leakage %
			00	1	2	3	4	5	
1	MTA	15	15	0	0	0	0	0	(0/15)*100 = 0
2	Vitremer	13	11	2	0	0	0	0	(2/13)*100 = 15.4
3	Negative control	05	05	0	0	0	0	0	(0/5)*100 = 0
4	Postive control	05	0	0	0	0	0	05	(5/5)*100 = 100

DISCUSSION

MTA is used as standard material for retrograde fillings.[6] From many studies, it is to be noted that MTA exhibits significantly lesser leakage than other materials like Amalgam, IRM.[6,7] In the present study, MTA was used to compare with RMGIC and both of them showed successfully same adherent and adaptability in terms of leakage.

Marginal leakage is the main dilemma of any dental restoration.[7] The perfect seal of a root-end filling material depends on the aspect of its adaptability.[14] For optimum adaptability, the material must have some kind of bond with tooth substance.[15] This bonding can be achieved either by chemical or mechanical means.[16] Glass ionomer cements (GIC) was invented in 1969 by Wilson and Kent.[17] Since then, it has been considered as one of the most adherent substances to the tooth structure forming chemical bond.[18] In the present study, resin modified glass ionomer cement (RMGIC) was used that not only formed chemical bond with tooth substance through its GIC component, but also contained resin part which is used to adapt to the tooth material by micro-mechanical tags formation.

Many comparative studies are made to understand if there is any statical difference among MTA, composite and amalgam, when used as retrograde filling material by using different methods of bacterial leakage.[9,19] No significant difference has been observed between MTA and amalgam as retrograde filling materials in a investigation having dye leakage model.[9] Similarly, scanned electron microscope has also been used to understand marginal gap in between material and tooth interface.[20] But dye penetration method is best known as more economical and convenient method to study microleakge.[21] In the present study, dye penetration method was used which proved to be economical, convenient and appropriate to estimate linear leakage by methylene blue dye.

The present study used to compare the sealing ability of two retrograde filling materials (MTA and RMGIC). However, the present study did not reveal any significant difference in the sealing ability of the two retrograde filling materials used. Therefore, RMGIC can be a more economical substitute for MTA in endodontic surgical application. However, further studies are required for comparing materials and techniques in order to create standard protocols for endodontic surgery.

CONCLUSION

MTA is equivalent to vitremer in preventing microleakage when used as retrograde filling material.

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Efficacy of Intense Pulse Light Therapy and Tripple Combination Cream Versus Intense Pulse Light Therapy and Tripple Combination Cream Alone in Epidermal Melasma Treatment

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Abstract

Objective: To compare the efficacy of intense pulse light therapy (IPL) and triple combination cream (TCC) versus intense pulse light therapy and triple combination cream alone in epidermal melasma treatment, downgrading MASI score to more than 10.

Study Design: Randomized controlled trial.

Place and Duration of Study: Dermatology Department, Lady Reading Hospital, Peshawar, from August 2014 to January 2015.

Methodology: Patients of 18-45 years were included in the study with Fitzpatrick skin type II-V. Sample of 96 patients was divided in to three groups of 32 each, through consecutive (non-probability) sampling method. Detailed history was taken, Woods Lamp Examination done, and melasma area and severity index (MASI) score was calculated. TCC had to be applied daily at night for two months by group A patients while group B was consigned for IPL therapy fortnightly, and those in group C were given both for two months. Efficacy was compared by recalculating MASI score at treatment end as well as at follow-up after 4 weeks, using Chi-square test with significance at $p < 0.05$.

Results: Male and female patients were 10 (31.2%) and 22 (68.8%) in group A, 7 (21.9%) and 25 (78.1%) in group B, while in group C were 12 (37.5%) and 20 (62.5%). The average age was 28.70 $\pm$8.70 years. MASI score reduction was achieved in 22 (68.8%) patients in group A; whereas, in 20 (62.5%) and 30(93.8%) patients in group B and C, respectively. Efficacy-wise distribution was significant (p=0.009).

Conclusion: Intense pulse light therapy and triple combination cream are more efficacious in epidermal melasma treatment than intense pulse light therapy and triple combination cream alone.

Key Words: *Intense pulse light. Triple combination cream. Epidermal melasma. Efficacy. MASI score. Melasma area and severity index.*

INTRODUCTION

Melasma, an aberrant and anomalous hyperpigmentation of face, is more common in women. It especially affects Asian and Hispanic natives as they abide in exquisite ultraviolet radiation sphere and having higher skin types (Fitzpatrick III, IV and V).[1-3] Major determinants of melasma are thyroid dysfunction, solar exposure, gestation, cosmetics and medicines, like oral contraceptives, phototoxic and antiseizures.[3-5]

Wide range of treatment is available with variable response. Existing options include sunscreens, topical steroids, retinoid, hydroquinone (bleaching agent), glycolic acid (peeling agent), Kojic acid, Azelic acid, physical modalities such as LASERS / intense pulse light therapy (IPL) and combination of above therapies.[6]

Topical therapy with triple combination cream (TCC) that is composed of hydroquinone 4%, tretinoin 0.05% and flucinolone acetonide 0.01%, has been established as the most yielding in melasma patients followed by glycolic acid peel and LASERS, like Q-switched or non-ablative fractional and IPL therapy. Spurting treatment options are tranexamic acid and glutathione.[7-10]

Hydroquinone arrests synthesis of melanin by inhibiting the enzyme tyrosinase, while tretinoin is a topical retinoid that stimulates keratinocytes turnover, decreases melanosomes transfer, inhibit tyrosinase transcription, prevents hydroquinone oxidation and allow its better penetration. Topical steroid inhibits synthesis of melanin by decreasing cellular metabolism along with diminishing irritability evoked by tretinoin and hydroquinone. IPL therapy is privileged to be reliable and competent in pigmentary lesions treatment, where thermal heating results in rapid differentiation of keratinocytes, leading to elimination of melanosomes with dead keratinocytes. Microcrusts are then removed from skin surface.[7]

Melanogenesis being a multi-stage and complex process, requires different agents to work at different steps of melanin formation, thus favoring combination of agents to perform in synergism to yield a superior therapeutic outcome.[11,12]

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The main objective of this study was to validate the effectiveness of intense pulse light therapy (IPLT) and TCC versus intense pulse light therapy and triple combination cream individually in patients with epidermal melasma.

METHODOLOGY

This randomized controlled trial was carried out at the Department of Dermatology, Lady Reading Hospital Peshawar, during August 2014 to January 2015.

A sample of 96 patients, divided by lottery method into three groups of 32 each, was figured out utilizing WHO software for estimation of sample size keeping power of test to be 90% and 5% level of significance. The efficacy in IPL group was taken as 77%[5] and in combination group of intense pulse light therapy and triple combination cream, was taken as 57%.[8] Age range of 18-45 years, Melasma duration of >3 months, having epidermal melasma, MASI score of $\geq$12 and skin types, Fitzpatrick II, III, IV, V were the benchmarks of study inclusion for patients incorporated through OPD. Patients were examined to exclude those having Fitzpatrick I or VI skin types and mixed or dermal melasma identified with the help of Woods Lamp. Those with recurrent herpes simplex infection history or received treatment of melasma in the preceeding three months were also excluded.

Hospital's Ethical and Research Committee granted approval to the study. It was ushered after revealing to patients its intents and favors; and attaining written informed consent. Patients' assortment in three groups was carried out after embarking history plus examination. TCC was applied at night daily for two months by group A, while group B had to undergo IPL therapy of four fortnightly sessions; and those in group C were given both. All patients were advised sunblock during treatment period. Filter of 560nm was used for IPL therapy, with double-pulses of 3.0 to 3.5ms, and a delay time of 20 or 30 for Fitzpatrick skin type II/III and IV/V, respectively. Fluence range was 14 - 18 J/cm^2, so that visible deepening of melanosis occurs. A fluence of 10% higher than the first session was employed for the next three IPL treatments. Patients were guided to discontinue TCC application 1 day before and on the day of IPL treatment. MASI scoring was done again to determine the efficacy in terms of improvement in minimum score of 10 in MASI at four weeks follow-up.

The observations, MASI assessments and IPLT were conducted under supervision of a single expert dermatologist.

A proforma was filled to register each patient's data. Paradigm of excluding and including patients was ensured.

Version 16 of SPSS was used to interpret data. Categorical variables were analyzed in terms of frequencies and percentages, whereas numerical variables were assayed as Mean ± SD. Comparison of efficacy in three groups was made employing Chi-square test, considering p-value $\leq$0.05 to be statistically significant. Efficacy in three groups was stratified among age, gender, Fitzpatrick skin type, duration of symptoms, and baseline MASI score. Results were presented in the form of tables.

RESULTS

Gender-wise distribution showed that there was female preponderance in all three groups. Male to female ratio was 0.43:1 (Table I).

Average age was 28.70 $\pm$8.70 years with range of 18-45 years (Table II).

Table I: Gender-wise comparison of the groups.

Groups	Gender		Total	p-value
	Male	Female		
A	10 (31.2%)	22 (68.8%)	32 (100.0%)	
B	7 (21.9%)	25 (78.1%)	32 (100.0%)	0.391
C	12 (37.5%)	20 (62.5%)	32 (100.0%)	
Total	29 (30.2%)	67 (69.8%)	96 (100.0%)	

Table II: Age-wise distribution in the groups.

Age (in years)	Groups			Total	p-value
	A	B	C		
$\leq$ 25.00	12 (37.5%)	8 (25.0%)	13 (40.6%)	33 (34.4%)	
26.00 - 40.00	17 (53.1%)	19 (59.4%)	17 (53.1%)	53 (55.2%)	0.588
41.00+	3 (9.4%)	5 (15.6%)	2 (6.2%)	10 (10.4%)	
Total	32 (100.0%)	32 (100.0%)	32 (100.0%)	96 (100.0%)	

Table III: Efficacy-wise distribution of patients in the groups.

Groups	Efficacy		Total	p-value
	Yes	No		
A	22 (68.8%)	10 (31.2%)	32 (100.0%)	0.009
B	20 (62.5%)	12 (37.5%)	32 (100.0%)	
C	30 (93.8%)	2 (6.2%)	32 (100.0%)	
Total	72 (75.0%)	24 (25.0%)	96 (100.0%)	

Table IV: Stratification of Fitzpatrick skin type, duration of symptoms and baseline MASI score over efficacy.

	Efficacy				p-value
	Yes		No		
	Count	%	Count	%	
Baseline MASI Score					
12- 18	53	74.6%	18	25.4%	0.893
19 +	19	76.0%	6	24.0%	
Duration of symptoms (in years)					
$\leq$5.00	7	100.0%	0	.0%	0.113
6.00+	65	73.0%	24	27.0%	
Fitzpatrick skin type					
II	21	80.8%	5	19.2%	
III	12	60.0%	8	40.0%	0.236
IV	18	85.7%	3	14.3%	
V	21	72.4%	8	27.6%	

Efficacy-wise distribution was significant with combination regimen as compared to either of them alone, with p-value = 0.009, using Chi-square test. This signifies that combination therapy has greater efficacy than the other two groups (Table III).

The most common Fitzpatrick skin type was type V followed by type II, IV and III with positive results. Baseline MASI score was in range of 12-18 in majority of patients and above 19 in few, in whom therapy was effective. Duration of melasma was more than 6 years in most of the patients (Table IV).

DISCUSSION

Melasma is a prevalent hypermelanosis, well known for treatment resistance and relapses on treatment discontinuation. There is not a single satisfactory treatment modality to date. Hence, every management protocol should be tailor-made. The consensus in international literature for melasma treatment is on using combinations of therapeutic agents that act in synergism to target multiple steps in melanogenesis for achieving maximum results.[11,12] The result of this study is in accordance with that; and has shown that the most effective treatment of melasma is combination of TCC and IPL. Goldman, *et al*. showed comparable outcome that sequential treatment with TCC and IPL is more effective in moderate to severe melasma followed by consecutive treatment with an inactive (control) cream and IPL.[13] The above mentioned study inducted 56 patients. IPL therapy was carried out for all patients at week 2 and 6, and IGA scale was applied to conclude that in TCC group, 57% of patients; while in inactive cream group, 23% were ameliorated. The success of TCC and IPL in combination in this study was in 93.8% of patients that can be rationalized due to increased number of IPL sessions that is fortnightly for two months, although the IPL parameters used were the same in both studies. There was also difference in assessment scales which was MASI in this study and IGA in referenced work. In addition, they have not stratified melasma in terms of epidermal, dermal, or mixed. While the included patients in this study had epidermal melasma, that is less treatment resistant.

Kligman and Willis first proclaimed propitious outcome of TCC.[14] Since plenty of studies have delineated reassuring endpoints. A multi-center trial was ordained on 260 South East Asians in 9 centers by Chan, in which TCC or 4% hydroquinone cream was used for 8 weeks. Employing static global assessment score, it was concluded that 50% in hydroquinone batch while 71% in TCC circle were contented with results (p=0.005).[15] Furthermore, in study by Taylor SC, 77% were disclosed to be clear of melasma at 8 weeks, using TCC.[16]

In this study, 68.8% patients in TCC group showed significant reduction in MASI score, which is compatible with the above mentioned work. This can be explained on the basis of fact that the concentration of components of combination cream method and duration of use were alike. Furthermore, an extension of 8 weeks trial using TCC conducted by Torok for a 12-month period revealed melasma clearance with 90% (N=327) rate, emphasizing the productiveness of TCC.[17]

Convincing evidence regarding IPL adequacy in melasma treatment has been revealed in clinical studies.[18] It was proclaimed in one of these that with IPL therapy, 77% of 89 Chinese patients had 50% betterment in melasma.[4] Another study noted 47% of patients having excellent response, while 29% were having good response out of 38 patients after therapy with IPL.[19]

In this study, 62.5% patients in IPL alone group showed significant improvement that was the least effectiveness as compared to the other two groups though analogous with the literature.

As far as demographic features are concerned, this study asserted that maximum patients were in age range of 26-40 years out of 18-45 years range of enrolled patients, with mean age of 28.56 years. These findings were comparable with the study of Silonie, *et al*.[20] and Achar that showed mean age of 27.5 and 33.45 years respectively.[21]

In this study, melasma was found to be commoner in females (69.8%) as compared to males (30.2%). Same results were manifested by most of the studies that showed male to female ratio of 1:3,[22] and a male to female ratio of 1:9.[23]

Age and gender distributions of melasma were similar in this study groups and in the referred studies. This can be explained on the basis of the fact that OCP consumption and pregnancy is most common in younger females, so both of them might have been the causative or aggravating factors for melasma in this section of sample population. In this study the common Fitzpatrick skin types were type II and V, followed by types III and IV. This was unlike the study of Safora *et al*. conducted at Mayo Hospital, Lahore,[24] Pakistan and Alka *et al*.[23] from Ludhiana, India that showed skin types IV and V to be 50% each. These differences are due to geographical variations in skin type in different ethnic populations. So it was observed that people of Peshawar, Khyber Pakhtunkhwa, were mostly of relatively lower or fairer skin types while patients from Punjab and Ludhiana are of higher skin types.

CONCLUSION

It is concluded, on the basis of results, that intense pulse light therapy and triple combination cream are more efficacious than intense pulse light therapy and triple combination cream alone in epidermal melasma treatment.

Disclosure: This study was part of a postgraduate dissertation.

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Non-Vascularized Autogenous Bone Grafts for Reconstruction of Maxillofacial Osseous Defects

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ABSTRACT

Objective: To determine the outcomes of non-vascularized bone grafts for reconstruction of maxillofacial defects.
Study Design: Case series.
Place and Duration of Study: Department of Oral and Maxillofacial Surgery, Armed Forces Institute of Dentistry, Rawalpindi, from January 2013 to December 2015.
Methodology: Descriptive analyses of 30 patients, who underwent maxillofacial reconstruction with non-vascularized bone grafts, were conducted. The demographic information, diagnosis, and type of graft harvested to reconstruct the defect were statistically analyzed. Outcomes of reconstruction with non-vascularized bone grafts were analyzed in terms of mouth opening, success of dental rehabilitation, and postoperative complications, i.e. surgical site infection and hardware loosening.
Results: A total of 30 patients ranging from 8 to 60 years (33.57 ±14.74 years) had maxillofacial defects reconstructed mostly due to gunshot injuries, followed by post-resection defects. Overall 15 cases (50%) were reconstructed with iliac crest cortico-cancellous bone graft, 11 cases (36.7%) with rib; while in four cases (13.3%), costochondral graft was used for reconstruction. In 26 cases (86.7%), graft was found to be successful. In three cases, re-operation for onlay bone graft was required to provide optimal dental rehabilitation; while in just one case, postoperative surgical site infection was observed.
Conclusion: Non-vascularized bone grafts provide a reasonable and effective modality for reconstruction of maxillofacial defects.

Key Words: *Maxillofacial osseous defect. Maxillofacial reconstruction. Non-vascularized bone grafts. Autogenous grafts. Guided bone regeneration. Maxillomandibular fixation.*

INTRODUCTION

There are various etiological factors for osseous defects in the maxillofacial region. The common causes are severe trauma, oncologic ablative surgery, large cystic lesions, osteoradionecrosis, and congenital defects like orofacial clefts.[1] Various injuries such as motor vehicle accidents, firearms, interpersonal assaults, burns, electrical flashes play their part in damaging the whole body in general and the maxillofacial region in particular.[2] Among these causes, gunshot and blast injuries present a major challenge to the reconstructive surgeon because of their extensive damage to both hard and soft tissues.

Maxillofacial reconstruction is a complex procedure with many available options. Anatomical, esthetic, and functional aspects must be addressed during reconstruction of maxillofacial region. Numerous reconstructive modalities are available to surgeons in current era including microvascular free flaps, autogenous grafts, alloplastic implants, bridging plates, genetically engineered bone growth method, and distraction osteogenesis.[3] Goals of reconstruction are to bridge the defect and to restore patient's function and form.[4] Success of reconstruction depends on its adequate blood supply, soft tissue coverage,[5] immobilization of transplanted tissue and overall health, and the nutrition status of the patient.

The first use of non-vascularized bone grafts (free bone grafts) goes back to the start of twentieth century when Skyoff,[6] used horizontal part of contralateral mandible to bridge a defect. During World War I, German surgeons Klapp and Schro¨der used various free bone grafts to bridge mandibular defects.[7] Earlier, until the end of 1970s, wire osteosyntheses were employed for fixation of these bone grafts. But since the improvement in osteosyntheses techniques (with miniplates, reconstruction plates and screws), the success rate of free bone grafts in case of maxillofacial reconstruction has improved markedly.

Sources of non-vascularized free bone grafts include calvarium, rib, ilium, tibia, fibula, scapula, and radius. Their usefulness has, however, been limited by early bone resorption and infection.[8] Reconstruction with vascularized grafts is now the state-of-the-art modality

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for bone replacement in the maxillofacial region as they are more reliable and better in volume to allow the placement of dental implants. They are relatively resistant to radiation, infection, and bone resorption.[9] Their disadvantages include high cost, the need for specialized training and equipment as well as relatively more donor site morbidity.[10]

Although many contemporary surgeons advocate the use of vascularized free flaps for reconstruction of maxillofacial defects, the role of non-vascularized grafts for maxillofacial reconstruction cannot be over-emphasized. The rationale of this study was to highlight the utility of non-vascularized bone grafts for reconstruction of various maxillofacial osseous defects, particularly in centers where facilities of free flaps are not available.

The aim of this study was to assess the outcomes of various autogenous non-vascularized bone grafts used for reconstruction of maxillofacial bony defects.

METHODOLOGY

This prospective case series was carried out at Oral and Maxillofacial Surgery Department, Armed Forces Institute of Dentistry, Rawalpindi, Pakistan from January 2013 to December 2015. Maxillofacial osseous defects, which required bone grafts, continuity defects, and maxillofacial bony defects requiring vertical and/or horizontal augmentation were included. The exclusion criteria were extremely small defects (like defects of alveolar cleft and other minor defects like alveolar ridge atrophy that required bone grafting or guided bone regeneration), extremely large defects (i.e. hemimandibulectomy defects), and defects that required corticotomy or osteotomy of grafts for optimal reconstruction (i.e. continuity defects of anterior mandible and maxilla). Patients with comorbid conditions like diabetes, end stage renal or liver disease, on immunosuppressive therapy, radiotherapy, bisphosphonates, and alcoholics were also excluded because these conditions can negatively affect the outcome of reconstruction with non-vascularized bone grafts. The demographic information, diagnoses and type of graft harvested to reconstruct the maxillofacial defects were statistically analyzed. Outcomes of reconstruction with non-vascularized bone grafts were analyzed in terms of mouth opening, success of dental rehabilitation and postoperative complications, i.e. surgical site infection and hardware loosening.

Reconstructions of defects by various bone grafts were performed under general anesthesia. In all patients, preoperative antibiotic prophylaxis was achieved with a single dose of co-amoxiclav (1.2 g in adults and 600 mg in children). Antibiotics were continued for a period of 7 days postoperatively. Maxillomandibular fixation was done in all patients with mandibular reconstruction for a period up to eight weeks; while for costochondral grafts, it was done only for 10 to 14 days to encourage early active and passive mouth opening exercises. Nutrition was achieved by nasogastric tube for initial post-operative period (up to seven days) and later by oral liquid diet until maxillomandibular fixation was removed.

Postoperatively, the bone grafts were evaluated by clinical and radiographic examinations. Clinical examination was performed at weekly intervals in first month; and monthly for further five months to evaluate the functional outcomes and signs of any complication. Radiographic evaluation was done immediately after the procedure; and later after three and six months post-operatively by plain radiographs.

Outcomes of reconstruction were assessed based on following three parameters: mouth opening, success of dental rehabilitation in terms of patient satisfaction, and complication rate in terms of postoperative infection and graft loosening.

Mouth opening was assessed after six months of surgical procedure and interincisal distance of 30 mm or more was considered adequate.

Dental rehabilitation was performed after three months of bone grafting with removable partial dentures or implant supported removable or fixed prosthesis. Success of dental rehabilitation was assessed in terms of patient satisfaction by verbal response scale after three months of dental rehabilitation. In a scale of 1 to 10, the value of 5 or less was considered a failure (unsuccessful dental rehabilitation). Patient's complaints regarding dental prosthesis were addressed accordingly.

Postoperatively, clinical and radiographic examinations evaluated the bone grafts to look for signs of surgical site infection and graft loosening. Surgical site infection was labelled when there was cellulitis or abscess at surgical site, fever (more than 38 degrees Celsius), and tachycardia.

Data was analyzed on SPSS version 17 by calculating the descriptive variables, i.e. frequencies and percentages for categorical variables and mean ±SD for continuous variables.

RESULTS

A total of 30 patients ranging from 8 to 60 years (33.57 ±14.74 years) of age were treated during the study period. Out of these 30 patients, 24 (80%) were males and 6 (20%) were females.

Reconstructions of maxillofacial osseous defects were achieved by different non-vascularized bone grafts, i.e. autogenous rib, costochondral bone, or corticocancellous iliac crest bone graft. Gunshot wound defects (n=13, 43.3% cases) were the most common defects and mandible was involved in most of the cases (n=25, 83.3%). Out of total 13 gunshot wound defects, nine

Table I: Descriptive statistics showing relationship of diagnosis with site of defect and graft used for reconstruction.

Diagnosis (cause of defect)	Freq. (n=30)	Site of defect		Type of graft		
		Mandible	Midface	Rib	Iliac crest	Costochondral
Gunshot injury	13 (43.3%)	11 (84.6%)	2 (15.4%)	4 (30.8%)	9 (69.2%)	0
Pathological lesion	8 (26.7%)	8 (100%)	0	5 (62.5%)	3 (37.5%)	0
Blast injury	5 (16.7%)	2 (40%)	3 (60%)	2 (40%)	3 (60%)	0
TMJ ankylosis	4 (13.3%)	4 (100%)	0	0	0	4 (100%)
Total	30	25 (83.3%)	5 (16.7%)	11 (36.7%)	15 (50%)	4 (13.3%)

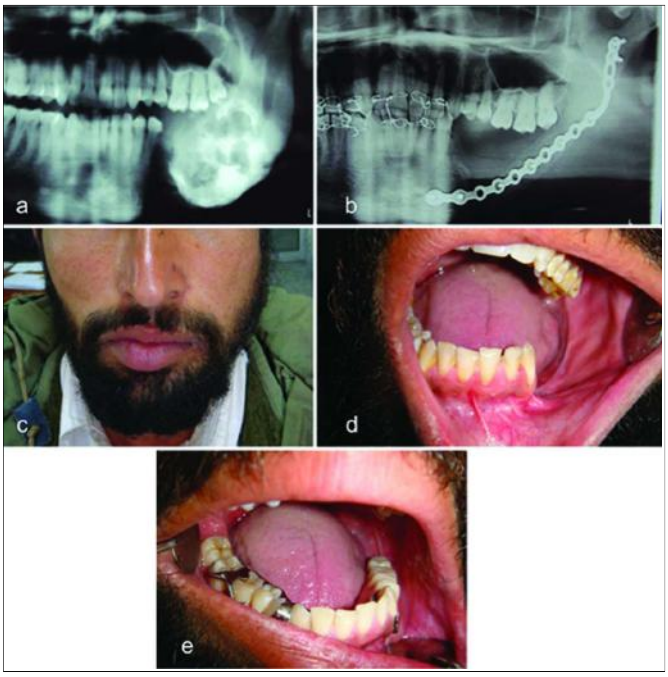

Figure 1: (a) Preoperative rediograph (b) Postoperative radiograph (c) Frontal view 6 months after surgery (d) Inraoral view 6 months after surgery (e) Intraoral photograph after dental rehabilitation.

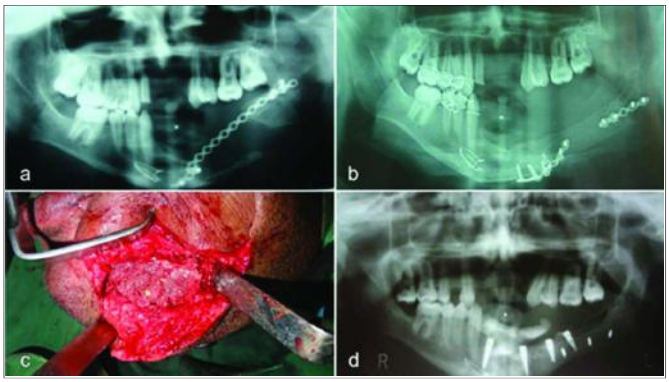

Figure 2: (a) Preoperative radiograph showing fractured miniplate across the continuity defect (b) Postoperaitve radiograph showing rib graft fixed with miniplates (c) Perioperative image of onlay iliac crest bone graft fixed with bicortical screws (d) Radiograph after implant placement for implant supported prosthesis.

were reconstructed with iliac crest cortico-cancellous bone, while other four (2 midface and 2 mandible) were reconstructed with rib bone graft. Post-resection defect due to odontogenic tumors (mostly ameloblastoma) was the second most common cause (26.7% cases) of continuity defects in this study and majority of these defects were reconstructed with free rib grafts. Five patients (n=5, 16.7%) in our study had blast injuries. Three victims of blast injury had orbital rim defects, among which, two had infraorbital rim defect and depression of zygomatic bone prominence, while one had supraorbital rim defect. There were four patients of temporomandibular joint (TMJ) ankylosis (n=4, 13.3%), which were operated for condylectomy followed by reconstruction with costochondral bone graft as all of them were below 12 years of age. Overall 11 cases (36.7%) were reconstructed by rib, 15 (50%) by iliac crest and 4 cases (13.3%) by costochondral graft (Table I).

Twenty-six (n=26, 86.6%) cases out of total 30 cases reconstructed with non-vascularized autogenous bone grafts were considered successful.

Three patients (n=3, 10%) were not satisfied with their removable dental prosthesis because of inadequate retention and stability. These patients were operated again to increase the horizontal dimension of bone with onlay bone grafts. Later, dental rehabilitation was performed with implant-supported prosthesis.

Postoperative surgical site infection was observed in just one case (3.4%), where reconstruction of supraorbital rim defect was carried out by iliac crest bone graft. Graft loosening and eventual failure of graft occurred in this particular case. The most probable cause of infection of graft was residual infectious focus in the orbit due to previous exenteration of orbit by oculoplastic surgeon. There was no other case of surgical site infection or graft/hardware loosening. No donor site morbidity was observed in any patient. All patients had adequate mouth opening (i.e. $\geq$30 mm), when evaluated 6 months after the reconstruction.

Outcomes of two typical cases are illustrated in Figures 1 and 2.

DISCUSSION

Reconstruction of orofacial defects poses a considerable challenge to oral and maxillofacial surgeons. The defects arising from oncologic ablative surgery, trauma, congenital anomalies and ballistic injuries have a significant impact on the patient's quality of life. Thus, the role of surgeon is to restore patient's functions and esthetics with minimum postoperative morbidity.[11]

In our experience, free (non-vascularized) bone graft is a useful reconstructive option. Prerequisites of the success of non-vascularized bone grafts are: healthy

recipient bed-free of infection, adequate soft tissue coverage of bone grafts, and adequate fixation of bone grafts. Free bone grafts are mainly beneficial in small structural defects[12-14] (5 cm to less than 9 cm in size) having well-perfused non-irradiated recipient sites. We emphasize adequate soft tissue coverage of the recipient site. Majority of cases with larger defects were addressed by extraoral approach without breaching oral mucosa in order to avoid infection. Literature also supports non-vascularized bone grafts, provided there is not much of soft tissue loss or where 2-layer watertight closure can be achieved intraorally and extraorally.[15] Rigid fixation and immobilization for a period of up to eight weeks, in case of mandibular reconstruction, was performed in this study. Firm fixation of graft to recipient site is essential for successful revascularization of graft as movement prevents vascular ingrowth in grafted bone.[16,17] In this study, the authors employed extraoral approach for reconstruction and used miniplates, reconstruction plates or bicortical screws to ensure stable fixation of bone grafts.

High cancellous content of iliac crest makes it suitable for early postoperative dental implant placement. Rib graft, on the other hand, is a good option for facial esthetics and contour restoration. It has low cancellous content and lesser thickness making it less reliable for dental implant placement but it provides adequate length of bone that can bridge large defects like hemimandibulectomy defects. Therefore, in some of our cases where reconstruction was performed by autogenous rib, onlay bone grafts were used to enhance the width of mandible before implant placement. In literature, autogenous rib grafts have been used for the restoration of mandibular continuity defects in newborns and young children. According to Ecardt and collegues, follow-up visits on a yearly basis is useful for early orthodontic treatment in case of growth deficits.[18] Further, corrective surgery with either bone augmentation or osseous distraction osteogenesis can be carried out after cessation of the facial growth.[18]

Defect of the midface, with or without the orbital involvement, is another challenging task for the oral and maxillofacial surgeons. For large maxillary and midfacial defects, the use of the prosthetic obturator with or without split thickness skin grafting is used in our center which is in accordance with other studies.[12,19] Both Iliac crest and autogenous rib were used for midface reconstruction in our study. Zygomatic contour defects are the ones most difficult to treat due to varying contours and difficulty in placement and fixation of grafts. Reconstruction with iliac arrest results in adequate restoration of ridge form which later helps in dental prosthesis placement. On the other hand, although rib grafts provide enough length and height, but adequate width is achieved only in few cases, i.e. when the harvested ribs were of thicker dimensions. Therefore, a second surgery for onlay bone grafts is used to augment the width of arch, particularly when dental implants are placed for dental rehabilitation.

Gunshot wound defect reconstruction at our center is usually carried out in two stages. In first stage, site is made free of infection by removal of foreign body, devitalization of teeth and necessary debridement is carried out followed by reconstruction in second phase. Penetrating, perforating and avulsive gunshot wounds were found infected in most of the cases. Then during next four to eight weeks, residual mandibular segments are kept stabilized by maxillomandibular fixation to preserve occlusion, and to prevent unnecessary pull of muscles that might result in displacement of fracture segments. Finally, in second stage, definitive reconstruction with non-vascularized bone grafts is performed once complete eradication of infection is ensured.

CONCLUSION

Non-vascularized bone grafts can be used successfully for reconstruction of maxillofacial bony defects. Size of defect, soft tissue coverage, condition of recipient site, and adequate fixation at the time of graft placement must be considered in case of reconstruction with non-vascularized bone grafts. However, it is important for the surgeon to be mindful of his surgical skills, patient's overall disease state, patient's expectations, and available resources before opting for a particular modality for reconstruction.

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Evaluation of Nitrate Reductase Assay for Early Detection of Multi and Extensively Drug Resistance Tuberculosis in our Setup

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ABSTRACT

Objective: To evaluate the performance of nitrate reductase assay on smear positive pulmonary specimens for detection of multi and extensively drug resistant tuberculosis simultaneously.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi from June to December 2016.

Methodology: Smear positive pulmonary samples were processed both by nitrate reductase method on Lowenstein Jenson medium and also inoculated on gold standard Bactec MGIT 960 TB system. All the specimens were first digested and decontaminated according to standard protocol before inoculation.

Results: Out of total 76 samples, three did not give color and, therefore, were excluded from the final data analysis. Among the remaining 73 samples, mycobacterial index was: 28 specimens were having 1+ (1-9 bacilli/100 fields), 26 samples were 2+ (1-9 bacilli/ field), and 19 samples were having 3+ index (>9 bacilli/field). The respective sensitivity and specificity were 84% and 100% for isoniazid (INH); 82% and 100% for rifampin (RIF); 67% and 100% for amikacin (AK); and both 100% for ofloxacin (OFX). Overall agreement in case of INH, RIF, AK, and OFX was 94.5%, 97.2%, 98.6% and100%, respectively. Overall average agreement was 97.5%.

Conclusion: Nitrate reductase assay is a reliable, low cost and accurate method that can be used for early for diagnosis of multi and extensively drug resistant tuberculosis.

Key Words: *BACTEC. Culture. Mycobacterium tuberculosis complex. Nitrate reductase assay.*

INTRODUCTION

Tuberculosis (TB) is a global health care concern that remains endemic in Pakistan. As per the statistics of World Health Organization (WHO), there were 10.4 million incident cases of TB. Pakistan is among the five countries that account for 60% of the new cases.[1] The increasing occurrence of multi drug resistant (MDR) and extensively drug resistant (XDR) isolates of TB have further contributed to the gravity of this deadly disease and stressed the significance of early identification and therapy.

In recent years, fully automated instruments, such as mycobacteria growth indicator tube (MGIT) 960 has substantially reduced the time for diagnosis and drug susceptibility testing (DST), and is considered as gold standard in many parts of the world. However, this system, being expensive, is difficult to be used as a reference method in resource-limited settings in developing countries. Conventional methods like indirect susceptibility testing on Lowenstein Jensen (LJ) and Middle Brook medium are slow and time consuming.

Therefore, the development of a rapid, reliable, inexpensive, and easy to perform modality is needed especially in developing countries. NRA, a colorimetric phenotypic method, has gained particular interest in the recent years with promising results.[2-6] *Mycobacterium tuberculosis* (MTB) has the ability to reduce nitrate into nitrite and this property is exploited in this technique. If *Mycobacteria* are present in the medium containing nitrate source, it will reduce nitrate into nitrite which can be dectected by adding a coloring reagent in the medium. Initially, this principle was used only to detect MTB in the medium; and later on, it was also utilized for drug susceptibility testing. Studies have shown that NRA gives rapid result as compared to conventional LJ medium for diagnosis and susceptibility testing of MTB.[3,4]

The objective of this study was to see the performance of NRA on LJ medium on smear positive sputum samples for susceptibility testing of two important first line anti-TB agents that is INH and RIF, and additional AK and OFX keeping BACTEC MGIT 960 system as gold standard.

METHODOLOGY

This cross-sectional analytical study was conducted in Microbiology Department, Armed Forces Institute of Pathology in collaboration with National University of

Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi.

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Medical Sciences, Pakistan. Permission from Institutional Ethical Committee was taken before the commencement of this study. A total of 73 specimens were dealt from June through December 2016. These 73 smear-positive specimens were treated as per the laboratory recommended protocols. All the samples were subjected to standard sodium hydroxide-N-acetyl-L-cysteine method for digestion and decontamination using 2% NaOH as final concentration.[7] After the digestion and decontamination, deposit was used to make a smear and stained by Ziehl-Neelsen (ZN) staining and subsequently marked according to the bacillary load index.[8] Inoculation into both media, that is NRA and MGIT 960 tube, was done from the remaining deposit which was mixed in 3 mL of sterile distilled water. We included all the smear positive specimens irrespective of their bacillary index. For quality control, *M. tuberculosis* H37Rv (ATCC 25177) was used as the susceptible control while an institutional reference MDR *M. tuberculosis* strain was used as resistant control.

This assay was performed on LJ medium as defined by Musa *et al*. with certain modifications in drugs' concentrations.[9] Two g KNO3 was mixed in 10 ml of sterile distilled water and thoroughly vortexed until dissolved. Five ml of stock solution was added to every 1000 ml of LJ medium and kept at 2-4°C for six months. A final concentration of 40 μg/ml was used for LJ medium containing rifampicin (RIF). For isoniazid (INH), amikacin (AK) and ofloxacin (OFX), critical concentrations of 0.2 μg/ml, 30 μg/ml and 2 μg/ml were used, respectively. The decontaminated specimen was diluted to 1:10 dilution in distilled water before NRA test. As per the protocol, the diluted suspension was used for inoculation of drug-free LJ bottles while undiluted/concentrated suspension was used for inoculation of drug containing LJ medium. 0.2 ml of the specimen was used for inoculation and all the bottles were incubated at 37°C.

The protocol for NRA assay was used as described previously by Angeby *et al*.[10] Twenty-five ml of 37% HCl was added into 25 ml of distilled water to make the stock solution for HCl, and 0.2 g of sulfanilamide was added in 100 ml of water to make the solution for sulfanilamide; 0.1 g of n-1-naphtylethylenediamine dihydrochloride was added in 100 ml of distilled water to make the stock solution. After completion of 14 days' incubation, reagent mixture (one part concentrated hydrochloric acid, two parts 0.2% sulfanilamide, and two parts 0.1% n-1-naphtylethylenediamine dihydrochloride) was prepared afresh and 0.5 ml of this mixture was added to one of the LJ bottle without drug. If any color developed, the mixture was added to all the corresponding drugs containing LJ bottles of that specimen. If no color appeared, that LJ bottle was discarded and remaining bottles were re-incubated. This step was repeated at incubation day-21 and finally at day-28. If the color change in the drug containing LJ bottle was equal or more than the growth control LJ bottle, the isolate was labelled as resistant to that agent. However, if no color appeared or the color is less in intensity as compared to growth control LJ bottle, the isolate was labelled as sensitive. The test was considered invalid if no color appeared in growth control LJ bottle at the end of test protocol that was incubation day-28.

Before incubation of MGIT 960 tubes in the system, growth enrichment supplement and antibiotics mixture named as MGIT PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) was added to all the MGIT 960 culture tubes. Half ml of the processed specimen was inoculated into each tube and was placed in the MGIT 960 instrument for incubation at 37°C. Once the system signaled a tube positive, it was removed from the system and then smear was made for confirmation of presence of AFB. After confirmation of AFB, susceptibility testing was setup according to the manufacturer's instructions. For each positive specimen, five tubes were prepared – one growth control and four tubes containing different drugs. The final drug concentrations for INH, RIF, AK and OFX were 0.4 μg/ml, 1.0 μg/ml, 1.0 μg/ml and 1.0 μg/ml, respectively.

Performance of NRA was determined by calculating sensitivity, specificity, positive predictive value, negative predictive value, and the total agreement on SPSS (Statistical Package for the Social Sciences) version 19. Sensitivity as the defined capability of a test to find true resistance in an isolate while specificity was the capability of a test to determine true sensitivity in an isolate. Average agreement was also calculated from all the agreements.

RESULTS

Three samples were invalid as they did not show any color at day 28 incubation. These specimens were however culture positive on MGIT and yielded growth of *M. tuberculosis*. Out of the 73 smear positive specimens, 28 samples were 1+ (1-9 bacilli/100 fields), 26 specimens were 2+ (1-9 bacilli/ field), and 19 specimens were 3+ (> 9 bacilli/field).

The sensitivity and specificity, and overall agreement is shown in Table I. Results were completely concordant in 66 out of 73 isolates. Overall average agreement was 97.5%. Out of the 73 specimens, 27 (37%) gave positive results on day 14, 43 (59%) specimens on day 21, and 3 (4.1%) specimens on day 28. Six isolates were MDR (resistant to INH and RIF), and one isolate was XDR (resistant to INH, RIF, OFX, AK). There were four isolates which were sensitive to INH by MGIT method but resistant by NRA method. Two isolates were sensitive to RIF by MGIT method but resistant by NRA. One isolate was resistant to AK by NRA method but was sensitive by MGIT. Out of the six MDR isolates, one

Table I: Comparison of results of MGIT 960 with results of sensitivity on LJ agar by NRA.

Drugs	MGIT	NRA %						
		R	S	Sensitivity	Specificity	PPV	NPV	Agreement
INH	R	21	0	84	100	100	92	94.5
	S	4	48					
RIF	R	9	0	82	100	100	97	97.2
	S	2	62					
AK	R	2	0	67	100	100	99	98.6
	S	1	70					
OFX	R	2	0	100	100	100	100	100
	S	0	71					

R = resistant; S = susceptible; PPV = positive predictive value; NPV = negative predictive value.

isolate was resistant to INH and RIF by NRA, but was susceptible to RIF by MGIT. By NRA method, 43 isolates were susceptible to all drugs, 16 isolates were only resistant to INH, one isolate was resistant to INH and OFX, one isolate was resistant to INH and AK, four isolates were resistant to RIF and one isolate was resistant to AK only. On MGIT 960 system, 49 isolates were sensitive to all the drugs, 13 were resistant to INH only, five were MDR, one was XDR, one was resistant to INH and OFX, two were resistant to RIF and one was resistant to INH and AK.

DISCUSSION

Drug resistant TB is becoming very common and for early initiation of therapy, early diagnosis and drug susceptibility testing is imperative. MDR TB may be defined as a strain that is resistant to two important first line agents that is RIF and INH, while XDR TB is defined as a strain that MDR plus having resistance to a fluoroquinolone and at least one injectable agent (AK, capreomycin, kanamycin).

The sensitivity and specificity of INH was found to be 84% and 100 % in our study, respectively. The sensitivity was slightly less as compared to other studies while specificity was comparable.[9,11-13] Kammoun *et al*. reported the sensitivity and specificity to be 92.6% and 98.2%, respectively.[11] Musa *et al*. reported the sensitivity and specificity to be 93% and 100%.[9] Solis *et al*. reported the sensitivity and specificity of INH to be 99.1% and 100%, respectively.[12] According to a systemic review and meta-analysis, the sensitivity of INH was found to be 96% and the specificity 99%.[13]

The sensitivity and specificity of RIF was 82% and 100%, according to our study. Sensitivity again, was found to be lower than in most other studies where sensitivities were mostly more than 90%.[9,12,13] However, our results were consistent with other studies.[14,15] These studies regarded NRA as a reliable method for the detection of MDR cases in smear positive specimens.

For OFX, sensitivity and specificity was both 100%. Results were comparable to several other studies. In a study conducted by Shrivastava *et al*., sensitivity and specificity was 100% and 96.3%, respectively. Huang *et al*. reported sensitivity and specificity of 97.4% and 100%, respectively.[16] Devasia *et al*. reported sensitivity and specificity to be 100% sensitivity and 98.7%, respectively.[17]

Sensitivity and specificity of AK in this study were 67% and 100%, respectively. This is the first reported study in which amikacin has been used in place of kanamycin. Huang *et al*. reported a sensitivity and specificity of 88.9% and 98.8% for kanamycin.[18] Martin *et al*. reported the accuracy of kanamycin to be 100%.[18] Ramos *et al*. reported a sensitivity and specificity of 94.6% and 99%, respectively.[19] In this study, sensitivity of amikacin was low. This might be due to less sample size study. However, further studies are needed with large sample size to know the sensitivity of AK by NRA method.

In this study, it was found that the turnaround time for NRA was shorter as compared to direct proportion method on LJ medium which takes 4-6 weeks. Out of 73 specimens, 67 (91.7%) specimens gave positive color within 21 days. This is in concordance with the recommendations of Centers for Disease Control and Prevention.[20] Majority of the isolates, that is 54.8%, gave positive color on the day 21 incubation. Only six specimens gave color on day 28. The mean turnaround time in our study was longer; 18.7 days (range 10 to 28 days) as compared to other studies using clinical isolates or strains and almost similar to studies conducted on smear positive sputum samples.[2,10,12]

In this study, there were only three samples bacillary load of 1+ which gave invalid results, as it did not produce any color even at incubation day-28. However, all the three samples yielded growth of *M. tuberculosis* on MGIT 960 system. The most likely justification for this might be due to the reason that about less than 1% of *M. tuberculosis* isolates do not have nitrate reductase activity resulting in production of negative color after adding the mixture. The other possibility might be due to slow metabolic activity of these mycobacteria on solid medium. However, further studies on large scale are needed to know its reliability on smear positive specimens.

CONCLUSION

NRA is a low cost, and accurate method for the diagnosis of MDR and XDR TB. It has a short turnaround time as compared to LJ medium and does not need special instruments or expertise. It can be utilized in resource-scarce developing countries for timely diagnosis of MDR TB case.

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ORIGINAL ARTICLE

Peripapillary Retinal Nerve Fiber Layer (RNFL) Thickness Measurements by Topcon SD-OCT in Myopic Patients

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ABSTRACT

Objective: To evaluate the effect of low-to-moderate myopia on peripapillary retinal nerve fiber layer (RNFL) thickness measured by Topcon SD optical coherence tomography (OCT).

Study Design: Cross-sectional study.

Place and Duration of Study: Ophthalmology Department, Shifa Foundation Falahee Clinic, over a period of one year starting from June 2015.

Methodology: A total of 43 eyes of 43 patients, having mild to moderate myopic refractive error, were enrolled in the study. Refractive error/spherical equivalent was calculated. RNFL thickness was obtained from all four peripapillary quadrants: temporal, superior, nasal, and inferior; and 12 sub-quadrants using Topcon SD OCT. Pearson correlation coefficients (r) were calculated to evaluate relationships between the RNFL thickness and spherical equivalent (SE) before and after adjustment for ocular magnification.

Results: The study included 51.2% females and 48.8% males. Mean age was 30.9 ±6.45 years. Mean axial length was 24.25 ±0.91 mm. Mean SE was -3.25 ±1.93 DS. Mean of average RNFL thickness (with Littmann's correction) was 97.28 ±8.15 µm. Correlation analysis among all subjects showed that the average, mean nasal quadrant, upper nasal, and inferonasal sub-quadrant RNFL thickness had positive correlation with spherical equivalent (r = 0.31, p = 0.045). However, correction of the magnification effect by applying Littmann formula eliminated this effect.

Conclusion: In low-to-moderate myopia, RNFL measurements vary with refractive error of the eye. Since ocular magnification significantly affects the RNFL measurement, it should be considered in diagnosing glaucoma.

Key Words: *Myopia. Optical coherence tomography. Refractive errors. Glaucoma. Retinal ganglion cells.*

INTRODUCTION

Myopia is one of the most prevalent disorders leading to decreased vision worldwide.[1] In the Beaver Dam Study, the prevalence of myopia in adults was 26.2%. Myopia is more prevalent in Asia. The National Blindness and Visual Impairment Survey (2006) reported myopia in 36.5% of adult Pakistani population.[2] In Singapore, 38.7% of adult Chinese are myopic.[3] Myopia and its related ocular problems are a major concern in Asian countries.

Glaucoma is the leading cause of irreversible blindness worldwide.[4] Myopes are at higher risk of developing glaucoma than non-myopes.[5] In early glaucoma, RNFL defects precede visual field defects; therefore, assessment of the RNFL is important in diagnosing and monitoring the progression of the disease.[6] The latest spectral domain optical coherence tomography (SD-OCT) provides reliable and reproducible high axial scanning resolution (<10 µm) measurements of the peripapillary RNFL.[7,8]

Studies have shown racial differences while investigating the relationship between the RNFL thickness and myopia.[9,10] Only few studies have been done in Pakistani population. This study was conducted to determine the relationship between myopia and the RNFL thickness in our clinical setting.

METHODOLOGY

This cross-sectional study was started in June 2015 after approval from Institutional Review Board and Ethics Committee. Patients were recruited from Ophthalmology OPD, Shifa Foundation Falahee Clinic. Participants were classified into two groups: low myopia SE -0.5 D to <-4.0 D and moderate myopia SE ≥ -4.0 D. Myopic patients of both genders, older than 18 years of age and having best corrected vision 6/6, were included in the study. Exclusion criteria were refractive errors other than myopia, astigmatism >2.00 D, amblyopia, retinal/optic disc diseases, corneal disorders, strabismus, glaucoma/ocular hypertension and a previous history of ophthalmic surgery/ocular trauma. Informed consent was taken from all the patients. Detailed history was obtained and complete ocular examination was performed including visual acuity, refractive error,

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intraocular pressure, anterior and posterior segment examination.

Canon RK-F1 autorefractor was used to measure refractive errors/spherical equivalent. An average of three measurements was taken. All OCT examinations were performed by single examiner. Cup/Disc area ratio was measured from disc topography parameters. RNFL thickness was obtained from four peripapillary quadrants: temporal, superior, nasal, and inferior; and also in 12 sub-quadrants, i.e. T=temporal, UT=upper temporal, LT=lower temporal, N=nasal, UN=upper-nasal, LN=lower-nasal, S=superior, ST=superotemporal, SN=superonasal, I=inferior, IT=inferotemporal, IN=inferonasal. Littmann formula (t=p×q×s) was used to correct ocular magnification induced by refractive error. When t was the actual fundus measurement, p was the magnification factor for the camera of the OCT used and remained constant, q was the magnification factor of the eye (calculated with the formula q=0.01306× (axial length-1.82), and s was the measurement obtained using OCT. All the data were entered in a proforma designed for the study.

Table I: Descriptive and clinical parameters of the groups (mean ±SD).

Characteristic	Low myopia (n=28)	Moderate myopia (n=15)	Total (n=43)	p-value
Age (years)	31.11±6.57	30.53±6.42	30.91±6.45	0.785
SE (diopters)	-2.12±1.16	-5.37±1.14	-3.25±1.93	0.000
Axial length (mm)	23.30±0.81	24.75±0.93	24.26±0.92	0.008
Ethnicity				
Punjabi	26 (93%)	13 (86.7%)	39 (90.7%)	0.505
Pathan	2 (7.1%)	2 (13.3%)	4 (9.3%)	
Gender				
Male	16 (57.1%)	5 (33.3%)	21 (48.8%)	0.137
Female	12 (42.9%)	10 (66.7%)	22 (51.2%)	

Statistical analysis was done using SPSS 20.0. Frequencies and percentages were calculated for gender and ethnicity, and were compared by Chi-square test. Mean ± SD was calculated for age, SE, axial length and RNFL thickness. Normality of data was checked by Kolmogorov-Smirnov test. Levene's test was used to assess the homogeneity of the variances. Independent sample t-test was used to compare the RNFL thickness with myopia among the two groups. A value of $p < 0.05$ was taken as statistically significant. Pearson correlation coefficients (r) were calculated to evaluate the relationship between the RNFL thickness and SE.

RESULTS

A total of 43 (21 right and 22 left) eyes of 43 patients were included in the study. There were 22 (51.2%) female and 21 (48.8%) male participants. Majority of them were Punjabi (90.7%). Mean age was 30.9 ±6.45 years, ranging from 22 to 47 years). Mean axial length was 24.25 ±0.91 mm. Mean SE was -3.25 ±1.93 DS (-0.5 to -7.37). Mean cup to disc ratio was 0.30 ±0.17. Mean IOP of study population was 13.58 ±2.38 mmHg. Mean IOP was 13.21 ±2.20 mmHg in low myopes and 14.27 ±2.63 mmHg in moderate myopes: slightly higher in moderate myopia group but not statistically significant (p=0.170).

The descriptive and clinical parameters of the groups are given in Table I. There were no statistically significant differences between the groups concerning age and gender. SE and axial length were significantly different among the two groups ($p<0.05$). When comparing means of average RNFL thickness with age, there was no significant difference (p=0.749). The mean values of the peripapillary RNFL thickness without Littmann formula are shown in Table II, and with Littmann formula in Table III.

Table II: Peripapillary retinal nerve fiber layer thickness (microns, mean ± SD) without Littmann formula.

Quadrant / Sub-quadrants	Low myopia	Moderate myopia	Total (n=43)		Correlation coefficient	
	(n=28)	(n=15)	Thickness	p-value	r	p-value
Average	95.92 ±6.85	93.06 ±9.65	94.93 ±7.95	0.266	0.31	0.045
Temporal quadrant	71.28 ±13.28	68.53 ±12.05	70.3 2 ±12.79	0.508	0.15	0.348
Superior quadrant	110.17 ±18.58	115.80 ±9.10	112.13 ±16.03	0.278	-0.02	0.920
Nasal quadrant	79.96 ±13.98	75.13 ±21.71	78.27 ±16.97	0.380	0.32	0.037
Inferior quadrant	118.57 ±9.38	118.0 ±17.79	118.37 ±12.73	0.890	0.14	0.387
T* Sub-quadrant	65.96 ±16.17	54.20 ±16.01	61.86 ±16.90	0.028	0.27	0.072
UT* Sub-quadrant	77.42 ±15.33	71.73 ±13.88	75.44 ±14.93	0.238	0.05	0.744
LT* Sub-quadrant	71.85 ±17.87	62.86 ±20.67	68.72 ±19.14	0.144	0.23	0.148
N* Sub-quadrant	70.21 ±22.00	71.73 ±24.27	70.74 ±22.54	0.836	0.19	0.230
UN* Sub-quadrant	94.46 ±19.23	87.66 ±13.59	92.09 ±17.61	0.232	0.37	0.014
LN* Sub-quadrant	72.67 ±19.56	76.80 ±21.78	74.11 ±20.20	0.530	0.13	0.418
S* Sub-quadrant	115.00 ±21.15	120.60 ±15.71	116.95 ±19.42	0.374	0.07	0.662
ST* Sub-quadrant	115.14 ±18.17	116.73 ±16.92	115.69 ±17.56	0.781	-0.14	0.381
SN* Sub-quadrant	106.35 ±18.07	109.60 ±15.57	107.48 ±17.12	0.560	0.01	0.927
I* Sub-quadrant	132.42 ±16.17	128.46 ±23.80	131.04 ±18.99	0.521	0.17	0.269
IT* Sub-quadrant	115.28 ±19.77	127 ±18.32	119.37 ±19.87	0.065	-0.26	0.920
IN* Sub-quadrant	108.07 ±14.70	99 ±31.40	104.90 ±22.06	0.203	0.30	0.049

** T=Temporal; UT=Upper temporal; LT=Lower temporal; N=Nasal; UN=Upper nasal; LN=Lower nasal; S=Superior; ST=Suprotemporal; SN=Supronasal; I=Inferior; IT=Inferotemporal; IN=Inferonasal.*

Table III: Peripapillary retinal nerve fiber layer thickness (microns, mean ± SD) with Littmann formula.

Quadrant / Sub-quadrants	Low myopia	Moderate myopia	Total (n=43)		Correlation coefficient	
	(n=28)	(n=15)	Thickness	p-value	r	p-value
Average	97.11 ±6.01	97.58 ±11.38	97.27 ±8.15	0.860	-0.02	0.881
Temporal quadrant	72.01 ±11.85	71.91 ±13.36	71.98 ±12.24	0.979	-0.02	0.899
Superior quadrant	111.54 ±18.82	121.52 ±12.57	115.02 ±17.42	0.073	-0.20	0.201
Nasal quadrant	80.84 ±13.28	78.59 ±22.53	80.06 ±16.85	0.682	0.20	0.191
Inferior quadrant	120.13 ±9.78	123.61 ±18.92	121.43 ±13.55	0.430	-0.11	0.474
T* Sub-quadrant	6.63 ±14.88	56.97 ±17.7	63.26 ±16.40	0.065	0.187	0.256
UT* Sub-quadrant	78.38 ±15.15	75.45 ±16.63	77.36 ±15.55	0.563	-0.10	0.542
LT* Sub-quadrant	72.66 ±17.44	66.11 ±22.81	70.38 ±19.47	0.299	0.122	0.437
N* Sub-quadrant	70.76 ±20.70	75.29 ±26.31	72.34 ±22.61	0.537	0.09	0.558
UN* Sub-quadrant	95.60 ±19.00	91.64 ±12.83	94.22 ±17.05	0.475	0.25	0.104
LN* Sub-quadrant	73.48 ±19.34	80.24 ±22.25	75.83 ±20.39	0.306	0.03	0.846
S* Sub-quadrant	116.40 ±21.02	126.72 ±19.76	120.0 ±20.90	0.125	-0.10	0.518
ST* Sub-quadrant	116.77 ±19.20	122.70 ±21.17	118.8 ±19.87	0.357	-0.30	0.059
SN* Sub-quadrant	107.76 ±18.39	114.66 ±15.43	110.17 ±17.55	0.224	-0.15	0.346
I* Sub-quadrant	134.14 ±16.30	134.61 ±25.25	134.30 ±19.58	0.924	-0.02	0.908
IT* Sub-quadrant	116.84 ±20.53	133.25 ±21.25	122.56 ±22.00	0.018	-0.34	0.009
IN* Sub-quadrant	109.50 ±15.31	103.45 ±32.16	107.39 ±22.55	0.406	0.18	0.243

** T=Temporal; UT=Upper temporal; LT=Lower temporal; N=Nasal; UN=Upper nasal; LN=Lower nasal; S=Superior; ST=Suprotemporal; SN=Supronasal; I=Inferior; IT=Inferotemporal; IN=Inferonasal.*

The RNFL thickness was lowest in temporal quadrant and highest in inferior quadrant. There was no statistically significant difference in mean thickness between two groups in any of the four major quadrants before and after applying Littmann formula. While comparing the sub-quadrants statistically, significant difference was found in mean thickness in temporal (T) sub-quadrant which disappeared after applying Littmann formula. However, in inferotemporal (IT) sub-quadrant, the difference became significant after application of Littmann formula (p = 0.018).

Correlation analyses among all subjects showed that the average, mean nasal quadrant, upper nasal and inferonasal sub-quadrant RNFL thickness had positive correlation with SE (Table II). However, correction of the magnification effect by applying Littmann formula eliminated the relationship both between RNFL thickness and SE in each quadrant and sub-quadrants (Table III).

The average RNFL thickness matched with the normative database in all 4 major quadrants. While looking at 12 sub-quadrants, 44.2% (n= 19) of eyes were identified as abnormal, based on the normative database provided in Topcon SD OCT. Of the abnormal eyes in 14% (n=6) 3 sub-quadrants and in 30.2% (n=13) 1-2 sub-quadrants were identified as outside normal limits and borderline, respectively.

DISCUSSION

In the present study, RNFL measurements were compared in a healthy non-glaucomatous group of Pakistani population. This research found an average RNFL lower than that found by most of other studies (mean 97.28 ±8.15 µm). In a study by Leung, average RNFL thickness in Chinese population with low to moderate myopia (mean SE -3.9 ±1.5) was 107.49 ±12.74 µm.[11] The mean RNFL thickness was 100.1 µm ± 11.6 in another study.[13] In a study by Murugan *et al*.,[12] the average global RNFL of all the participants was 105 ±9 µm. In this study, the myopic SE for black participants was -1.48 D ±1.13 D and for Indian participants was -2.42 D ±2.22 D.

Kang and colleagues conducted a study on normal subject of Korean origin (mean SE -2.52 ±2.30 D). The mean of average peripapillary RNFL thickness was 98.25 ±8.59 µm.[9]

In a study by Vernon and colleagues, the mean RNFL thickness was 81.4 ±13.7 µm, which is much lower than the present findings.[13] But their sample had a mean age of 48.1 years and mean SE of -7.7 D, i.e. higher than the presently reported. The differences in RNFL thickness in these studies may be due to ethnic variation and difference in mean SE. Differences in study sample sizes and OCT device algorithms (software used) could also account for the variation observed.

On comparing with normative data base, the RNFL measurement is classified as "outside normal limits" (red), if it is less than 99% of the age-matched RNFL thickness normogram and as "borderline" (yellow), if falling between the 95% - 99%. In this study, done on Topcon SD OCT 44% of the cases were below 99%. Our findings match closely with Leung *et al*.,[11] they found that 16.5% and 28.7% of the myopic eyes were identified as outside normal limits and borderline respectively, on comparing with normative data base of Stratus OCT. Zhao and colleagues found a similar percentage (43.4%) of myopes as outside the normal limits.[14] In another study, Rauscher found that eleven

subjects (40%) had RNFL thickness scans outside normal limits on the Stratus OCT.[15] Patients with RNFL outside normal limits need evaluation by Visual field analysis. All these studies also considered visual fields of the study population to classify them as normal. This study lacks data on visual fields but OCT scan was repeated at three months to document that RNFL findings were stable. For analyzing RNFL in myopic subjects, the normative database alone may be misleading, and refractive error should always be considered in the interpretation of RNFL measurements.

RNFL thickness varies in different peripapillary quadrants. In the studied population, RNFL was thinnest in temporal quadrant and thickest in inferior quadrant. Murugan *et al*. found similar results.[12] They found that in both black and Indian participants, the inferior quadrant was the thickest, followed by the superior, nasal and temporal quadrants. Kang and Rauscher found that myopic eyes showed thicker temporal RNFL and thinner superior and inferior RNFL.[9,15] This is against our findings. In another study by Ramakrishnan *et al*.,[16] RNFL thickness was reported to be greatest in the superior quadrant. They used a time-domain Stratus OCT 3000 which may account for the variations observed in the quadrant with the greatest thickness.

Gender seems to have no effect on average RNFL thickness. No statistically significant gender related differences in global RNFL thickness were found (p=0.137) in this study, which is in agreement with earlier studies by Murugan (p = 0.79), O'Rese and Alasil.[12,17,18]

The effect of myopia on the average RNFL thickness has been studied for many years and is still debatable. Various studies have reported significant correlations between average RNFL thickness and myopic SE. However, Sony *et al*. and Rao *et al*. reported no significant association between these two clinical variables.[19,20] In this study, correlation analyses among all subjects showed that the average RNFL thickness had positive correlation with SE (r=0.31, p=0.045). However, correction of the magnification effect by applying Littmann formula eliminated this relationship. Not many studies used Littmann formula to correct the effect of ocular magnification caused by refractive error. Among the studies discussed below, only Kang and colleagues used Littmann formula and found results similar to this study.[9]

In a study by Murugan and colleagues,[12] RNFL thickness was compared in 80 black and Indian myopic students; ages from 19-24 years. They found positive and significant association between myopic SE and global RNFL thickness for the total sample (r = 0.36, p = 0.00) and for Indians (r = 0.33, p = 0.04) but not for the black (r = 0.25, p = 0.13) participants. Similarly, Leung studied 115 subjects with myopia (age range, 22-60 years) with mean SE of -7.31 ±3.04 D and found that there was a linear correlation between RNFL thickness and SE.[11] The average RNFL thickness decreased with negative refractive power (r=0.291, P=0.002). Rauscher found a significant relationship between SE (mean -5.40) and RNFL thickness in a population with a racial breakdown of: 63% Caucasian, 15% Hispanic, 15% Asian, and 7% Black.[17] According to this study, RNFL thickness decreased with higher SE.

Zhao and colleagues studied healthy Chinese myopic individuals and found that when the degree of myopia increases, the RNFL thickness, measured by 3D-OCT including the average and superior, nasal, inferior sectors decreases.[14] In a local study conducted by Tayyab and colleagues, no association was found between the RNFL thickness and refractive errors.[21] Their study was different from our study in several aspects: use of HRT to measure RNFL thickness; effect of ocular magnification was not taken into consideration; mean of all types of refractive errors was taken and amblyopic eyes were also included.

Kang and colleagues conducted a study on a large number of normal subjects of Korean origin.[9] In that study, before adjusting for the ocular magnification, it was found that the average RNFL thickness decreased with SE. However, after ocular magnification adjustments, the average RNFL thickness exhibited no correlation with the SE. These findings strongly agree with this study results.

The strength of this study include the use of a high-resolution spectral domain OCT, standardized examination techniques, and a relatively uniform sample. Possible limitations include the lack of visual field analysis and relatively small sample size. However, the study provides baseline data on RNFL thickness measurements in Pakistani population. Therefore, it is recommended that studies with a larger sample size and a wider range of myopic SE be conducted in future.

CONCLUSION

The myopic refractive error apparently influences peripapillary RNFL thickness as measured by Topcon SD OCT. However, this is due to the ocular magnification effects associated with refractive error, and are corrected after the application of the Littmann formula. So the current database should be improved by taking refractive error into account. We also noticed that Pakistani population has thinner average RNFL measurements.

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ORIGINAL ARTICLE

The Potential Therapeutic Role of Peroxisome Proliferator-Activated Receptors Agonist in Preeclamptic Pregnant Rats

Hayam Ibrahim Gad Allam and Abeer AbdulMoati Al Masri

ABSTRACT

Objective: To investigate the role of peroxisome proliferator-activated receptor (PPAR)-γ agonist on renal functions and blood levels of vasoregulatory peptides by administering rosiglitazone-a PPAR-γ agonist- to nitric oxide (NO) synthase lacking pregnant rats as a creature model of preeclampsia.

Study Design: Experimental study.

Place and Duration of Study: Medical College at King Khaled University Hospital, Riyadh, Saudi Arabia, from July 2016 to May 2017.

Methodology: One hundred female rats were divided into four equal groups; virgin non-pregnant, pregnant saline-received, pregnant treated with N^G-nitro-L-arginine-methyl-ester (L-NAME) to produce preeclampsia and the last group received L-NAME+rosiglitazone. Some renal function tests and blood level of vasoregulatory factors were measured on day 13 and day 20 of pregnancy. Pup weight was also measured.

Results: L-NAME treated rats exhibited all classic hallmarks of preeclampsia. Rosiglitazone treatment from days 14 to 20 ameliorated hypertension, improved renal function, decreased endothelin-1 (ET-1), angiotensin II (Ag II) and interleukin (IL-6), while it increases NO level and pup weight.

Conclusion: Administration of rosiglitazone can improve some of the pathophysiological trademarks related with preeclampsia and may prove useful for the prevention of the development and progression of this condition. However, human trials are necessary to prove this hypothesis.

Key Words: *Preeclampsia. Rosiglitazone. L-NAME. Renal functions. Endothelin-1. Interleukin-6.*

INTRODUCTION

The key process in the establishment of successful pregnancy is trophoblast invasion into maternal uterine tissues together with remodeling of maternal spiral arteries, a process regulated by a large number of autocrine and paracrine factors which play pivotal roles in degradation of basement membranes and extracellular matrix.[1] Preeclampsia is a pregnancy-particular disorder, perceived as a main source of maternal and perinatal mortality. Shallow endovascular cytotrophoblast intrusion into the maternal arteries and endothelial cell damage are included in its pathogenesis. Decreased placental perfusion, secondary to abnormal placentation, causes release of cytotoxic factors and maternal endothelial dysfunction.[2]

Peroxisome proliferator-enacted receptors (PPARs) are expressed in placental trophoblast and vascular cells. Accumulating evidence supports their role in placental development, trophoblast invasion, and maternal adaptation to fetal nutritional requirements.[3] A diminution in the placental expression of PPAR-activators has been exhibited in ladies who had serious preeclampsia.[4] In addition, administration of a PPAR-γ antagonist to pregnant rats resulted in the development of all classic hallmarks of preeclampsia.[5] As a result, significant accentuation has been put on illustrating the mechanisms for its activation and, therefore, the potential for its utilization as a pharmacological focus for the treatment of muddled pregnancies.

NO is an important messenger in inducing vasodilation in normal pregnancy. Many reserachers revealed the hindrance of NOS with L-NAME caused hypertension, proteinuria, fetal development impediment, and expanded fetal mortality, and in this way showing the characteristics of preeclampsia.[6] Along these lines, rats treated by L-NAME can be utilized as a model of preeclampsia.

There is, as of now, no powerful pharmacological intercession accessible for the treatment of preeclampsia and, thus, pregnancies are frequently conveyed preterm for maternal advantage, forcing the difficulties of iatrogenic rashness on the fetus. It was hypothesized that PPAR-γ agonist (rosiglitazone) may be helpful as adjuvant treatment for preeclampsia, utilizing pregnant rats received L-NAME as a creature model of pre-eclampsia.

The aim of this study was to investigate the role of peroxisome proliferator-activated receptor PPAR-γ

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agonist on renal functions and blood levels of vaso-regulatory peptides by administering rosiglitazone-a PPAR-γ agonist- to NOS lacking pregnant rats as a creature model of preeclampsia.

METHODOLOGY

The present research was done at King Saud University, Medical College in Riyadh, Saudi Arabia. This study was performed as per Institutional Review Board (IRB) of College of Medicine at King Khaled University Hospital. Wistar rats got free access to water ad libitum and housed in a controlled situation. Their initial weight ranged from 220 to 240 gm. Two or three cycling female rats were combined with male rats and tried every day by vaginal smear for the nearness of vaginal plug or sperms as confirmation of sex. Those dams that coupled between the second and fourth days in the wake of matching with guys were additionally examined. The nearness of sperms in vaginal smears was considered as the very first moment of pregnancy. One hundred female rats were haphazardly partitioned into four equivalent gatherings: virgin non-pregnant rats, pregnant rats that were given saline (50 ml/Kg body weight/day in drinking water) day by day beginning from day-1 to day-20 of pregnancy, and pregnant rats that were treated with L-NAME (catalogue No. N5751, provided from Sigma) at a measurement of 50 mg/Kg body weight/day broke dissolved in drinking water and every day beginning from day-1 to day-20 of pregnancy to make a creature model of preeclampsia.[7] The fourth group was pregnant rats who were dealt with by both L-NAME (a similar measurement and duration as specified for the third group) and PPAR-agonist (rosiglitazone, index No. R2408-50MG provided by Sigma), in a measurement of 5 mg/kg every day in drinking water beginning from day 14 to day 20 of pregnancy.[8]

Arterial blood pressure of all rats was measured on day 13 of pregnancy, using blood pressure system model IITC Biotech.[9] Also we measured urinary creatinine and 24-hour urinary albumin excretion (UAE).[10] Then, blood samples were drawn from the eye ball of each rat, using capillary tubes and collected in plain and EDTA tubes containing aprotinine at 0°C. Blood samples were centrifuged at 1600 g for 16 minutes. Plasma or serum samples were investigated for creatinine, and creatinine clearance was calculated as a measure of glomerular filtration rate, endothelin-1 (ET-I), utilizing the parameter ET-1 immunoassay kit provided from R&D System Inc. USA, Minneapolis, MN. (catalog No. BBE 5),[11] angiotensin II (Ag II) utilizing the competitive EIA kit given from Peninsula Laboratories Inc. San Carlos, California. Index No. S-1133 (EIAH7002),[12] total serum NO (as the total of nitrite and nitrate) utilizing total nitric oxide assay (catalog No. KGE001) produced by R&D System Inc. USA, Minneapolis, MN,[13] and interleukin-6 (IL-6) utilizing Quatinkine, rat IL-6 immunoassay (catalog No. R6000B), made by R&D Systems, Inc. USA, Minneapolis, MN.[14]

On day-20 of development (i.e. 19 days after L-NAME treatment in the third group, and 6 days after the start of rosiglitazone treatment in the fourth group), blood pressure was measured and 24-hour urine were collected. The rats were sacrificed and blood was gathered by means of cardiac puncture. Plasma or serum tests were utilized for estimation of the previously mentioned parameters. Pups for each rat were weighed and the mean of weight was calculated (total weight/number of pups). Control non-pregnant rats were kept separately and exposed to the same environment. Control non-pregnant rats were kept independently and presented to a similar situation. Blood and urine samples of non-pregnant rats were taken on the same day as for the other pregnant rats.

Data were expressed as mean ± standard deviation (X ± SD). Statistical analysis was completed utilizing the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) program for Windows version 21. Paired t-tests were performed to estimate within-group changes (on day 13 and 20 of gestation for the same group). One-way analysis of variance (ANOVA) was performed to appraise the changes between various groups with 95% confidence intervals, followed by Tukey's multiple comparison tests. Differences were considered to be statistically significant at $p<0.05$.

RESULTS

Treatment of pregnant rats with rosiglitazone significantly reduced L-NAME-induced hypertension (93.52 ±3.86 versus 160.68 ±3.51 mmHg for groups IVb and IIIb, respectively, where $p<0.001$ and 93.52 ±3.86 versus 145.60 ±9.50 mmHg for groups IVb and IVa, respectively, where $t=-28.06$, $p<0.0001$, Table I).

After rosiglitazone treatment, creatinine clearance was significantly increased on day-20 when contrasted with L-NAME treated pregnant rats around the same time of pregnancy (2.640 ±0.376 vs. 0.936 ±0.297 ml/minutes where $F=162.359$, $p<0.001$) and rosiglitazone treated rats on day 13 (2.640 ±0.376 vs. 1.056 ±0.258 ml/minutes where $t=19.868$, $p<0.001$, Table I).

Treatment with rosiglitazone significantly ameliorated 24-hour UAE when contrasted with L-NAME treated rats on day-20 (69.48 ±4.993 vs. 140.08 ±6.11 mg/dl, $F=3965.13$, $p<0.001$) and rosiglitazone treated rats on day 13 (69.48 ±4.99 vs. 140.08 ±4.821 mg/dl, $t=-56.53$, $p<0.001$, Table I).

Plasma ET-1 level was significantly decreased after rosiglitazone treatment from day-14 till day-20 of pregnancy (0.32 ±0.06 vs. 0.45 ±0.07 pg/ml for groups IVb and IIIb, respectively, $p<0.001$); (0.32 ±0.06 vs. 0.44 ±0.06 pg/ml for groups IVb, and IVa, respectively, where

Table I: Mean values ± SD of the measured parameters in control rats (group I), non-treated pregnant rats on day 13 (group IIa) and day 20 (group IIb), L-NAME-treated rats on day 13 (group IIIa) and day 20 (group IIIb) and rosiglitazone-treated pregnant rats on day 13 (group IVa) and on day 20 (group IVb).

	Group (controls)	Non treated		L-NAME-treated		Rosiglitazone-treated		F1-value (Gp I, IIa, IIIa, IVa)	F2-value (Gp I, IIb, IIIb, IVb)	P- value
		Group IIa (day 13)	Group IIb (day 20)	Group IIIa (day 13)	Group IIIb (day 20)	Group IVa (day 13)	Group IVb (day 20)			
Mean arterial blood pressure (mmHg)	90.28±5.73	89.76±6.84	90.20±5.82	144.20±6.93*	160.68±3.51*	145.60±9.50*	93.52±3.864	460.904	1282.25	<0.0001
		T= -0.463, P=0.648		T= 10.422, P=0.000#		T= -28.067, P=0.000£				
Creatinine clearance (ml/min)	2.68±0.384	2.78±0.354	2.80±0.330	1.06±0.280*	0.94±0.297*	1.06±0.258*	2.64±0.376	223.993	162.359	<0.0001
		T= 0.537, P=0.597		T= -9.436, P=0.000#		T= 19.868, P=0.000£				
24 h urinary albumin excretion (mg/dl)	24.1±2.682	24.2±1.958	25.3±2.304	139.7±5.683^	140.1±6.116^	140.1* ± 4.821^	69.48±4.993^	6708.306	3965.127	<0.0001
		T= 1.899, P=0.07		T= 0.228,P=0.822		T= -56.525,P=0.000£				

** Significance was considered at p<0.05, 95% confidence interval.*
(*) Significant increase in groups IIIa, IVa as compared to I, IIa and in group IIIb as compared to groups I, IIb.
(^) Significant increase in groups IIIa, IVa as compared to I, IIa and in groups IIIb, IVb as compared to I, IIb.
(#) Significant changes between groups IIIa and IIIb.
(£) Significant changes between groups VIa and VIb.

Table II: Mean values ± SD of the measured parameters in control rats (group I), non-treated pregnant rats on day 13 (group IIa) and day 20 (group IIb), L-NAME-treated rats on day 13 (group IIIa) and day 20 (group IIIb) and rosiglitazone-treated rats on day 13 (group IVa) and day 20 (group IVb).

	Group (controls)	Non-treated		L-NAME-treated		Rosiglitazone-treated		F1-value (Gp I, IIa, IIIa, IVa)	F2-value (Gp I, IIb, IIIb, IVb)	P- value
		Group IIa (day 13)	Group IIb (day 20)	Group IIIa (day 13)	Group IIIb (day 20)	Group IVa (day 13)	Group IVb (day 20)			
Plasma endothelin-1 (pg/ml)	0.314±0.055	0.321±0.057	0.319±0.057	0.437±0.060*	0.445±0.068*	0.440±0.059*	0.324±0.064	36.238	26.515	<0.0001
		T=-1.225,P=0.233		T=1.445,P=0.161		T=-8.567,P=0.000£				
Plasma angiotensin II (ng/ml)	1.116±0.352	1.192± 0.341	1.224± 0.280	2.128± 0.325*	2.164± 0.351*	2.196±0.252*	1.196±0.369	83.097	52.998	<0.0001
		T=1.281,P=0.212		T=2.221,P=0.036^		T=-12.769,P=0.000£				
Serum nitric oxide (µmol/l)	249.1±9.390	249.2±10.015	249.6±8.827	171.7± 7.765*	171.1±7.566*	171.4± 8.271*	248.6±10.251	632.204	462.619	<0.0001
		T=0.501,P=0.621		T= -1.664,P=0.109		T= 40.356,P=0.000£				
Pup weight (g)		2.22±0.600		1.21±0.344$		2.27±0.278		48.416		<0.0001

** Significance was considered at p<0.05, 95% confidence interval.*
(*) Significant increase in groups IIIa, IVa as compared to I, IIa and in group IIIb as compared to I, IIb, IVb.
(^) Significant changes between groups IIIa and IIIb.
(£) Significant changes between groups VIa and VIb.
($) Significant decreases in group III as compared to groups II and IV.

t=-8.56, p<0.001). When the effects of rosiglitazone treatment on plasma Ag II level, were statistically analyzed, significant decrease of its plasma level was found on day-20 of gestation (1.19 ±0.36 vs. 2.16 ±0.35 pg/ml for rosiglitazone and L-NAME treated rats respectively, p<0.001); (1.19 ±0.37 vs. 2.19 ±0.25 pg/ml for rats received rosiglitazone after and before treatment respectively where t=-12.77, p<0.001, Table II).

A significant decrease in serum total NO products (nitrite + nitrate) levels were identified in L-NAME treated pregnant rats on days 13 and 20 of development when contrasted with controls and non-treated pregnant rats on that days of pregnancy (p<0.001). Rosiglitazone treatment produced significant increase of serum total NO products levels (248.56 ±10.25 vs. 171.40 ±8.27 µmol/l before and after treatment, p<0.001, Table II).

L-NAME and rosiglitazone treated pregnant rats on day-13 of pregnancy displayed a significant increment of serum IL-6 levels when contrasted with controls and non-treated pregnant rats around the same time of growth (141.60 ±7.77 and 142.16 ±8.08 vs. 110.00 ±7.56 and 111.44 ±6.36 ng/l, p<0.001). Treatment with rosiglitazone from day-14 till day-20 of pregnancy significantly reduced IL-6 levels (116.08 ±4.62 vs. 142.16 ±8.08 ng/l after and before treatment respectively, p<0.001, t=-15.568, Figure 1).

Regarding pup weight, a significant decrease was detected in L-NAME treated rats as compared to controls and rosiglitazone treated rats (1.21 ±0.34 vs. 2.22 ±0.60 and 2.27 ±0.28 g, respectively). Rosiglitazone treatment attenuated the decrease in pup weight seen in L-NAME treated rats (p<0.001, F=48.42, Table II).

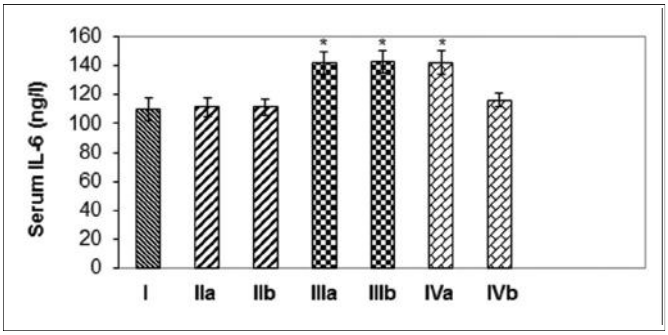

Figure 1: : Mean values ± SD of serum interleukin-6 (IL-6) ng/l in control rats (group I), non-treated pregnant rats on day 13 (group IIa) and day 20 (group IIb), L-NAME-treated rats on day 13 (group IIIa) and day 20 (group IIIb) and rosiglitazone-treated rats on day 13 (group IVa) and day 20 (group IVb). (*) Significant increase in groups IIIa, IVa as compared to groups I, IIa and in group IIIb as compared to groups I, IIb, IVb.

DISCUSSION

Many studies revealed the cardinal role of the placenta in the pathogenesis of preeclampsia together with an imbalance of proangiogenic and antiangiogenic factors. In the present study, administration of L-NAME to pregnant rats resulted in elevation of arterial blood pressure and 24-hour UAE, and reduction of creatinine clearance. Moreover, L-NAME treated rats exhibited all classic hallmarks of preeclampsia, i.e. elevated levels of ET-1, Ag II and IL-6, as well reduced NO level.

The neurovascular components of hypertension is recommended to be because of placental hypoxia that advances the arrival of biological active variables which fortify contraction of vascular smooth muscles, together with enhanced sympathetic tone.[15] Previuosly, we have examined the role of anti-tumor necrosis factor-alpha antibodies in preeclamptic pregnant rats.[16] In the present study, rosiglitazone ameliorated hypertension, improved renal function as evidenced from increased creatinine clearance and reduced the elevated 24-hour UAE, providing attenuation the degree of renal dysfunction and consistent with other studies.[17] It has been accounted for that independent of its blood glucose-bringing down impacts, PPARγ shows pleiotropic gainful consequences for vasculature as inhibition of Ag-II type 1 receptor expression and Ag-II-mediated signaling pathways, which may bring about inhibition of the renin-angiotensin system and prompt lowering of blood pressure.[18] The renoprotective effects of rosiglitazone could be due to attenuation of collagen deposition, increase the tubular uptake of albumin, reduction of tubular cell production of the inflammatory and profibrotic cytokines or by its influence on the renin angiotensin system.[19]

The present work exhibited increased ET-1 and Ag II levels in L-NAME treated pregnant rats, predictable with past investigations.[20,21] The increased generation of ET-1 from the placental or fetal tissue because of cell hypoxia or expanded dispersion into the maternal blood flow may clarify the increment in ET-1 levels found in preeclampsia. In the present study, treatment by rosiglitazone decreased plasma levels of ET-1 and Ag II in comparison with L-NAME treated pregnant rats. It is postulated that rosiglitazone acts directly on the vascular wall with reduction in local tissue Ag II concentration, down regulation of Ag II type 1 receptors.[22] These findings of diminished plasma ET-1 and Ag II levels and the related lessening in the mean ABP in rosiglitazone treated pregnant rats demonstrate the participation of these contracting factors in the pathogenesis of preeclampsia.

The present work demonstrated a significant abatement in serum NO levels in L-NAME treated pregnant rats. Therefore, the unopposed impacts of ET-1 and Ag II could support hindered uteroplacental blood stream. Emerging evidence suggests that preeclampsia targets and down regulates the expression of multiple G-protein-coupled receptors involved in NO regulation that might lead to impaired placental NO release and contribute to the imbalance of the homeostatic mechanisms involved in vascular tone regulation. In the present study, rosiglitazone treatment increased NO levels to normal. Previously, PPARs stimulation has been shown to modulate vascular tone and induces low blood pressure by means of a mechanism that involves the participation of NO release from vascular endothelial cells.[23] Taken together, it is feasible that rosiglitazone treatment via increased NO bioavailability prevented the L-NAME-induced rise in arterial blood pressure.

Reliable confirmation showed endothelial cell expression of IL-6 messenger RNA by placental trophoblast cells because of brooding with fetal plasma from placental vascular illnesses because of oxidative stress or hypoxia. IL-6 production from preeclamptic pregnancies was correlated with the severity and onset of preeclampsia.[24] The outcomes indicated increment in IL-6 levels in non-treated preeclamptic rats. Rosiglitazone treatment was associated with a significant reduction of serum IL-6 levels which demonstrates its conceivable advantageous impact in decreasing oxidative stress. Several preliminary findings suggest the mechanism(s) of reduction of serum IL-6 levels by rosiglitazone treatment due to inhibition of the apoB-48 receptor in macrophages or increased apoptosis of macrophages. Taken together, PPAR-γ agonists display promising beneficial effects in controlling conditions associated with increased IL-6 production.

Many data suggested basal PPAR-γ expression for proper placental development and revealed two-fold higher PPAR-γ expression in placentas from appropriate or large for gestational age infants.[25] Therefore, it was not surprising to find reduction in pup weight seen in preeclamptic rats. Along these lines, it was not shocking to discover prevention the decrease in pup weight seen

in preeclamptic rats by rosiglitazone treatment. It seems that PPARγ plays an essential role in the control of uteroplacental vasodilatory function during pregnancy.

CONCLUSION

Administration of rosiglitazone can improve some of the pathophysiological trademarks related with preeclampsia and may prove useful for the prevention of the development and progression of this condition. Research into the wellbeing and adequacy of rosiglitazone amid pregnancy and preeclampsia is justified. Therefore, human trials are necessary to prove this hypothesis.

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ORIGINAL ARTICLE

Relationship among Hypovitaminosis D, Maternal Periodontal Disease, and Low Birth Weight

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Abstract

Objective: To determine if low birth weight is associated with hypovitaminosis D and periodontal disease among a sample of Pakistani women residing in district Jhelum, Punjab.

Study Design: Cross-sectional study nested in a large community-based longitudinal study.

Place and Duration of Study: Tehsil Pind Dadan Khan, District Jhelum, Pakistan from August 2012 to October 2015.

Methodology: Women during 12-16th week of pregnancy were selected. Dental examination was performed. Probing depth ≥3 mm was labeled as periodontal disease, whereas serum level <20.0 ng/mL was taken as hypovitaminosis D. Mothers of low birth weight babies (<2500 g) were compared to mothers who gave birth to normal weight (≥2500 g) babies. Odds ratio was applied to measure the strength of association of low birth weight with maternal hypovitaminosis D and also for maternal periodontal disease.

Results: There were 62 participants in the study. The mean age of mothers was 26.7 ±4.5 years. It was alarming to observe that 53 (85%) participants had vitamin D deficiency. However, periodontal disease was only seen in four participants (6%). Out of the 62 mothers, eighteen (29%) gave birth to low birth weight babies. None of the variables were found to be associated with the low birth weight.

Conclusion: The present study did not find any significant association of low birth weight with hypovitaminosis D or maternal periodontal disease in the studied sample.

Key Words: *Low birth weight. Periodontal disease. Pregnant women. Vitamin D.*

INTRODUCTION

In the developing world, over 20 million infants (>15% of all live births) are born annually with low birth weight (LBW). According to the World Health Organization (WHO) global estimates on LBW, the prevalence of LBW in the developed part of the world is around 7%; whereas, in developing countries it is nearly 16.5%.[1] Highest prevalence of LBW is reported in South Asia, i.e. 27%. The prevalence of LBW in Pakistan ranges between 12-25%.[2] The WHO report estimates it to be at least 19%.[1]

Periodontal disease is a persistent source of bacterial infection that can induce systemic inflammation, which in turn, exacerbates the risk of adverse pregnancy outcomes.[3,4] Considerable research has been done to study this relationship but to date the findings on this relationship have been varied. A systemic review done on 25 studies showed that 18 studies, showing the risk of adverse pregnancy outcomes, is associated with periodontal disease [odds ratio: 1.10 - 20.0], while seven studies indicated no evidence of such association [odds ratio: 0.73 - 2.50].[5]

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Adverse pregnancy outcomes are also linked with maternal hypovitaminosis D.[6] A systematic review, performed on 31 studies, indicated that hypovitaminosis D is significantly associated with LBW but the substantial heterogeneity among studies made the reliability of that association questionable.[7] Another systematic review and meta-analysis, conducted on 24 studies, clearly demonstrated that pregnant women with vitamin D levels <20 ng/mL had a higher risk of developing preeclampsia [odds ratio: 2.09]; pre-term birth [odds ratio: 1.58]; small for gestational age [odds ratio: 1.52]; and gestational diabetes [odds ratio: 1.38].[8]

As hypovitaminosis D is a prevalent condition among pregnant in south Asian countries, particularly India and Pakistan,[9-11] a large proportion of pregnant women (35-100%) are affected with periodontal disease,[12] and one out of every five live-births in Pakistan is a LBW.[1] Thus, it is imperative to study the factors related to the LBW.

The objective of the present study was to assess if low birth weight is associated with maternal hypovitaminosis D and periodontal disease.

METHODOLOGY

A cross-sectional study that was nested in a large longitudinal study was done from August 2012 to October 2015 at Tehsil Pind Dadan Khan, District Jhelum, Pakistan. The Women and Child Health Division of the Aga Khan University with the help of Pakistan

Initiative for Mothers and Newborns has established an outreach community research center in that area. The study participants were pregnant women who were resident of that area. Participants were selected using convenience sampling that was done within the sampling frame of the primary study whose objectives were different from the present study. The present study focused on the mothers who gave birth to low birth weight babies (<2500g). These mothers were compared with the mothers with newborns of normal weight (birth weight ≥2500g).

Subjects with less than 20 standing teeth or history of dental surgery or antibiotic consumption within six weeks of delivery were excluded. Similarly, mothers whose vitamin D status were not available or could not be measured were also excluded. The study protocol was approved by the ethics review committee (ERC) of the Aga Khan University, Ref # 147-Ped-ERC-2010.

The sample size was calculated using WHO calculator by Lwanga and Lemeshow. [13] A population prevalence of 20% was taken for low birth weight babies with an absolute precision of 10% at 95% level of confidence to reach a final sample size of 62 mothers.

The interviews of the study participants took place at the outreach community data collection center and at the study subjects' residence. All study participants underwent a physical examination during 12-16th week of pregnancy. The study participants were dentally examined in daylight by a trained dentist using sterilized dental examination instruments. The subjects were examined while seated on a house chair. A standard periodontal probe (William's probe with graduated marking at 1mm interval) was used for recording following parameters: clinical attachment loss (AL), periodontal probing depth (PD), and gingival bleeding on probing (BoP). A six-point probing approach was followed for AL and PD readings (only Ramfjord's teeth were included). Thus, of 36 probing sites per individual were obtained. The periodontal readings were rounded off to the nearest millimeter. The oral hygiene was assessed using Silness-Loe Index,[12] and dental caries was assessed using DMFT index on the six teeth identified above. Serum samples (10 ml) were also obtained for assessing the hemoglobin levels for anemia and vitamin D levels (analyzed using DiaSorin kit, USA). The samples were later tested at the Aga Khan University, nutrition research laboratory, Karachi.

Table I: Characteristics of study participants (n=62).

Variables	Range	Mean	SD
Age (years)	19-36	26.8	4.4
*Vitamin D level (ng/mL)	4-27.3	10.8	IQR: 8.2-15.2
*Weight in Kg (12-16th week of pregnancy)	38-101.3	58.7	IQR: 49.8-66.7
Height (in cm)	145-170	155.3	5.2
Hb (gm/dL)	6.5-15	10.2	2.9
Number of teeth	23-32	30.1	2.3
Probing depth (mm)	1-3	1.8	0.6
Attachment loss (mm)	0-4	1.2	0.9
Birth weight (kg)	2.0-5.5	2.9	0.6
Gestation period (weeks)	37-44	38.4	1.7
Parity status		n	%
<2		43	69.4
≥3		19	30.6

**Owing to lack of normal distribution of vitamin D levels and weight of the study participants, the median and interquartile ranges (IQR) were reported rather than the mean and standard deviation.*

Table II: Variables associated with low birth weight among study participants (n=62).

Variables	Categories	n	LBW count (%)	Odds ratio (95% CI)	p-value
All	-	62	18 (29.0)	-	-
Age in years	<26	32	6 (18.75)	1.0	0.35
	≥26.1	30	12 (40.0)	0.35 (0.11-1.09)	
Vitamin D levels	<20.0 ng/mL	53	16 (30.1)	1.51 (0.29-8.10)	1.00
	≥20.1 ng/mL	9	2 (22.2)	1.0	
School education	<5 years	22	7 (31.8)	1.23 (0.40-3.83)	0.78
	≥6 years	40	11 (27.5)	1.0	
Parity status	<2	43	12 (27.9)	0.84 (0.26-2.72)	0.77
	≥3	19	6 (31.6)	1.0	
Anemia	<10.0 gm/dL	40	11 (27.5)	0.81 (0.32-1.81)	0.81
	≥10.1 gm/dL	22	7 (31.8)	1.0	
Bleeding on probing	Absent	29	6 (20.6)	0.46 (0.15-1.43)	0.26
	Present	33	12 (36.4)	1.0	
Probing depth	<3 mm	55	14 (25.4)	0.25 (0.05-1.29)	0.18
	≥3.1 mm	7	4 (57.1)	1.0	
Attachment loss	<2 mm	44	13 (29.5)	1.0	1.00
	≥2.1 mm	18	5 (27.8)	1.09 (0.32-3.69)	
DMFT index	<4	55	16 (29.1)	1.0	1.00
	≥4.1	7	2 (28.5)	1.03 (0.18-5.85)	
Oral hygiene	high plaque ≥1.1	22	6 (27.3)	0.87 (0.28-2.78)	1.00
	low plaque <1	40	12 (30.0)	1.0	

Odds ratio was calculated. LBW refers to low birth weight (≤2500 gm).
DMFT index was used for assessing decayed, missing and filled teeth (on Ramfjord teeth only) Silness-Loe Index was used for the assessment of oral hygiene.

For case definitions, newborns with known birth weight <2500 g were labeled as LBW. Study participants exhibiting at least one periodontal site with probing depth ≥3 mm were signified as having periodontal disease. For vitamin D levels, the cutoff value of <20.0 ng/mL was taken for hypovitaminosis D.

Data was analyzed on SPSS version 19.0 and Graph Pad Prism 6.0. The frequency distribution and percentages of the categorical variables and means and SD of continuous variables were computed. Odds ratio was applied to determine the strength of association between LBW and other variables including maternal periodontal disease and hypovitaminosis D. Logistic regression was planned to be applied while keeping the LBW as an outcome variable; whereas mother's age, periodontal disease, and vitamin D status were taken as independent variables. The level of significance for the statistical test was kept at 0.05.

RESULTS

There were 62 mothers in the study. The mean age of the participants was 26.7 ±4.5 years. All participants were otherwise healthy; no diabetics or smokers were reported in the study sample. Similarly, none of them has ever received a dental implant or fixed bridge. Nearly one-third of the participants (n=22) had no formal education beyond secondary school. Sixteen mothers (25.8%) were primi-para and 19 mothers (30.6%) had a parity status of three or more. The demographic details of the participants are shown in Table I.

Fifty-three (85%) participants were vitamin D deficient. However, periodontal disease was only seen in four (6%) subjects. Out of the 62 mothers, eighteen (29%) gave birth to low birth weight babies. None of the variables (vitamin D status, age, education, anemia, parity status, periodontal probing depth, bleeding on probing, clinical attachment loss, oral hygiene and DMFT status etc.) were found to have any association with the LBW (Table II). Hence, logistic regression analysis could not be performed.

DISCUSSION

Birth weight of the newborn is primarily determined by the health and nutrition of the female before conception and during pregnancy. The other risk factors known to be associated with LBW are poor maternal nutrition, anemia, smoking, primi-parity, low socioeconomic status (SES), mental stress during pregnancy, abuse in family, lack of antenatal visits, short maternal height and low maternal weight etc.[14] The frequency of LBW in the present study turned out to be 18/62 (29%). This proportion is alarmingly high. A probable explanation would be the fact that the studied sample belonged to a rural setting, which has a number of risk factors simultaneously present for LBW. These include low education, low SES, lack of antenatal care, and above all anemia. It's imperative to note that two third of the mothers in the study were anemic (Table I).

The present study was attempted to explore a possible relationship of LBW with maternal hypovitaminosis D and periodontal disease. In addition to serve as an essential micronutrient for calcium balance, vitamin D is a modulator of immunity (through induction of defensins such as Cathelicidin LL-37) and through its bone mineral density effect.[15] Thus, it can potentially play a part in the inflammation caused by periodontal disease.

Considerable amount of research has been done on exploring the relationship of adverse pregnancy outcome and periodontal disease. Ide and Papapanou inferred in a meta-analysis that low birth weight and preterm birth are significantly linked to maternal periodontitis.[16] Despite the substantial evidence in favor of that association, the presence of substantial heterogeneity among the studies warranted the researchers to interpret the evidence with caution.

The relationship between adverse pregnancy outcomes and hypovitaminosis D has also been established.[6] Aghajafari *et al*. carried out a systematic review on 31 studies and reported that hypovitaminosis D in mothers is associated with adverse pregnancy outcomes such as preeclampsia, small for gestational age, gestational diabetes, and low birth weight.[7] Wei *et al*. in their meta-analysis of 24 studies confirmed that maternal hypovitaminosis D is linked with adverse outcomes such as preeclampsia, pre-term birth, small for gestational age and gestational diabetes.[8]

Nearly 85% mothers in the present study had vitamin D deficiency. Presence of maternal hypovitaminosis D among pregnant is a disturbing situation. Vitamin D deficiency or insufficiency is now recognized as a public health problem. A local study reported that >90% Pakistani females of child bearing in certain geographical areas of country, are deficient in their plasma vitamin D concentration.[17]

It is interesting to note that although periodontal disease has been identified as a risk factor for low birth weight,[18-20] but the effectiveness of periodontal treatment such as non-surgical periodontal scaling on improving the birth outcomes has been inconsistent.[8,20] This has made the management of periodontal diseases, among pregnant women, a challenge for dentists, obstetricians and related public health professionals.

Females with periodontal disease or hypovitaminosis D are at a higher risk of delivering low birth weight babies. Moreover, evidence also suggests that periodontitis and maternal hypovitaminosis D are associated with each other.[21] Therefore, finding a simple and cost-effective nutritional intervention that could improve the pregnancy outcomes as well as periodontal and bone health would be a highly desirable endeavor. In this context, attempts have been made to use vitamin D supplementation among pregnant to improve periodontal,[22] or maternal outcomes,[23] but without any major success.

No significant association of LBW with other variables could be detected in the present study (Table II). This is

probably due to low frequency of periodontal disease in the present sample. This is the most important limitation of the present study. Out of the 62 participants in the study, only seven had periodontal disease, and of which only four exhibited LBW. This can be explained by the fact that study participants were selected irrespective of their periodontal status. Other limitation of the present study includes a cross-sectional study design where no biological plausibility of maternal hypovitaminosis D or periodontal disease could be detected on the occurrence of LBW.

As an ethical responsibility of providing oral health education and required dental care to the study participants, a two-week dental camp was set up by the investigators at the study site after the conclusion of the study. Basic clinical dental care, such as teeth extraction and scaling, was provided free of cost to the study participants by trained dentist. The details of those services are documented elsewhere.[24]

CONCLUSION

No association was observed between low birth weight and hypovitaminosis D among study participants. Similarly, maternal periodontal disease was also not found to be associated with the low birth weight.

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ORIGINAL ARTICLE

Determination of Specificity and Pattern of Antinuclear Antibodies (ANA) in Systemic Rheumatic Disease Patients Positive for ANA Testing

Hamid Nawaz Tipu, Muhammad Mukarram Bashir and Waseem Iqbal

Abstract

Objective: To determine probability of finding antinuclear antibodies (ANA) and anti extractable nuclear antigens (ENA) positive samples and associating ANA patterns with anti-ENA reactivities among a consecutive cohort of samples of systemic rheumatic disease patients referred for ANA testing.

Study Design: Prospective cohort study.

Place and Duration of Study: Immunology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from January to June 2016.

Methodology: All the samples referred for ANA testing with clinical suspicion of systemic rheumatic disease were included. After screening, ANA positive samples were subjected to anti-ENA antibodies testing (including anti-SSA, anti-SSB, anti-Sm, anti-RNP, anti-SCL-70 and anti-Jo-1 antibodies) and ANA pattern and titer determination.

Results: Of 4,347 samples received, 397 were positive for ANA (9%). Of 397, 96 (24%) samples positive on ENA screen were tested for anti-ENA reactivity. Anti-SSA antibodies were found in 59 samples. Commonest ANA patterns were coarse and fine speckled (43 and 22 samples of 81 tested), while majority of samples carried ANA in titers of 1:40 and 1:80 (22 and 18 samples of 81 tested). No specific ANA pattern was associated with any particular anti-ENA reactivity.

Conclusion: Among samples/patients referred for investigations of autoimmune disorders, probability of finding positive ANA is approximately 9%. Of these 9%, about 24% also show reactivity against ENA. Commonest ANA pattern is coarse speckled and majority of such patients carry ANA in titers ranging from 1:40 to 1:80. Commonest ENA reactivity was against SSA.

Key Words: *Antinuclear antibodies. Indirect immunofluorescence. Systemic rheumatic disorders.*

INTRODUCTION

Autoantibodies to different cellular antigens are the characteristic finding in several organ specific and non-organ specific autoimmune (AI) disorders.[1] Among several different autoantibodies, antinuclear antibodies (ANA) are considered to be the commonest and hallmark of systemic rheumatic disorders like systemic lupus erythematosus (SLE).[2] ANA have traditionally been carried out by indirect immunofluorescence (IIF) using HEp2 cells as substrate. However, solid phase assays are now increasingly replacing IIF.[3] ANA may also be found in non-rheumatic subjects like in pregnancy, advanced age, family history of AI diseases, cancers and infections. ANA test results affect a patient's diagnosis, evaluation and treatment; therefore, particular attention should be paid to careful determination of ANA pattern and titer.[4,5] After an initial ANA positive screen, the next step is usually the determination of its specificity called extractable nuclear antigen (ENA) testing. This ENA determination helps in discrimination of different systemic autoimmune disorders and carries diagnostic and prognostic significance.[6,7] ENA encompasses several antigens: Sjogren's syndrome antigen A (SSA/Ro), Sjogren's syndrome antigen B (SSB/La), Smith (Sm), ribonucleoprotein (RNP), histidyl tRNA synthetase (Jo-1), and topoisomerase (Scl-70).

ANA testing is done on HEp2 cells, but it has its own limitations. Assay standardization is difficult due to inter-manufacturers variations and subjective nature of indirect immunofluorescence. SSA, Ro52 and Jo-1 may not be detectable on HEp2 cells despite being present in high titers. Automated pattern recognition systems have recently become available that perform at par with manual immunofluorescence; however, pattern recognition and interpretation still remain a problem.[8] So far, indirect immunofluorescence remains the gold standard for ANA testing, titration, and pattern determination.[9] A positive ANA test in sufficiently high titer is usually followed by specificity determination by ENA testing. This helps establish or exclude the diagnosis of a particular connective tissue disorder (CTD). ENA was initially carried out using gel-based techniques like double immunodiffusion (DID) and later

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with its improvement, counter current immunoelectrophoresis (CIEP).[10] However, due to technical difficulties and low sensitivity, the practical approach is to screen with high sensitivity technique like ELISA followed by high specificity technique like CIEP or immunoblot (IB).[7]

Previously, the authors have determined ANA prevalence of about 3% in healthy subjects.[11] The aim of the present study was to determine the probability of finding (i) positive ANA among large number of referred serum samples along with its titer and patterns, (ii) positive anti-ENA antibodies among positive ANA samples, and (iii) determine the association of different ANA patterns with specific ENA antigens.

METHODOLOGY

This prospective cohort study was carried out at the Immunology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan from January to June 2016 after formal approval of institutional ethical committee. Sampling was done through non-probability consecutive sampling. All the samples referred to us for ANA testing were analyzed by indirect immunofluorescence, using rat liver at a dilution of 1:10 in phosphate buffer saline (PBS). There were no exclusions. The tissue sections were incubated with diluted serum samples in moist chamber and then washed. It was followed by incubation with prediluted fluorescene isothiocyanate (FITC) conjugated anti-human immunoglobulin G (IgG) (Bioscientifica SA, Argentina), again washed and mounted with 1,4-diazabicyclo 2.2.2 octane (DABCO) medium. Two independent observers observed the slides on Olympus BX51 fluorescent microscope. These rat liver ANA positive samples were then screened for ENA using ENA ELISA kit (Euroimmun, Germany) according to manufacturer's recommendations. Cut off was 20 RU/ml as recommended. Samples positive on ENA screening were subjected to ENA specificity determination (SSA, SSB, Sm, RNP, Jo-1 and Scl-70) using enzyme immunoassay (GenBio ImmunoDot Autoimmunity Screening Panel 6, California, USA), according to manufacturer's recommendations. This was a line immunoassay and results were recorded as positive or negative. Indirect immunofluorescence on HEp2 cells (Immco Diagnostics, United States) was carried out by using same staining procedure as for rat liver. Pattern and titer were recorded by two independent observers. Where disagreement existed, the samples were again observed together and agreement reached. Where ANA results were equivocal or weak positive, samples though were included in analysis but were not graded for titer. Results were recorded in Statistical Package for Social Sciences (SPSS) version 23.0 and analyzed for frequency and percentage.

RESULTS

Over a course of six months study period, the authors received a total of 4,347 serum samples for ANA testing, out of which 397 (9.13%) were found positive on initial screening on rat liver cells. Out of these 397 samples, only 96 (24.2%) were positive for anti-ENA antibodies on initial ENA ELISA screen. These 96 samples were then included in final analysis and subjected to indirect immunofluorescence on HEp2 cells and ENA specificity determination, using enzyme immunoassay. The age of these 96 patients ranged from 1-80 years, and included 78 females (81.2%) and 18 males (18.8%). Of these 96 analyzed samples, 81 were clearly positive for ANA, while 15 were either equivocal or weak positive. These 15 samples were included in anti-ENA specificity determination though not graded for fluorescence titer or pattern on HEp2 cells.

Table I shows different ANA patterns seen against ANA titers. Coarse speckled was the predominant pattern

Table I: Relationship of different ANA patterns with ANA titers.

ANA Pattern	ANA titer							Total
	Not determined (Equivocal/weak positive)	10	20	40	80	160	320	
Not determined	15	0	0	0	0	0	0	15 (16%)
Coarse speckled	0	11	5	9	9	5	4	43 (44%)
Fine speckled	0	2	2	9	4	2	3	22 (23%)
Homogenous	0	0	2	4	5	1	2	14 (15%)
Peripheral	0	0	0	0	0	1	1	2 (2%)
Total	15 (16%)	13 (14%)	9 (9%)	22 (23%)	18 (19%)	9 (9%)	10 (10%)	96 (100%)

Table II: Distribution of different ENA specificities among different ANA patterns observed.

	SSA	SSB	Sm	RNP	Jo-1	Scl-70
Not determined	14 (23.7%)	1 (4.2%)	1 (3.7%)	1 (3.3%)	0 (0%)	1 (9.1%)
Coarse speckled	21 (35.6%)	12 (50%)	10 (37%)	16 (53.3%)	2 (100%)	6 (54.5%)
Fine speckled	10 (17%)	5 (20.8%)	11 (40.8%)	9 (30%)	0 (0%)	3 (27.3%)
Homogenous	12 (20.3%)	4 (16.7%)	5 (18.5%)	4 (13.4%)	0 (0%)	1 (9.1%)
Peripheral	2 (3.4%)	2 (8.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	59 (100%)	24 (100%)	27 (100%)	30 (100%)	2 (100%)	11 (100%)

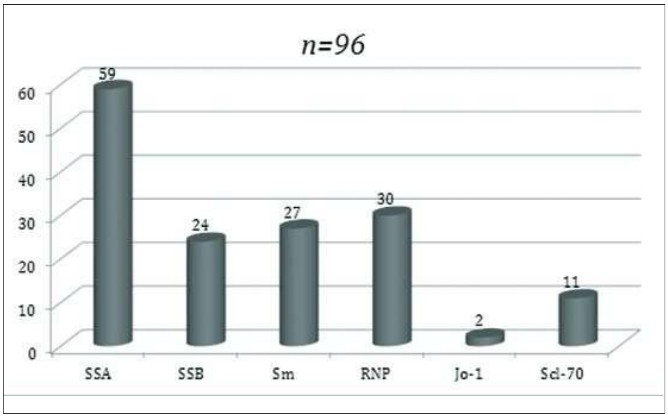

Figure 1: Total number of samples positive against ENA.

found in 43 cases (44.8%), followed by fine speckled pattern in 22 cases (23%), while peripheral pattern was rare, only 2 cases (2%). 22 samples (23%) carried a titer of 1:40, while 18 samples (19%) had a titer of 1:80.

Figure 1 shows number of samples positive for each antigen. SSA was the antigen detected in maximum samples, 59 (61.4%) while Jo-1 was detected in only 2 samples (2%). Fifty-eight samples showed reactivity against single antigen only, while remaining 38 were reactive against more than one antigen.

Table II shows the probability of finding specific anti-ENA antibodies against different ANA patterns observed, in terms of percentages. It is evident that reactivity against SSA, SSB, RNP, Jo-1 and Scl-70 are observed most often when ANA pattern is coarse speckled while anti-Sm antibodies are most often present when ANA pattern is fine speckled.

DISCUSSION

ANA being the characteristic laboratory finding in most of the CTDs, carry important diagnostic significance. Previously, the authors had determined ANA prevalence in healthy subjects to be 3%;[11] while in this study, it was 9.1%. However, in current study sample population consisted of cases referred for investigations of CTDs, in contrast to previous study where these were healthy subjects. Minz, *et al*. determined ANA prevalence of 12.3% in samples screened for AI disorders in India; whereas, it is 5.92% in China in general population.[12,13] Ahmed, *et al*. determined 72.3% samples positive for ANA testing in Bangladesh. Their high results might be due to referral only by rheumatologists and small number of samples (n=152).[14] This knowledge of ANA prevalence is important since it is common to find ANA in healthy population especially in elderly, and in disorders other than CTDs.

Prevalence of anti-ENA antibodies among ANA positive samples was 24.2%, in concordance with Stamouli *et al*. who have determined that among positive ANA samples, 21.36% of samples react against ENA.[15] The positive samples were fine tested on HEp2 cells for titer and pattern determination and these parameters were correlated with anti-ENA specificity. Of these 96 samples, 15 were equivocal or weak positive and their titers and patterns were not determined on HEp2 cells; however, these were tested to detect if these contained any anti-ENA specificity. Speckled pattern was highest (both coarse and fine speckled), in 65 of 81 (79%) samples tested for pattern, in concordance with Ahmed *et al.* who determined it to be 50.8%.[14]

Regarding anti-ENA antibodies, 58 samples showed reactivity against single antigen, while remaining 38 were positive against more than one antigen. Commonest reactivity was against SSA where 59 samples were positive, followed by RNP (30), Sm (27) and SSB (24). Jo-1 was least reactive antigen, against two samples only. Reactivity against SSA is the commonest finding in patients of AI disorders as determined by Peene, *et al*.[16] Although their ANA prevalence (23.5% vs. the present 9.1%) was high but commonest ANA pattern (speckled) and anti-ENA positivity (theirs 21.1% vs. the present 24.2%) were similar. Our reactivities were SSA (61.4%), SSB (25%), Sm (26%), RNP (31%), Jo-1 (2%) and Scl-70 (11%) in contrast to Peene *et al*. who showed these to be 10.5%, 6.7%. 1.8%, 2.7%, 0.2%, 1.2%, respectively and Ahmed *et al*. findings which were 14.2% (SSA), 5.7% (SSB), RNP (25.7%) and Scl-70 (20%). Difference in ENA reactivities is due to inclusion of anti dsDNA antibodies in their analysis, accordingly reducing positive percentage of ENA reactivities and large number of ANA positive samples. In dogs however, anti RNP and anti Sm antibodies are more frequent as compared to other ENA antibodies among speckled ANA positive group.[17]

Speckled pattern (Table II) was highest among all ENA specificities since it was present in 65 of 81 samples tested for pattern. Coarse speckled patterns was associated with most of the ENA specificities but, in fact, any pattern may be found with any anti-ENA antibody and more than one ANA patterns may be present in one sample.[18] The distinction between them then requires sufficient sample dilution to clearly distinguish between two patterns.

The data from this study is useful in estimating approximate probabilities of detecting positive ANA and anti-ENA antibodies, along with commonest ANA pattern and their anti-ENA antibodies association.

CONCLUSION

Among samples/patients referred for investigations of autoimmune disorders, probability of finding positive ANA is approximately 9%. Of these 9%, about 24% also show reactivity against ENA. Commonest ANA pattern is coarse speckled and majority of such patients carry ANA in titers ranging from 1:40 to 1:80. Commonest ENA reactivity was against SSA.

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Epidemic of *Kala Pathar* (Paraphenylene Diamine) Poisoning: an Emerging Threat in Southern Punjab

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ABSTRACT

Objective: To assess cases of the spectrum of *Kala Pathar* poisoning in all age groups.

Study Design: Cross-sectional study.

Place and Duration of Study: Combined Military Hospital (CMH) Bahawalpur and Bahawal Victoria Hospital (BVH), Bahawalpur, from January 2016 to April 2017.

Methodology: All the cases of *Kala Pathar* (Paraphenylene diamine (PPD)) poisoning, admitted and treated at the study places during said period were included in the study. The assessed variable included gender, age, education status, socioeconomic status, reason of poisoning and mortality. Chi-square was applied for qualitative variables with p-value less than 0.05 was considered significant.

Results: A total of 1,258 cases of PPD poisoning were included in the study; 814 (64.7%) females and 444 (35.3%) males. Their age ranged from 5 - 63 years, with median age 21 (IQR 4). Sixty-six (5.2%) were children and the rest 1,192 (94.8%) were adults. In adults 1,125 (94.37%) cases of PPD poisoning were suicidal and 62 (5.20%) accidental cases; only 5 (0.42%) adults were intentionally poisoned. On the other hand, only one child took it with suicidal intent, 54 (81.81%) ingested it accidentally and 11 (16.66%) children were given poison deliberately with the intent to murder. The overall mortality was 24.08% – 22.81% in adults, and 46.96% in children.

Conclusion: *Kala Pathar* (PPD) is a lethal substance when ingested. PPD poisoning is not limited to adults; many cases of pediatric poisoning are also being reported in Southern Punjab. Mortality due to *Kala Pathar* is high. Tracheostomy should be done immediately in all such cases; and high intensive multidisciplinary approach is required.

Key Words: *Deliberate self-harm. Hair dyes. Kala Pathar. Paraphenylene diamine. Poisoning.*

INTRODUCTION

According to World Health Organization (WHO), the incidence of suicide is increasing every year, making it a public health concern.[1] Most common way for this deliberate self-harm is poisoning. Lack of knowledge about the toxicity of available poisons and paucity of medical services lead to high mortality in developing countries.[2] In Pakistan, although pesticide poisoning remains the leading cause, but poisoning with paraphenylene diamine (PPD), locally known as '*Kala Pathar*', is emerging as important means of intentional self-harm.[3-5]

Kala Pathar (black stone) is a low-cost and readily available hair dye in Pakistan. Its chemical ingredient PPD is a toxic and lethal substance when ingested.[6] PPD is a coal tar derivative, used as hair dying agent after mixing with hydrogen peroxide and ammonia. It is also used for tattooing due to its darkening effect when it is mixed with henna (Lawsonia Alba).[7,8]

PPD can be ingested accidentally, can be taken orally for suicidal purpose or can also be given to someone for attempted murder.[7] PPD ingestion is a common, low-priced and easy way of poisoning, becoming an emerging trend of self harm in adults in many developing countries of Asia, including Pakistan.[6] However, its poisoning in children is almost always accidental or with intent to murder with a very high mortality.[6,7] Unfortunately, it is easily accessible in our region.[6,8]

PPD can cause contact dermatitis in susceptible individuals.[9] However, major systemic toxicity occurs after ingesting it.[10] After ingestion, PPD causes edema of face, neck, tongue, pharynx and larynx. Its poisoning also causes angioneurotic edema, rhabdomyolysis and renal failure.[11] PPD is known to cause multiple organ dysfunctions. It can cause death within 6-24 hours because of angioneurotic edema or fatal cardiac toxicity.[12,13] The outcome depends mainly on the amount ingested. The exact lethal PPD dose is not known, estimates vary from 7-10 grams.[11,13] There is no antidote available for this toxic substance. The mainstay of management is conservative.[14]

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The purpose of this study was to evaluate and assess the age-wise cases of PPD poisoning who were admitted and treated at two main hospitals of Bahawalpur.

METHODOLOGY

This cross-sectional study was carried out at the Combined Military Hospital Bahawalpur and Bahawal Victoria Hospital, Bahawalpur after taking the permission from the administration of both the hospitals. All the cases of *Kala Pathar* PPD) poisoning who were admitted and treated in emergency, pediatric or medicine ward of these two hospitals from January 2016 to April 2017 were included in the subject study. The patients having history of mixed poisoning or patients with other medical comorbidities were excluded from this study.

Data assessed included gender, age, education status, socioeconomic status, mode of poisoning (accidental, suicidal or with intent of murder), tracheostomy operation, mortality, mortality within first 48 hours etc. Patients aged less than 12 were grouped as children and those above 12 were grouped as adults. Education status was divided into different categories as; illiterate (no formal education), primary, matriculate, intermediate and bachelor and above. Individuals with family income less than Rs. 20,000 per month were termed "low socioeconomic class", families with income > Rs. 20,000 and less than Rs. 100,000 per month were termed as "middle class", and those with income > Rs. 100,000 rupees per month were classified as "high class". The groups (children and adults) were compared in terms of age, gender, education, socioeconomic background, reason of poisoning, and mortality.

All the data were entered and analyzed in statistical package for social sciences (SPSS) version 20. Chi-square test was applied for qualitative variables and T-test was applied for quantitative variables. Frequencies with percentages were given for qualitative data; while mean with standard deviation or median with inter-quartile range was given for quantitative data. P-value less than 0.05 was considered significant.

RESULTS

In the present study, 1,258 cases of *Kala Pathar* poisoning were assessed. There were 814 (64.7%) females and 444 (35.3%) males. Their age range was from 5 - 63 years, with overall median age 21 (IQR 4.0). Out of which, 66 (5.2%) were children (<13 years) and rest 1,192 (94.8%) were adults (>13 years). In adults, median age was 21.0 (IQR 4) years, and among children, median age was 11 (IQR 3) years (Table I).

Among children, there were 43 (65.15%) males and 23 (34.84%) females. In contrast, among adults the majority were females i.e. 791 (66.36%), and 401 (33.64%) were males.

Table I: Demographic profile.

Demographic variables	Group		Total (N=1,258)	P-value
	Adults (N=1192)	Children (N=66)		
Socioeconomic status				
Low	758 (63.59%)	60 (90.90%)	818 (65.02%)	<0.001
Middle	417 (34.98%)	6 (9.09%)	423 (33.62%)	
High	17 (1.43%)	0 (0%)	17 (1.35%)	
Education				
No formal education (illiterate)	746 (62.58%)	63 (95.45%)	809(64.31%)	<0.001
Primary	350 (29.36%)	3 (4.54%)	353 (28.06%)	
Matric	58 (4.86%)	0 (0%)	58 (4.61%)	
Intermediate	28 (2.34%)	0 (0%)	28 (2.23%)	
Bachelor and above	10 (0.84%)	0 (0%)	10 (0.79%)	

Table II: Mortality of intoxicated patients .

Mortality	Group		Total (N=1,258)	P-value
	Adults (N=1192)	Children (N=66)		
Overall Mortality				
Yes	272 (22.81%)	31 (46.96%)	303 (24.08%)	<0.001
No	920 (77.18%)	35 (53.03%)	955 (75.99%)	
Mortality within first 48 hours				
Yes	211 (17.70%)	25 (37.87%)	236 (18.75%)	<0.001
No	981 (82.29%)	41 (62.12%)	1022 (81.24%)	

Among adults 1,125 (94.37%) cases were due to suicidal ingestion, followed by 62 (5.20%) accidental cases. On the other hand, only one child took *Kala Pathar* with suicidal intent, 54 (81.81%) cases ingested it accidentally. Five (0.42%) adults and 11 (16.66%) children were given this poison deliberately by others with ill intent (reportedly murder).

As far as socioeconomic status of the cases is concerned, n=818 (65.02%) belonged to poor back-ground, n=423 (33.62%) were middle class cases and n=17 (1.35%) belonged to high class. The group-wise distribution is shown in Table I. Education status of the cases has been shown in Table II. Majority of poisoning cases n=809 (64.31%) were illiterate (had no formal education). Other demographic profiles are also given in Table I.

The mortality caused by this low-cost and easily available poison was 24.08% (n=303). In adults, mortality was 22.81% (n=272) compared to 46.96% (n=31) in children (Table II). Majority of cases died within first 48 hours in hospital. Overall 18.75% (n=236) cases expired in this time period. In adults, 17.70% (n=211) cases died within first 48 hours compared to 37.87% (n=25) children as shown in Table II.

Tracheostomy was carried out in these cases to relieve breathing difficulty and impending suffocation because this poison causes gross swelling of neck, tongue and intra-oral tissue, causing dyspnea and stridor. Around n=1173 (98.01%) cases underwent tracheostomy operation.

DISCUSSION

Kala Pathar (PPD) poisoning is emerging means of intentional self-harm with high mortality in Pakistan.[2,6] Easy availability, low cost, and harmful effects of this hair dye ingestion makes it a frequent choice for committing self-harm.[6,13] It is used to commit suicide or can be ingested accidentally. It can also be mixed in edibles with criminal intentions and given to innocent victims.

This study findings show females being the primary victims of this poisoning (64.7%) as compared to 35.3% males. The male to female ratio in adults is almost similar to that shown in some regional studies by Qasim *et al*. and Shakuntala *et al*.[14,15] However, some studies conducted in India and African countries show much more female victims than this study findings.[15,16] The female preponderance explanation could be its easy availability as hair dye and because of the gender inequalities and the social pressure of the society. However, in children the ratio of male to female was opposite to that in adults, as majority were males. The reason for majority of males in children is because parents let the male children roam free, more often. They also develop habit of doing errands with the fellow friends of the same age unsupervised.

This study highlights young age group (24 ±9 years) with predominantly unmarried females to be the main victims of PPD poisoning. This is consistent with almost all the studies conducted in this region and African countries.[14,17] Moreover, these findings are also in accordance with the WHO self-harm report for middle and low income countries.[1,14] However, results of a study conducted in Sindh are in contradiction to these findings, where majority of females (57%) were married. The possible explanation given by the author was the practice of early marriages in Sindh.[14] As seen in the present study, PPD ingestion was chiefly for deliberate self-harm/suicide in adult population. On the other hand, in children the chief cause of intake was accidental ($p<0.001$). There were 66 cases of PPD poisoning in children, which is an alarming sign. There was no study with such a large number of cases in pediatric population of this poisoning. Another alarming situation was the use of PPD in many children (16.66%) by strangers or other people with intent to murder.

Majority of the cases belonged to lower socioeconomic class and few cases in middle socioeconomic back-ground. As far as education status of cases was concerned, majority (64.31%) had no formal education or till primary (28.06%). Only few were bachelors or highly qualified individuals among the affected. It showed a reciproid relation between the level of education and socioeconomic class and *Kala Pathar* poisoning; the lower the education and socioeconomic status, more frequent were the cases of *Kala Pathar* intake. Results of other studies conducted in Pakistan and India, were in agreement with ours.[14,17]

The mortality caused by *Kala Pathar* was 24.08% in this study. The mortality in this study was almost similar to that shown by Qasim *et al*. (21%).[14] Similarly, Khan *et al*. reported 20% mortality due to PPD ingestion.[18] However, Khuhro *et al*. documented a higher mortality in their study, which was 37.5%.[13] Akbar *et al*. had reported even higher mortality rate i.e 47.4%.[6] As PPD poisoning results in gross cervicofascial and neck swelling, it causes severe shortness of breath and stridor; so, tracheostomy is carried out to relieve suffocation.[19] The higher mortality rates in the later studies were due to lack of proper and immediate care to these intoxicated patients including late or no tracheostomy. However, in this study, 98% patient underwent tracheostomy. Tracheostomy was done in 60% of cases in the study by Akbar *et al*.[6] and in 87.5% cases in the study done by Khuhro *et al*.[13]

Government should ban/curtail this hair dye, being responsible for so many deaths each month, and easily available at a low cost. Laws should be framed pertaining to injudicious supply to people/shops where every person can get it.

CONCLUSION

Kala Pathar (PPD) is a lethal substance when ingested. In Southern Punjab, large number of cases present every month with this poisoning. This poisoning is not limited to adults, many cases of pediatric poisoning are also reported in this region, which is an alarming sign. Mortality due to *Kala Pathar* is high. Tracheostomy should be done immediately in all such cases, and high intensive multidisciplinary approach is provided.

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Demographics and Outcome in Paediatric Non-Hodgkin Lymphoma: Single Centre Experience at The Children Hospital, Lahore, Pakistan

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ABSTRACT

Objective: To describe the patient demographics and outcome analysis in paediatric non-Hodgkin lymphoma (NHL) patients.

Study Design: An observational study.

Place and Duration of Study: The Hematology/Oncology Unit of The Children's Hospital and Institute of Child Health, Lahore, from January 2012 till December 2014.

Methodology: Demographics including age, gender, histopathology, stage and outcome data, in biopsy proven NHL patients were analyzed. Burkitts/B Cell and Diffuse Large B Cell lymphoma patients were treated with MCP 842 Protocol while T/B-cell lymphoblastic lymphoma (LL) patients were treated with EURO-LB 02 protocol.

Results: Ninety-one patients were treated during the study period at CHL. Data was insufficient in 18 patients, so they were excluded from the study. Patients included were 73. Males were 53 (72.6%). Thirty-seven (50.7%) were 5-10 years of age, and 22 (30.1%) 10-16 years old. Abdominal mass was the commonest presentation seen in 32 (43.8%), lymphadenopathy in 27 (37%), intussusception in 5 (6.8%), while intestinal obstruction, obstructive uropathy, nasopharyngeal mass, gastric mass, primary bone lymphoma, pericardial effusion, jaw swelling, cheek swelling and paraspinal mass present in one (1%) each. Histopathological subtypes consist of Burkitt's lymphoma (BL) in 32 (43.8%), B cell NHL in 10 (13.7%), lymphoblastic lymphoma (LL) in 26 (35.6%), diffuse large B cell lymphoma (DLBCL) in 2 (2.8%), and anaplastic large cell lymphoma (ALCL) in 1 (1.4%). Sixty-seven (91%) presented in stage III, and six (8.4%) in stage IV. Forty-eight (65.8%) patients had completed treatment and are well to date, 16 (21.9%) died, 5 (6.8%) left against medical advice (LAMA), and 4 (5.5%) patients relapsed.

Conclusion: Burkitt's lymphoma was the commonest type of NHL seen in this cohort that predominantly presented with an abdominal mass. Children usually presented in advanced stage with delayed diagnosis. Better supportive care can improve the prognosis significantly. Training of pediatricians is equally important along with increasing parental/family knowledge about the disease symptoms so that they can seek early medical care, and earlier diagnosis is possible.

Key Words: *Non-Hodgkin lymphoma. Abdominal mass. Lower-middle income countries. Burkitt's lymphoma.*

INTRODUCTION

Lymphomas constitute 10-15% of childhood malignancies.[1] Non-Hodgkin lymphoma (NHL) accounts for approximately 60% of all lymphomas in children and adolescents.[2] Childhood NHL occurs predominantly in males than females, except primary mediastinal B-cell lymphomas, which have the same prevalence for both males and females.[3] They constitute heterogeneous group of malignant tumors of lymphoid tissues derived from mature B or T cells. Clinical presentation of NHL varies tremendously, depending upon the histological subtype and the areas of involvement. Majority of patients present in advanced stage especially in the developing countries. Despite this, there is significant improvement in prognosis with survival rates for localized disease reaching upto 90-95% and 60-90% with advanced stage disease.[4] Majority of chemotherapy protocols use high dose methotrexate, which has significant toxicity and poor tolerance in developing countries. There is paucity of data from developing countries on pediatric NHL.

The aim of this study was to describe the patient demographics and outcome in pediatric non-Hodgkin lymphoma (NHL) patients at a tertiary care hospital in Pakistan.

METHODOLOGY

This was an observational study done in the Pediatric Hematology/Oncology Department at The Children's Hospital and Institute of Child Health (ICH), Lahore, from January 2012 to December 2014. Approval was taken from the Hospital's Institutional Review board. Inclusion criteria consist of both males and females, aged 1 to 16 years with biopsy proven NHL. Children with relapsed NHL or unclear histopathological diagnosis were

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excluded. Data was recorded from the patients' notes. Data analyzed include age, gender, histopathological subtypes, disease stage, and final outcome. Patients were treated as per MCP 842 guidelines for Burkitts, Burkitt's-like, and B cell NHL. EURO-LB 02 protocol was utilized for T-cell and B-cell LL. Surgery and radiation therapy was given, if required as per protocol guidelines.

Data was entered and analyzed by using SPSS 20.0. P-value of less than 0.05 was considered significant. Percentages and frequencies were calculated. Chi-square test was used to review relationship of age, gender, histology, symptoms and stage with diagnosis, and histological types with outcome.

RESULTS

Ninety-one pediatric patients with biopsy proven NHL were managed at the Hospital during the study period. Eighteen patients had insufficient data, so were excluded from the study. Total 73 patients fulfilled the inclusion criteria. Males were 53 (72.6%, p=0.95). Thirty-seven (50.7%) were 5-10 years of age, 22 (30.1%) 10-16 years of age, (p=0.28). Commonest presentation was abdominal mass seen in 32 (43.8%), lymphadenopathy 27 (37%), intussusception 5 (6.8%), intestinal obstruction, obstructive uropathy, nasopharyngeal mass, gastric mass, primary bone lymphoma, pericardial effusion, jaw swelling, cheek swelling, and paraspinal mass seen in one patient each. In the histology, 32 (43.8%) had BL, 10 (13.7%) had B cell NHL, 26 (35.6%) had LL, two (2.7%) had DLBCL, and one (1.3%) patient had ALCL. The histology was inconclusive in two (2.7%) cases. Predominantly, patients presented in advanced stages III, (n=57, 78%) and IV (n=15, 20.5%, Table I).

Forty-eight (65.8%) patients completed treatment and are well to date, five (6.8%) left against medical advice 16 (21.9%) expired, and four (5.5%) relapsed (Table II).

DISCUSSION

Non-Hodgkin lymphomas (NHLs) are tumors arising from the lymphoid tissues. Most childhood and adolescent NHLs are high-grade tumors and usually show aggressive clinical behavior in comparison to NHL in adults, which usually are low- to intermediate-grade indolent tumors. NHL constitute 7.5% of childhood cancers in the advanced world.[5] According to the National Cancer Institute (NCI), pediatric NHL can be

Table I: Patient characteristics.

Parameters	Histopathological diagnosis					Total	P-value
	Burkitts	B cell NHL	LL	LCL	Misc		
Age							
1-5 years	5	4	5	0	0	14 (19.2%)	
5-10 years	18	5	9	3	2	37 (50.7%)	
10-16 years	9	1	12	0	0	22 (30.1%)	0.28
Gender							
Male	10	3	6	0	0	53 (72.6)	
Female	22	7	20	3	2	20 (27.4%)	0.95
Presentation							
Abdominal mass	22	7	1	1	1	32 (43.8%)	
Lymphadenopathy	1	1	23	1	1	27 (37%)	
Intussusception	4	0	0	0	0	5 (1.4%)	0.00
Intestinal obstruction	1	0	0	0	0	1 (1.4%)	
Misc	4	2	2	0	0	8 (10.9%)	
Stage							
Stage I	0	0	0	0	0	0	
Stage II	1	0	0	0	0	1 (1.36%)	
Stage III	22	10	22	3	2	57 (78%)	0.93
Stage IV	9	0	4	0	2	15 (20.5%)	
Bone marrow involvement							
Not Involved	23	10	22	3	0	58 (79.4%)	
Involved	9	0	4	0	2	15 (20.5%)	

Table II: Outcome analysis.

Diagnosis	Outcome				Total	P-value
	Expired	LAMA	Relapse	Treatment completed		
Burkitts	10	4	1	17	32 (44%)	
B NHL	0	1	0	9	10 (13.6%)	
LL	4	0	2	20	26 (35.6%)	0.02
LCL	0	0	1	2	3 (4.1%)	
Misc	2	0	0	0	2 (2.7%)	
Total	16 (21.9%)	5 (6.8%)	4 (5.5%)	48 (65.8%)	73 (100%)	

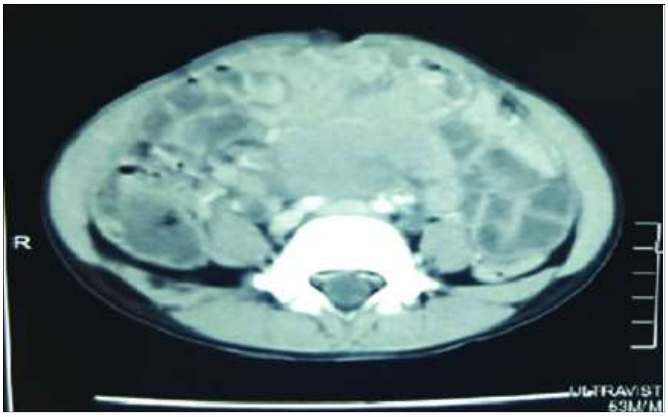

Figure 1: Typical CT scan findings in abdominal Burkitt's lymphoma.

categorized into lymphoblastic lymphomas, small non-cleaved cell lymphomas (SNCCLs, Burkitt lymphomas and Burkitt-like lymphomas) and large cell lymphomas (LCLs) and staged according to extent of dissemination.[6] The clinical features of pediatric and adolescent NHL vary, based primarily on pathological subtype and involvement sites. At present, 80 to 90% of children with NHL in developed countries, are cured by intensive, risk-adapted chemotherapy. However, in developing countries, data is lacking due to absence of national cancer-based registry. The study place is the largest referral center in the public sector hospital in Pakistan.

NHL incidence in children and adolescents depends upon age, histopathology, gender and race. Childhood NHL is more common in males as compared to females in this study as evident from other studies also.[7,8] Majority of children presented are between 5 to 10 years of age, which correlates with other studies.[9,10] Geographical variations exist in the presentation of NHL.[11] BL is the most common histological type in the Asian countries as mentioned in literature and was also our observation in this study. Commonest presentation (Figure 1) of BL was with an abdominal mass,[12,13] and evident in this study as well (p=0.001). Whereas, in literature, endemic BL is by far the commonest type primarily being found in equatorial Africa,[14] and other developing countries. It usually present with jaw swelling which was not seen in this study and in other studies from developing countries. Lymphomas are rapidly growing tumors and as is the case in other developing countries, patients present late with widespread disease,[15] majority of patients in this study also presented in advanced stage i.e. in stage, 3 or 4. LL was seen in 35.6 % of patients in this study, and commonly presented with a mediastinal mass. LLs are mainly of the T-cell type, with only 10-20% being of the B-cell.

In the developed nations, predominant types of NHL are BL, DLBCL, LTC or BCL, and ALCL. Other types (e.g., DLBCL PBL follicular lymphoma (FL), primary bone lymphoma (PBL), marginal zone lymphoma) are less common. DLBCL, FL and PBL were also rare in this cohort of patients, seen in only one patient.[16]

Worldwide, prognosis of B cell non-Hodgkin lymphoma has been reported to be upto 80-90% in the developed countries,[17,18] and is only slightly lower for LL and ALCL, using intensive chemotherapy regimens. However, in underdeveloped countries prognosis is still not the same. Overall survival in this study was 65% which is comparable with data from developing countries.[19] In this study, 16 patients (21.9%) died, a situation similar to many developing countries (23.8%). Marked difference was observed in the outcomes with different histological subtype in this study. LL and BL had better prognosis compared to other histological types. Mortality rate is high as compared to developed world and is usually due to delayed presentation, lack of supportive care services, life-threatening infections, Malnutrition and side effects of chemotherapy.[20] A significant number of patients in this study abandoned treatment or were lost to follow-up after completing therapy, which is a common problem when compared with studies from other developing countries.[21,22] Among the factors effecting outcome, lower educational level of patients, myths pertaining to diagnosis of cancer and socioeconomic condition of the patients must be taken into account that may effect treatment compliance.

However, this is a single center study involving small number of patients. There is a dire need of multicenter studies involving large number of patients to determine exact disease behavior in our country, so as to tailor therapies, which are less toxic and more effective.

CONCLUSION

BL presenting with an abdominal mass was the most common type of NHL seen in our study. Majority presented in advanced stage. Chemotherapy was usually well tolerated by the patients. Better supportive care, family counseling and adopting protocols that are more tolerable in local setup with limited toxicities can improve the prognosis. There is a dire need to in the developing countries of establishing shared care units to provide treatment near patients' hometowns and to prevent therapy abandonment. There is a further need for larger multicenter studies to document exact disease behavior in our country.

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Spectrum of Gynecological Malignancies at Jamshoro

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ABSTRACT

Objective: To describe the frequency and spectrum of different types of gynecological malignancies.

Study Design: An observational study.

Place and Duration of Study: Department of Obstetrics and Gynaecology, Liaquat University of Medical and Health Sciences, Jamshoro, from January 2014 to December 2015.

Methodology: All patients with gynecological malignancies, admitted at the study place, whether previously diagnosed or need to establish the diagnosis on the basis of EUA, biopsy, staging and for surgery during the study period were included. All patients with benign gynecologic masses were excluded. Studied variables were age, marital status, parity, education, socioeconomic status, clinical presentation, clinical diagnosis, tumor site and surgical procedure. The diagnosis and type of malignancy was confirmed on the histopathology report of the specimen taken.

Results: There were a total of 65 patients (6.39%) with gynaecological malignancies. The median age was 15 years (46.26%). Ninety-four percent were married. Seventy-one percent had a poor socioeconomic status. Cervical cancer was most common as seen in 27 (41.5%) followed by ovarian malignancy in 21 (32.3%) of cases, uterine caners in nine (13.8%), vaginal cancer in six (9.2%), and vulval cancer in three (4.6%) of cases. The common histopathological type of cervical cancer was moderately differentiated squamous cell carcinoma (n=19, 29.2%), papillary serous cyst adenocarcinoma in ovarian (n=13, 20.0%), and squamous cell carcinoma in vagina and vulva. There was no case of primary fallopian tube malignancy.

Conclusion: Gynecological malignancies are common in younger age group. The need is to strengthen screening practices, making it easy and cost-effective for all the general and at risk population, so that early detection and treatment can be possible to control the female genital tract cancers.

Key Words: *Gynecological malignancy. Frequency. Pakistan. Cervical cancer. Uterine cancer. Squamous cell carcinoma.*

INTRODUCTION

Gynecological cancers are a leading cause of cancer-related deaths worldwide. Malignancies related to female genital tract encompass cervical, ovarian, uterine, endometrial, gestational trophoblastic tumors, vulva, vagina, and fallopian tubes malignancies. In developing countries, the epidemiology of these cancers is different from those in developed countries.[1] Cervical cancer is the most common female genital tract malignancy in underdeveloped countries.[2] Cervical cancer in USA is ranked the fourth and in England it is at the seventh position.[3] In India, overall 10 to 15% of gynaecological malignancies diagnosed each year; amongst all cervical cancer is the most common.[4]

According to WHO, in developing countries routine cervical screening still needs to be established due to discrepant low resource setting. There is a lack of awareness of cervical cancer among the populations, healthcare providers, and policy-makers with absence or poor quality of screening programs, limited access to healthcare services, and lack of functional referral system.[5] On the other hand, in industrialized world the routine screening and treatment policy for premalignant cervical lesion has dramatically reduced the incidence of cervical cancer related deaths in last five decades.[6] Ovarian cancer is the most common and poor prognostic cancer in gynecological malignancies; its five-year survival rate is only 30% in the United Kingdom.[7]

Almost 75% of ovarian cancers present in advanced stages due to non-specific symptoms and deep situation in the pelvis leading to failure in early detection of tumor. Sub-optional screening methods, especially in low resource countries also affect.[8] Although endometrial cancer is supposed to be the least challenging because of its early detection, good prognosis and cure rates; but in developed countries, ovarian and endometrial malignancies are still the leading forms of female genital tract cancers.[7,9,10] Vulval and vaginal cancers are the rare forms of gynaecological malignancies.[7]

Despite the high frequencies of gynecological malignancies in Pakistan, still there is paucity on this subject and lack of availability of accurate population-based statistics to reliably measure the incidences of different female genital tract cancers. Therefore, the present study was conducted to observe the frequency and spectrum of different types of gynecological malignancies in our community.

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METHODOLOGY

This study was conducted at the Department of Obstetrics & Gynaecology, Unit IV, Liaquat University of Medical and Health Sciences, Jamshoro, during the two years period from January 2014 to December 2015. Sampling technique was non-probability convenience sampling in which all the patients with gynecological malignancies, admitted in Department of Obstetrics & Gynaecology, Unit IV, whether previously diagnosed or need to establish the diagnosis on the basis of examination under anesthesia (EUA), biopsy, staging and surgery during this study period were included. These women were enrolled after taking informed written consent and approval from Institutional Ethical Research Committee. A predesigned proforma was filled by the Unit doctors. Studied variables were age, marital status, parity, religion, education, socioeconomic status, clinical presentation, clinical diagnosis, tumor site and surgical procedure. The diagnosis and type of malignancy was confirmed on histopathology of the specimen taken. All patients with benign gynecologic masses were excluded.

Data was analyzed on statistical package of social sciences (SPSS) Version 16. The results in the forms of frequencies and percentages for categorical data and median (IQR) were calculated for continuous data.

RESULTS

A total of 65 patients (6.39%) with gynaecological malignancies were admitted, out of the total gynae admissions (1,017) in a 2-year period. Regarding their sociodemographic analysis, the maximum age recorded was 90 and minimum was 16 years. The median age was 46.26 years. Most (59, 90.7%) were Muslims and remaining 7 (10.7%) were non-Muslims, almost all 94% of women were married and 6% were unmarried. Seventy-one percent of them were poor and 29% belong to good family status. These demographic characteristics are shown in Table I.

The most common gynecological malignancy seen was cervical cancer in 27 (41.5%) of study population; and the second common was ovarian malignancy in 21 (32.3%) of cases, uterine caners were seen in 9 (13.8%), vaginal in 6 (9.2%) of cases and vulval cancers in 3 (4.6%) of carcinomas were recorded.

The age of women with cervical cancer range between 29 - 75 years, 25 - 60 years in ovarian. The youngest girl of 16 years suffered from primary vaginal cancer and a 90 years lady from uterine malignancy.

In this study, all gynaecological malignancies were confirmed on histopathology report of samples taken through EUA biopsy and surgery. In cervical cancer, the most common histopathological type observed was moderately differentiated squamous cell carcinoma seen in 19 (29.2%), and well differentiated squamous cell carcinoma in six (09.2%) women. In ovarian malignancy, the papillary serous cyst adenocarcinoma (n=13, 20.0%) was seen most commonly; and in vagina and vulva, the squamous cell carcinoma was noted. There was no case of primary fallopian tube malignancy. The description of histopathological types is given in Table II.

Table I: Demographic characteristics of study women.

Characters	Number (Total = 65)	Percentages
Age in years		
Median	46.26	15
Minimum	16	1
Maximum	90	1
Marital status		
Married	61	93.8
Unmarried	4	6.2
Education		
Uneducated	53	81.5
Primary	12	18.5
Religion		
Muslim	59	90.7
Non-Muslim	7	10.7
Socioeconomic status		
Poor	46	70.8
Well off family	19	29.2

Table II: Distribution of tumor site and histopathological types.

Tumor site	Histopathological type	Number Percentage (Total = 65)
Cervix	-Squamous cell carcinoma, moderately differentiated	19 (29.2%)
	-Squamous cell carcinoma, well differentiated	06 (9.2%)
	-Squamous cell carcinoma, poorly differentiated	02 (3.1%)
Ovaries	-Papillary serous cystadenocarcinoma of ovary	13 (20%)
	-Pappilary mucinous cyst adenocarcinoma	06 (9.2%)
	-Granulosa cell tumor of ovary	02 (3.1%)
Uterus	-Adenocarcinoma uterus	08 (12.30%)
	-Choriocarcinoma	01 (1.5%)
Vagina	-Squamous cell carcinoma vagina well differentiated	04 (6.1%)
	-Clear cell adenocarcinoma of vagina	02 (3%)
Vulva	-Squamous cell carcinoma vulva well differentiated	03 (4.6%)

DISCUSSION

The incidence of gynecological malignancies is related with age.[1] We have seen wide range for age between 16 to 90 years in this study, the young girl was suffering from primary vaginal cancer and the oldest from uterine malignancy. Cervical cancer was most common in the present study, 27 (41.5%), and frequently seen in

between 29 to 50 years of age, less number of cases were seen in older age groups. Similarly, cervical cancer was documented as the commonest genital tract cancer in studies from Africa; 274 (69%) of carcinoma of cervix were observed in a study at Sokoto Hospital, Benin city of Nigeria.[1,11,12]

The same results were seen in a local study from Karachi, Pakistan.[13] On the contrary, a study conducted at Tehran showed low prevalence of cancer of cervix (13.6%); this decreased prevalence was due to wide spread screening program in their country.[14] While the lack of awareness and decreased practices of screening are important contributing factors of cervical cancer observed in this study, with a lack of vaccination against HPV in young girls. HPV infection is a cause in 99.7% of cervical cancers in developed countries. Therefore, implementation of HPV vaccination in the developed world reduced the 75% incidence and mortality of cervical cancer over the past 50 years.[15]

Ovarian malignancy was the second commonest in this study. In a Nigerian study, it was seen on the third number and the histological type reported was serous cystadenocarcinoma as seen in the present study results. In comparison, ovarian tumor was seen most frequent in some other studies from Pakistan.[16]

The endometrial carcinoma found the third place in this study, while it is the commonest gynecological malignancy in developed countries.[17,18] Two cases of choriocarcinoma were recorded in present study; the cure rate of choriocarcinoma is high even with chemotherapy alone. Nndi, *et al.* mentioned it as second most common tumor in their study.[1]

Primary cancer of vagina is rare, usually secondary or metastasis of other tumors are present. In this study, the authors observed a relatively high number (n=06, 9.3%) of cases, and the onset was at younger age in this study, compared with 4.36% in a 14-year review of vaginal cancers among all gynecological malignancies in Nigeria.[1] Three cases (4.6%) of vulval cancers were reported in these patients; almost similar results (3.6%) were found by Tcokeke *et al*.[8] There was a ten-year study conducted at Faisalabad Pakistan, in which vulva/vagina cancers were reported in (9.9%), of cases and the squamous cell carcinoma was the most frequent type, like these results.[19]

The outcome of patients with malignancy was out of scope of this study. However, it was seen that ovarian cancers diagnosed early because of easy access to ultrasound. Post-surgery, they received chemotherapy in collaboration with oncology department. Those with ovarian cancer fared better; while patients with cervical cancer presented to the hospital late in advanced stage, responded poorly.

Gynecological malignancies are common in developing countries. Therefore, it is needed to increase awareness about HPV vaccination in young girls before the start of their sexual activity. Strengthening our screening practices, makes it easy and cost-effective for the general and the at-risk population. So that early detection and treatment can be possible to control female genital tract cancer.

CONCLUSION

The overall frequency of gynecological malignancy was 6.39% with 41.5% cervical cancer, 32.3% ovarian cancer, 13.8% uterine cancer, 9.2% vaginal cancer, and 4.6% vulval cancer.

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The Prognostic Value of Heart-Type Fatty Acid Binding Protein in Patients with Acute Coronary Syndrome

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Abstract

The effect of heart-type fatty acid binding protein (H-FABP) remains unclear on the quality of the prognoses of patients with acute coronary syndrome (ACS). The authors searched the EMBASE, PubMed, Cochrane Library, and Medline electronic databases from their respective inceptions throughout June 2017. Odds ratios were pooled from individual studies, and conducted heterogeneity analysis, quality assessment, and publication bias analysis. A total of four studies with 1,994 patients were included (554 positive patients; 1,440 negative patients). Pooled analysis of these studies showed that patients positive for H-FABP had higher incidence for mortality (OR, 6.39; p<0.01) and cardiac events (OR, 3.94; P<0.01) than negative patients, but myocardial infarction (MI) (OR, 1.43; P=0.32) showed no significant differences. ACS patients positive for H-FABP had higher rates in cardiac events and mortality. The incidence of MI showed no significant difference, possibly because this study was based on limited data from randomized studies. Additional substantial randomized controlled trials certainly needed to verify these discovery.

Key Words: *Acute coronary syndrome. Heart-type fatty acid binding protein. Prognosis. Meta-analysis. Cardiac event. Mortality.*

INTRODUCTION

The effect of heart-type fatty acid binding protein (H-FABP) on the value of the prognoses given to patients with acute coronary syndrome is unclear, and there is still some controversy surrounding its use. Risk-stratification is significant to the management of acute coronary syndrome (ACS) in patients.[1] The Global Registry of Acute Coronary Events (GRACE) and Thrombolysis in Myocardial Infarction (TIMI) risk scores are current ways of evaluating risk-stratification. These methods use the status of elevated biomarkers as risk factors.[2] H-FABP is a cytoplasmic protein that is primarily involved in the co-transport of long-chain fatty-acids and increase rapidly in serum during myocardial injury.[3,4] Studies have shown that incorporating H-FABP into the risk scores improves risk prediction.[5,6] We found H-FABP to be closely associated with the prognosis of acute coronary syndrome.[7] In this way, H-FABP can be used to predict the prognosis of ACS.[8-11]

This meta-analysis was performed to probe into the differences in ACS result in individuals positive and negative for H-FABP.

METHODOLOGY

The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement guidelines were used in this meta-analysis.[12] English-language entries in the PubMed, EMBASE, and the Cochrane Library databases were systematically searched throughout June 2017. MeSH terms and keywords used to identify articles included STEMI or ST elevation myocardial infarction, NSTEMI or non-ST elevated myocardial infarction (MI) or myocardial infarction, or acute myocardial infarction (AMI), or acute coronary syndrome (ACS), and heart-type fatty acid binding protein or H-FABP.

Randomized controlled trials of ACS patients (included STEMI, NSTEMI, and UA) who were positive for H-FABP were evaluated in comparison with those negative for H-FABP. Only randomized controlled trials performed in ACS patients whose follow-up data regarding H-FABP levels were measured at the least one month during index admission were included in the study. Non-cohort studies, those covering chronic stable angina, and those included no follow-up results were excluded.

The data independently extracted from each study were study design, the first author, sample size, location of study, clinical baseline characteristics, proportion of PCI patients, and duration of follow-up. The outcomes assessed included all-cause mortality, cardiac events, the primary endpoint or re-hospitalization for MI or heart failure, and length of stay. The primary endpoint was defined as death or MI. Only two studies had covered MI.[9,11]

Individual risk of bias was evaluated using Cochrane's risk assessment tool for every type of study bias.[13] Data analysis was based on the Cochrane Collaboration and the PRISMA Statement.[12] Review Manager 5.1 (RevMan) was used for meta-analyses. Heterogeneity between studies was assessed using Chi-square tests

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of heterogeneity and I^2 statistics of inconsistency. Low, moderate, and high heterogeneity were defined as I^2 values of 25%, 50%, and 75%, respectively.[14] The Mantel-Haenszel method was used to calculate pooled estimates of their 95% confidence intervals (CIs) and odd risks (ORs). The results were defined as significant at $p<0.05$, and the reported values are two-tailed. A funnel chart, the Begg's log-rank test, and the Egger's test were used to assess publication bias and the small study effect.

RESULTS

A total of 713 articles were obtained from the PubMed, Medline, EMBASE, and Cochrane databases. After a careful evaluation, 212 duplicated articles were eliminated. Among the remaining 501 articles, we removed a further 457 because they were not related to our topic. Forty-four full-text articles were assessed for eligibility. Of these, 40 articles were eliminated because they were either meta-analyses or commentaries or letters to editors or did not report adverse clinical outcomes. Finally, four studies were included in this meta-analysis.[8-11]

This meta-analysis identified a total of 4 retrospective trials. A total of 1,994 patients with ACS, 554 positive for H-FABP and 1,440 negative for H-FABP, were ultimately included in the meta-analysis. The average age was 64.5 years in the study population. The baseline characteristics of studies are represented in Table I. The population characteristics in every study are given in Table 2. In the view of quality evaluation, all studies were found to be at low risk of bias.

The incidence of cardiac events was 18.6% in patients positive for H-FABP *vs*. 6.6% in patients negative for H-FABP. From the three studies the data available for cardiovascular events were extracted, there was little heterogeneity among the results ($p=0.41$, $I^2=0\%$). The group positive for H-FABP showed higher incidences of cardiovascular events than the group negative for H-FABP (OR, 3.94; 95% CI, 2.85-5.44; $p<0.001$, Figure 1).

The incidence of all-cause mortality during the follow-up period was 13.3% among patients positive for H-FABP and 3.3% among patients negative for H-FABP. Negative results for H-FABP were associated with significantly lower incidences of mortality than positive results (OR: 6.39, 95% CI: 4.06-10.07; $p < 0.00001$; $I^2 = 0\%$, Figure 2).

The incidence of myocardial infarction was 3.9% in the group positive for H-FABP *vs.* 3.1% in the negative group. There was no significant difference between the two groups with respect to MI (OR:1.43, 95%CI:0.71-2.89; $p=0.32$; $I^2= 0\%$, Figure 3).

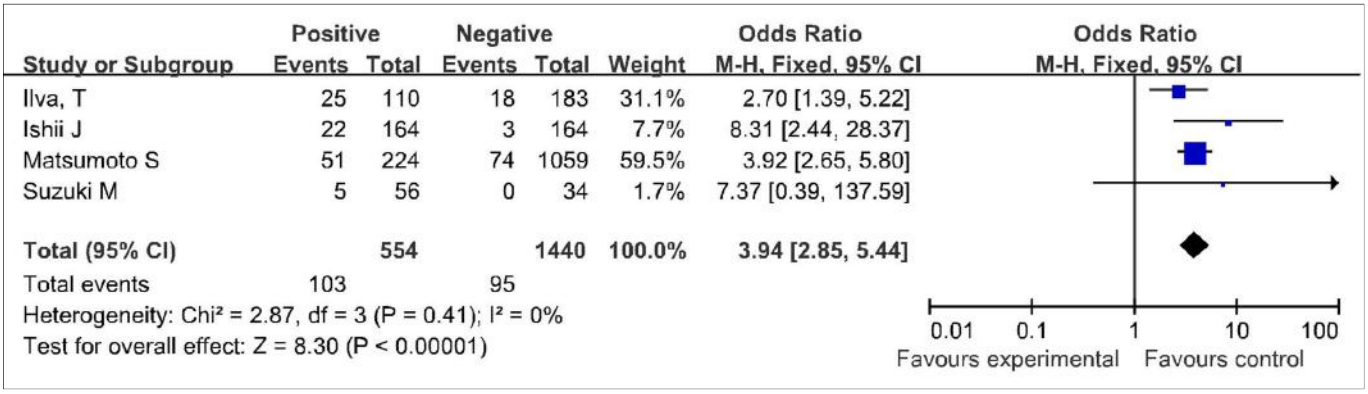

Figure 1: Fixed-effect meta-analysis for cardiac events. The figure shows the number of events, number of patients in the positive and negative groups, odds ratio (OR), and 95% confidence interval (CI) for each trial, overall OR estimate with 95% CI and P-value for association test, P-value for heterogeneity test, and (I^2) measures of trial inconsistency.

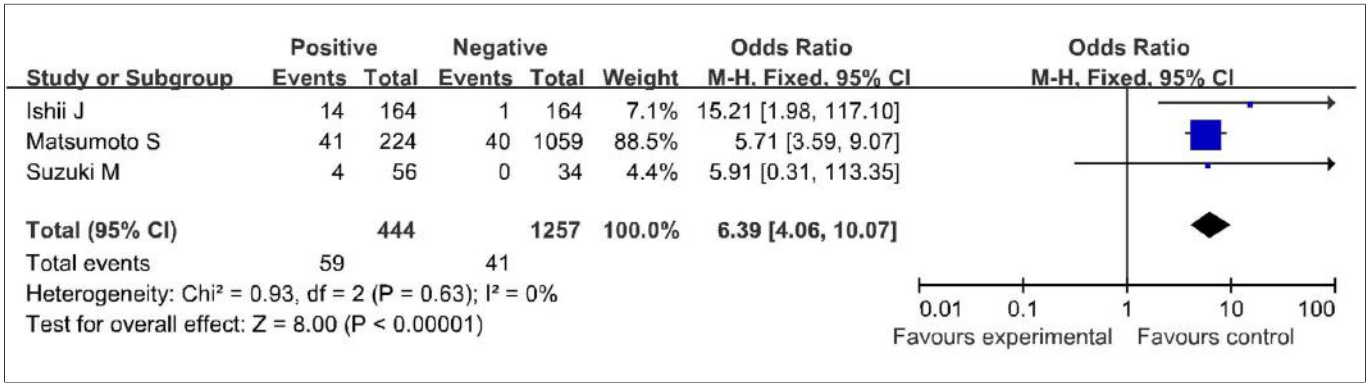

Figure 2: Fixed-effect meta-analysis for all-cause mortality.

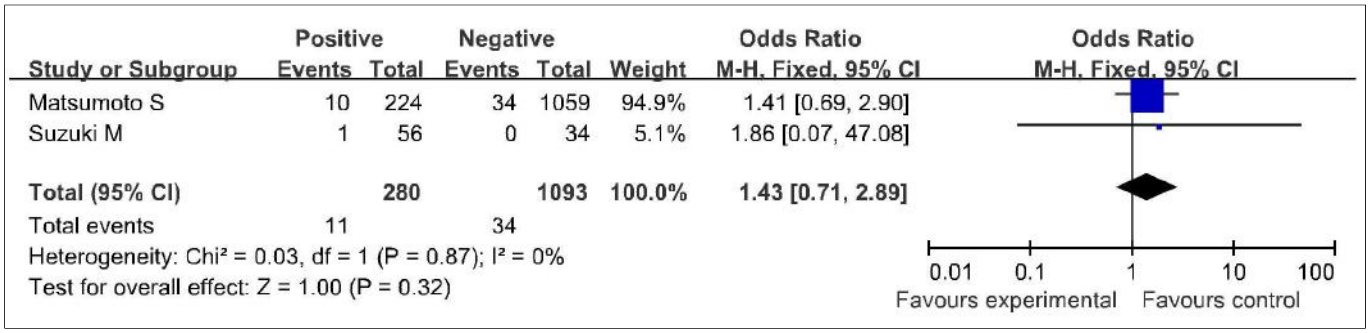

Figure 3: Fixed-effect meta-analysis for myocardial infarction.

Table I: Baseline characteristics of randomized studies.

Randomized studies	(Year)	Sample size Positive	Sample size Negative	Inclusion criteria	Exclusion criteria	Endpoints	Mean follow-up period
Suzuki M.	(2005)	56	34	ACS; consisting of ST-segment elevation, ST-segment depression, T-wave inversion, normal tracing, and electrocardiographic confounders within 6 h before	Lack of H-FABP data and incomplete clinical information.	Major adverse cardiac events were death or relapse of ACS.	30 days
Ishii J.	(2005)	164	164	admission, an episode of resting anginal pain lasting 10 min and at least one of the following: ST-segment elevation of at least 0.05 mV, ST-segment depression of at least 0.05 mV, T-wave inversion of at least 0.1 mV in at least 2 contiguous leads, or presumed new left-bundle branch block.	History of coronary revascularization within the preceding 6 months, serum creatinine concentration of 15 mg/L.	Cardiac events were defined as cardiac death or subsequent non-fatal AMI. Cardiac death was defined as any death for which there was no clearly documented non-cardiac cause. Sudden death occurring outside the hospital for which no other cause was assigned was also considered cardiac death.	6 months
Ilva T.	(2009)	110	183	ACS, evaluation of chest pain or equivalent symptoms, suggesting myocardial ischemia. All patients were diagnosed and treated according to local routine clinical protocols. Blood samples and ECG were collected at the time of enrollment, and in the case of hospitalized patients, at 6-12 and 24 h thereafter.	Uncertain delay or more than 24-h period from symptom onset to admission, incomplete cardiac injury marker results.	Combination of total mortality and myocardial reinfarction.	6 months
Matsumoto S.	(2013)	224	1059	Myocardial infarction (post-AMI) patients; discharged alive between 2000 and 2005; provision of a blood sample before or within 14 days of discharge.	Other	All-cause mortality; non-fatal MI; readmission for heart failure.	5 years

Table II: Patient characteristics in each randomized trial.

Demographics	Suzuki M. (90)	Ishii J. (328)	Ilva T. (293)	Matsumoto S. (1283)
Age, mean	66.8	64.9	67.1	63.6
Male sex (n)	67	264	181	991
Smoking history (n)	33	178	55	877
Prior CAD (n)	57	58	129	141
Hypertension (n)	44	180	128	738
Diabetes mellitus (n)	38	105	51	398
Hyperlipidemia (n)	39	145	171	617

DISCUSSION

The results of the Egger's test did not differ significantly with respect to patient outcomes studied, and the funnel chart analysis showed symmetry consistent with publication bias. The effect of H-FABP was evaluated in the meta-analysis by means of a prognostic biomarker in ACS patients. To the authors' knowledge, no previous analysis has been attempted for the purpose of elucidating the potential clinical value of H-FABP as a prognostic biomarker in ACS. So far there is not sufficient evidence to assess its serviceability for use as a prognostic marker of ACS.

H-FABP is a soluble cytosolic, small-molecule protein involved in the transportation of long-chain fatty-acids in cardiomyocytes. It may pass through the endothelium to enter the blood directly because of its small size. This allows it to be rapidly released into the circulation upon cardiomyocyte injury. Because of its solubility, H-FABP can be released more quickly than cardiac markers such as cardiac troponins, which makes it suitable as an early marker of myocardial injury and for ruling out the combination of myocardial infarction and troponins).[15]

The prognostic features of suitable biomarker of atherosclerotic cardiovascular disease have been established in some previous studies.[16] Though H-FABP seems to aid the clinical assessment of patients, it is not yet clear whether H-FABP can facilitate the treatment of their conditions or improve outcomes. This meta-analysis showed that group positive for H-FABP had a higher incidence of mortality and cardiovascular events during the follow-up period than ACS patients. Although the heterogeneity was low, ACS patients positive for H-FABP had significant differences in the incidence of mortality and cardiovascular events. However, there were no statistically significant differences in the incidence of myocardial infarction between positive and negative patients.[8,11] Elevated H-FABP levels were found to be associated with an increased risk of acute coronary ischemia, but there is insufficient evidence of myocyte necrosis (negative cardiac troponin). With respect to prognosis, patients positive for H-FABP were at greater risk of both cardiac events and mortality. For this reason, its use as a prognostic indicator for ACS, particularly unstable angina pectoris, has been proposed.[10] Overall, the studies in this meta-analysis indicate that H-FABP does offer some prognostic information for ACS patients in terms of cardiac events and mortality; however, there were no statistically significant differences in the incidence of myocardial infarction because this meta-analysis included only two studies that addressed myocardial infarction. Only rarely did H-FBAP provide information about myocardial infarction in patients with ACS.

There are several limitations that must be considered when interpreting the results of this study. First, because there is a lack of specialized RCTs focusing on this topic to date. We only extracted data from observational studies, which contributed to inherent bias, such as selection bias, design bias, treatment bias, and publication bias. We also used summarized published data regarding the incidence of events for each study instead of level data for individual patients, which may have introduced confounding and selection bias, and some clinical outcomes may be subject to inter-study heterogeneity. Finally, we were unable to analyze the specific medical treatments used. However, despite these limitations, our data indicate an urgent need for comprehensive comparison between the two outcomes with respect to myocardial markers, which may provide a basis for our risk stratification and assessment of the prognosis. This may facilitate informed decision-making that could allow patients and physicians to select an optimal strategy for treatment of ACS.

CONCLUSION

ACS patients that were positive for H-FABP had higher incidences of all-cause mortality and cardiovascular events. This might contribute to a better risk stratification and facilitate the planning of individualized treatment regimens. It may help achieve the best medical treatment and decrease health care costs, which would improve patient quality of life and reduce re-hospitalization rates and medical expenses. If H-FABP can be incorporated into current methods for risk stratification, including the thrombolysis in myocardial infarction (TIMI) and Global Registry of Acute Coronary Events (GRACE) risk scores, they may play an important role in clinical prognosis and improve the accuracy of current methods of evaluating risk stratification. The limited data from these randomized studies showed no significant differences in the incidence of MI.

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Do We Need Cardiac Rehabilitation in Heart Failure?

Abdulhalim Jamal Kinsara

ABSTRACT

Cardiac rehabilitation is a well established therapy for heart failure patients. Discussion is made of the different types, modalities, time and number of sessions and whether it is restricted to systolic heart failure or younger patients only. Elaboration will be made on how to make it more accessible to a larger proportion of population.

Key Words: *Cardiac rehabilitation. Heart failure. Home-based rehabilitation. Centre-based rehabilitation.*

Heart failure (HF) is a highly prevalent cardiac disease. Older adults are increasingly more at risk of HF. Efforts are ongoing to find a therapy that decreases morbidity and mortality.

Cardiac rehabilitation (CR) is an all-encompassing lifestyle change required to improve cardiac health, general health, psychological and general health in patients with HF.

A robust body of research demonstrating improved clinical outcomes supports CR use in HF. Despite this evidence, CR referral and attendance remains low; and interventions to increase its use need to be developed. Despite these salient benefits, fewer than one-tenth of eligible patients with HF are referred for exercise-based CR (EBCR) after hospitalization.[1]

Medicare beneficiaries and veterans conducted a retrospective study; very few HF patients participated in CR. There were 2.3% - 2.6%, respectively, who attended ≥1 sessions of outpatient CR. Among them, men were more likely than women to participate in CR. Characteristics associated with participation in CR in both groups included younger age, white race, and history of ischemic heart disease.[2]

A study of 2,054 CR in 69 centers in 12 European countries was undertaken. The aim of this study was to ensure guideline adherence and treatment quality standards. Of the 2,054 patients, 74% were male. The research found diverse CR programmes of varying duration (3-24 weeks) and, therefore, varying numbers of sessions per patient (30 - 196). After admission to a CR programme, 85% of patients were found to be compliant. However, the electronic case report form (ECRF) follow-up data exchange was felt to be incomplete, and there was only a minor change in the patient cardiovascular (CV) risk profile. Increased exercise capacity >25W was seen in younger patients and those in employment. However, results varied from country to country. It was found that only 9% of patients were admitted to a post CR programme.[3]

The benefits of CR are improvement in muscle power in peak oxygen consumption (VO_2), physical function and in general wellbeing. It also reduces the hospital admission time and has been found to be a safe mode of treatment.

HF patients in general have reduced tolerance to exercise measured as decreased peak aerobic power or what is known as peak oxygen consumption, and thereby affecting the life quality and overall survival. It is of paramount importance to select the optimal exercise training intensity to improve peak VO_2.[4]

Abnormal blood pressure (BP) can respond positively to CR, i.e., it normalizes. Two parameters were independently predictive of failure to normalize the BP response: female gender (odds ratio 3.5; 95% confidence interval 1.4-9.0) and decreased systolic function (odds ratio 3.2; 95% confidence interval 1.0-9.4). A 93% normalization rate was found in patients with hypertrophic cardiomyopathy vs. 62% in those with HF from other causes (P=0.03). CR exhibited an improvement in average exercise capacity (4.7 to 6.4 metabolic equivalents), ejection fraction (35.4% to 37.7%), and percentage with New York Heart Association class 3-4 (50% to 43.4%).[5]

CR works on both systolic and diastolic HF. The meta-analysis exercise training studies that investigate different indices of diastolic function in patients with HF with preserved or reduced systolic function, showed a significant reduction in the ratio of early diastolic transmitral velocity (E) to early diastolic tissue velocity (E') (E/E' ratio) with exercise training, exercise versus control.[6]

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The incidence of diastolic HF has been found to be increasing among the older generation. CR has shown to improve patients' general wellbeing, functioning, symptoms, general quality of life, mortality and morbidity.[7]

The benefit was demonstrated as early as 2 weeks after an acute episode of cardiac decompensation. Cardiac hemodynamics: left ventricular ejection fraction (LVEF), cardiac output, and stroke volume were all improved as assessed by radionuclide ambulatory ventricular function monitoring. Four weeks' exercise training has shown to improve cardiac performance indices and pulmonary function in both middle-aged and elderly HF patients.[8]

Combined exercise/inspiratory muscle training (IMT) should be included in CR programmes, as it has proved to be efficacious in the rehabilitation of patients with HF, improving exercise tolerance, respiration and, therefore, general quality of life. The combined exercise/IMT was found to result in greater IMT mean differences and Minnesota Questionnaire when compared with conventional exercise. In addition, no serious adverse incidents were noted.[9]

The most useful form of CR will be the one that is adjusted to the patient's medical condition, patient's own capability and individual needs.

In congestive heart failure (CHF) patients, the practice of moderate-intensity aerobic continuous exercise training (ET) is the most efficient training modality. Alternatively, repeated sessions of high-intensity exercise interchanging with recovery periods, known as high-intensity interval exercise, had resulted in superior enhancement in exercise capacity with no significant safety concerns. It is yet for the high-intensity to demonstrate the positive effect on endothelial function, heart size and function.[10]

Meta-analysis and review of randomized trials of home-based vs. centre-based CR programmes for adults with HF has found benefits in both programmes to be similar in terms of clinical and health-related quality of life, cost, exercise capacity, cardiac event mortality and smoking. Differences identified were lower cholesterol and diastolic blood pressure in the centre-based patients, but higher adherence in the home-based patients.[11]

The hesitancy in referral is multi-factorial; reimbursement is no longer one of them. Education is an important factor to encourage referral to CR. Home services will make it more accessible.

In summary, only a few therapies received class 1 indication, and CR is one of them, being an established treatment for HF patients associated with impaired ejection fraction <35%.

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CASE REPORT

Huge Gastric and Ileal Trichobezoars Causing Small Bowel Obstruction

Muhammad Imran[1], Ume Sughra[2] and Aurangzeb Khan[1]

ABSTRACT

The bezoar is the accumulation of undigestible food or fibre in the digestive tract, most commonly in stomach and proximal small bowel. Trichobezoar is an accumulation of hair. They may present as an independent mass in small bowel or with or without an extension into the small bowel. The trichobezoars are well described, in terms of surgical, diagnostic, and therapeutic procedures. However, there are very few reports on psychiatric literature and a dual presentation of gastric and ileal trichobezoars. The authors present a case report of a gastric with an ileal trichobezoar that is rarely reported in literature. A young girl with a history of trichophagia presented with features of small bowel obstruction. On physical examination, there was abdominal distension with a huge mobile mass on left side of umbilicus extending up to the epigestrium. There were dilated small bowel loops on abdominal radiograph. Ultrasound-abdomen also showed fluid-filled dilated small bowel loops. She was further investigated with CT scan abdomen, which showed a large mass occupying whole of the stomach and another mass in distal ileum causing small bowel obstruction, suggestive of trichobezoar. Through anterior wall gastrotomy, huge trichobezoar was removed from stomach; and through distal ileal entrotomy, ileal trichobezoar was removed.

Key Words: *Trichobezoars. Stomach. Obstruction. Small bowel obstruction. Trichotillomania.*

INTRODUCTION

Bezoars are defined as foreign bodies in the gastro-intestinal tract which increase in size by accumulation of nonabsorbable foods or fibers. Trichobezoars, a ball of hair in the proximal portion of gastrointestinal tract, are often a rare condition commonly occuring in young female psychiatric patients.[1] A trichobezoar develops in approximately 1% of patients with trichophagia.[2] The first case of trichobezoar was reported in 1779.[3] This ball of hair can lead to gastrointestinal bleeding and ulceration, perforation or obstruction.[1] In most of the cases, they are confined and limited in the stomach; but in few of the cases, it may extends up to the pylorus and enters into the jejunum, ileum or may even lead to colon. This condition is called Rapunzel syndrome and was first mentioned by Vaughan *et al*. in 1968.[4] Sometimes, last portion of trichobezoar breaks off and migrates to small intestine, resulting in intestinal obstruction.[5,6] A small number of cases reported simultaneous gastric and small bowel trichobezoars causing obstruction.

Trichobezoar should be kept in mind while making a differential diagnosis of non-tender abdominal mass and abdominal pain in young females. The most common site of trichobezoar is stomach. Gastric and ileal trichobezoars, occurring simultaneously, are very rare. Treatment of such cases is conventional laparotomy and thorough exploration of stomach and small bowel. Psychiatric evaluation and treatment is an essential part of management to avoid recurrence.

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CASE REPORT

A 21-year girl came in the Emergency Department and presented with complaints of abdominal pain, vomiting and constipation for 3 days. On examination, her abdomen was distended, soft and non-tender. There was a large mobile mass in the left lumbar region, extending up to the left hypochondrium and epigestrium. Rest of the physical examination was unremarkable.

Her laboratory tests were within normal limits. Her abdominal radiograph showed distended small bowel loops. Ultrasound abdomen also showed distended fluid-filled small bowel loops. CT scan abdomen showed a mass occupying whole of the stomach, distending it to maximum and another mass in distal ileum with massively distended proximal bowel, suggestive of trichobezoars (Figure 1). On inquiring again from patient's relatives, they admitted her habit of trichophagia. They told that 10 years back, she had rapid hair fall and was treated as hair-fall disease by a local doctor.

Exploratory laparotomy revealed distended stomach and proximal small bowel loops. Two masses were identified, one in stomach and another in distal ileum 20 cm proximal to ileocaecal junction. The ileal trichobezoar

was removed through distal ileal entrotomy, whole of the small bowel was decompressed and entrotomy closed. Then, anterior wall gastrotomy was done through longitudinal incision and huge trichobezoar removed from the stomach (Figures 2-4). Her postoperative recovery was uneventful except that she developed superficial surgical site infection which was managed conservatively. She was discharged after psychiatrist opinion.

DISCUSSION

A bezoar is collection of undigestable food or fibres in gastrointestinal tract.[7] Bezoars are of mainly three types: phytobezoars (vegetable or fruit fibres), lactobezoars (milk curds), and trichbezoars (hairs).[8] First case of trichobezoar was reported in 1779 by Baudamant.[9] It is a rare condition and is more common in young females. Ninety percent of these cases have a psychiatric disorder (trichotillomania). In trichbezoars, the swallowed hair and undigested food particles mostly accumulate in stomach. However, the trichobezoar can extend, in some cases, through the pylorus into small gut like jejunum, ileum or even colon. Mostly, cases of trichobezoar present late due to the low level of suspicion by the healthcare professionals. Patients present with symptoms like anorexia nervosa, low hemoglobin level, loss of weight, GI bleeding and repeated bouts of abdominal pain or more serious conditions like intestinal obstruction and perforation.[10,11] The complication that occur mostly is perforation of either the intestine or stomach.

The diagnosis is made on detailed history taking and careful clinical examination. An abdominal radiograph may show an intragastric mass outlined by gas in the distended stomach. Ultrasound abdomen shows a thick, echogenic rim with clear posterior shadowing in the epigastrium. CT abdominal shows a mass that is mobile and intragastric with a mixed pattern in density because of the presence of trapped air and food materials. It also reveals the extension of the trichobezoar and localization of bowel obstruction. Upper GI endoscopy remains the gold standard procedure for diagnosis. Treatment may include endoscopic removal but this must be limited to small trichobezoars because of risk of bowel perforation. Other methods including chemical dissolution, mechanical fragmentation, and explosive technique, which are laser ignited, were also used successfully.[12] Laparoscopic techniques have also been used with little success but can be tried for cases depending upon the expertise. Open surgery still remains the gold standard for the removal of large trichobezoars, specifically if it is extented up to the bowel, which may otherwise be missed with other modes of treatment. Psychological support and psychiatric treatment is essential for preventing recurrence.[13]

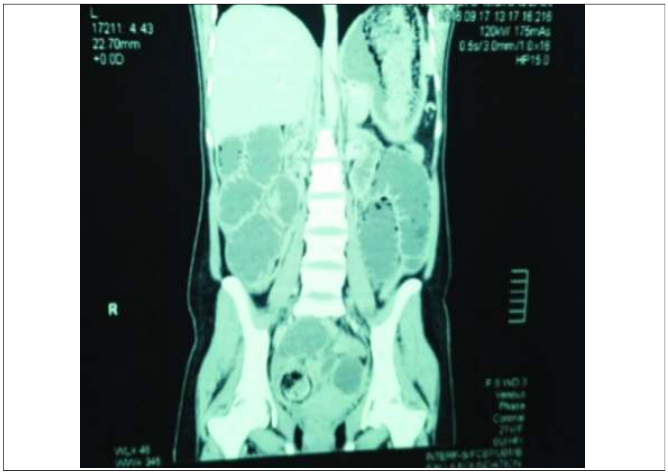

Figure 1: CT scan abdomen showing gastric and ileal trichobezoars.

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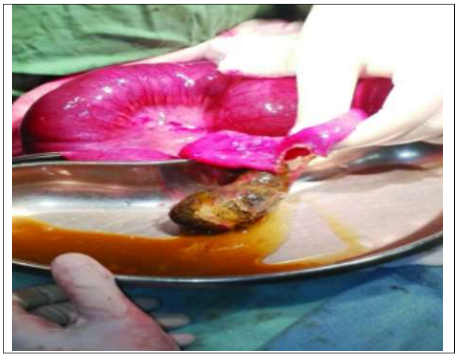

Figure 2: Ileal trichobezoar removed through entrotomy.

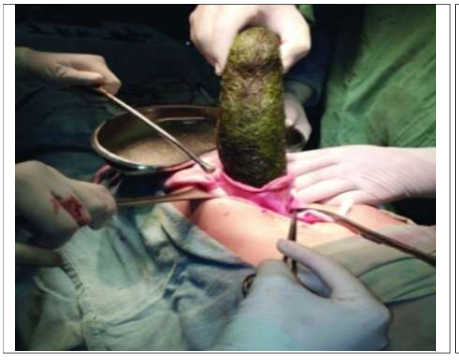

Figure 3: Removal of huge gastric trichobezoar through gastrotomy.

Figure 4: Removed huge gastric and small ileal trichobezoars.

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Primary CNS Lymphoma vs. Tumefactive Multiple Sclerosis: A Diagnostic Challenge

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Abstract

Primary CNS (central nervous system) lymphoma is a rare condition with the incidence of less than 1% of all non-Hodgkin lymphomas (NHLs) and approximately 2% of all primary brain tumours. Diagnosis can be challenging and necessitates brain biopsy for definitive diagnosis. A 41-year male presented with history of impaired cognition, facial asymmetry, visual impairment and left sided body weakness. MRI brain demonstrated multiple enhancing lesions with one larger lesion in right basal ganglia with surrounding oedema and mass effect. These findings suggested the differential diagnoses of tumefactive multiple sclerosis (MS), primary CNS lymphoma (PCNSL) and tuberculosis. The patient had normal CT chest, abdomen and pelvis, normal CSF examination and cytology, negative CSF oligoclonal bands (OCBs) and negative HIV screening. It was impossible to differentiate between tumefactive MS and PCNSL without undertaking brain biopsy. Diffuse large B cell lymphoma (DLBCL) was the final diagnosis. Diagnosing PCNSL can be challenging and brain biopsy should not be delayed for definitive diagnosis and targeted treatment.

Key Words: *Primary CNS lymphoma. Diffuse large B cell lypmphoma (DLBCL). Tumefactive multiple sclerosis.*

INTRODUCTION

The occurrence of tumour-like demyelination is reportedly rare, being estimated at 1-2/1000 cases of multiple sclerosis.[1] When demyelinating disease does not present with classical presentation and its imaging is indistinguishable from neoplasms then it is described as tumefactive demyelinating lesions (TDLs) which are generally defined by pseudotumoral plaques larger than 2 cm, with or without associated mass effect and perilesional edema.[2] Primary CNS lymphoma (PCNSL) is a rare brain tumour with the incidence of less than 1% of all non-Hodgkin lymphomas (NHLs) and approximately 2% of all primary brain tumors.[3]

We present one such rare case where it was impossible to differentiate between tumefactive MS; and PCNSL and brain biopsy confirmed the diagnosis.

CASE REPORT

A 41-year male presented in the Hospital Emergency Department with history of impaired cognition that lasted for few hours and then resolved completely by itself. After three weeks of the initial attack, he noticed facial asymmetry with difficulty in swallowing solids. Four days after this episode, he developed sudden left sided weakness, blurred vision and urinary incontinence that gradually worsened over a period of two days. On examination, he had left partial third nerve palsy, left seventh nerve palsy, UMN type. Fundi showed bilateral disc pallor. Left hemiparesis with power of 4/5. Initially his MRI brain was done which showed multiple enhancing lesions in infra- and supratentorial region with surrounding oedema and mass effect, being the larger one in right basal ganglia showing heterogeneous mixed signal on T1 and T2WI with a smaller central relatively lower T1 and brighter T2 and FLAIR signal without any enhancement, suggestive of necrosis. It approximately measured 32x26x20 mm as shown in Figure 1.

The larger lesion had a significant mass effect and surrounding edema extending into right temporal lobe causing mild compression of ipsilateral lateral and third ventricles with subtle midline shift towards left. The lesions were present in the anterior body and genu of corpus callosum, left fronto-parietal lobe, bilateral cerebral peduncles, mid brain, pons, right middle

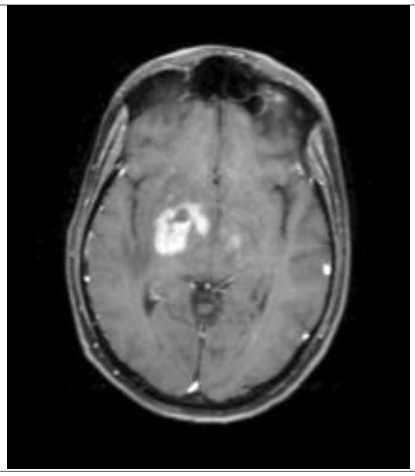

Figure 1: MRI brain with contrast showing large right basal ganglia lesion.

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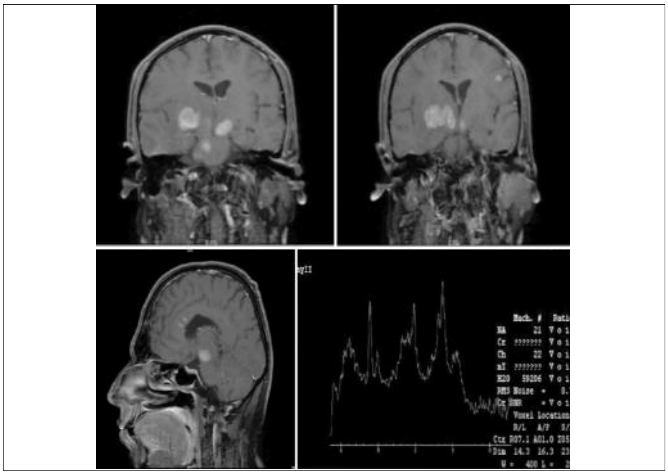

Figure 2: MRI brain and MRS. MRI brain showing multiple lesions in pons, left parietal region and corpus callosum. MRS showing raised lipid, lactate and choline peak and decreased NAA

cerebellar peduncle and bilateral cerebellar hemispheres as shown in Figure 2. On GRE images, there is no signal to suggest intra cranial haemorrhage. On MR spectroscopy, there was raised lipid lactate peak with slight decreased NAA and increased choline peak. NAA/choline ratio ranges between 0.4 and 0.9 at different locations in right basal ganglia lesion.

Further workup showed normal CRP and ESR. CSF showed slightly raised proteins in CSF and normal cells and glucose and CSF cytology was negative for malignant cells. Mycobacterium tuberculosis polymerase chain reaction (MTB PCR) came out to be negative in CSF. Visual evoked potential was suggestive of bilateral severe optic pathway dysfunction with no recordable waveforms in the right eye. CSF oligoclonal bands (OCBs) were negative. HIV screening was negative. A series of other investigations were carried out to find out the primary source of the lesion which included bone marrow biopsy and CT chest abdomen and pelvis which came out be non-conclusive. Slit lamp examination of the eye for vitreous involvement was also normal.

He was given high dose of intravenous corticosteroids for five days and it showed improvement in bladder function, but overall condition of the patient deteriorated over the time. Three sessions of plasmapheresis were also carried out after intravenous corticosteroids, thinking of aggressive multiple sclerosis but did not prove to be beneficial for the patient. Then he was referred for brain biopsy for definitive diagnosis. Morphology and immunohistochemistry of brain specimen were consistent with the diagnosis of DLBCL (activated B cell immunophenotype) and thus the patient received cranial radiation therapy for DLBCL.

DISCUSSION

The incidence of primary CNS lymphoma is increasing for unknown reasons in immuno-competent patients. It is a rare brain tumour with the incidence of less than 1% of all NHLs and approximately 2% of all primary brain tumors.[3]

Primary CNS lymphoma is an extranodal malignant lymphoma of the CNS in the absence of lymphoma outside the nervous system and in majority of the cases it is DLBCL.[4] According to WHO classification of hematopoietic tumours, CNS DLBCL is the preferred term used for primary CNS lymphoma.[5]

It is a rare condition, representing less than 1% of all NHLs and approximately 2% of all primary brain tumours. It occurs at all ages with a male to female ratio of 3:2 and mainly 70-80% presents with focal neurological signs that are related to the location of the lesions in CNS. About 60% of the lesions are supratentorial and may present with single or multiple intraparenchymal lesions, while 13% of the primary CNS lymphoma involves posterior fossa. Only a minority of the patients presents with diffuse leptomeningeal involvement that requires further workup for the systemic lymphoma.[6] It is a highly aggressive tumour with a median survival rate of nine months. Poor prognostic factors include age greater than 60 years, poor performance status, elevated initial LDH, high CSF protein and involvement of deep regions of the brain.[7]

Its diagnosis is often delayed due to limited diagnostic value of CSF examination and non-specific findings of MRI; and its differentiation from other diseases like demyelinating lesions and glioblastoma can be difficult. Prior administration of corticosteroids can be a major diagnostic challenge because of the high sensitivity of the lymphoma cells to corticosteroid induced apoptosis and can mask the morphological appearance of the tumour. Corticosteroids may also reduce the contrast enhancement of MRI and is highly suspicious for CNS lymphoma but the suspicion of demyelinating lesions cannot be ruled out; hence, stereotactic brain biopsy is the current gold standard method for establishing the tissue diagnosis of CNS lymphoma.[4] Though stereotactic brain biopsy is the gold standard,[4] this patient underwent open brain biopsy and tolerated it well.

In this patient due to his rapidly deteriorating condition and little improvement with corticosteroids and repeated sessions of plasmapheresis, he was suspected of having PCNS lymphoma. Further, his CSF findings and MRI and MR spectroscopy raised the suspicion of demyelinating lesions with no conclusive evidence of CNS infection. The differentiation between demyelination and PCNSL, especially in young immunocompetent patients, was very challenging. In such scenario, there is no way these two can be differentiated without undertaking brain biopsy. Furthermore, his extensive workup was performed for systemic malignancy and infection, but all came out to be negative. His open brain biopsy confirmed the diagnosis of primary DLBCL.

PCNL can be a diagnostic challenge for a neurologist on the basis of clinical, laboratory, and radiological examination in an immunocompetent patient; and it may be necessary to perform early brain biopsy in such patients for definitive diagnosis and targeted management.

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Progression of Fibroadenoma to Malignant Phyllodes Tumour in a 14-Year Female

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ABSTRACT

Phyllodes tumours are uncommon breast tumours which account for less than 1% of all breast neoplasms. High-grade malignant phyllodes tumour is a very rare but aggressive breast malignancy and forms approximately 15-30% of all phyllodes tumours. The transformation of a benign fibroadenoma into a malignant phyllodes tumour in a teenaged female is even rarer. We report here an interesting case of malignant phyllodes tumour in a 14-year female patient who was operated twice previously with the diagnosis of complex fibroadenoma in the same breast. There was a large tumour involving whole of the breast and infiltrating the skin. The patient was operated and total mastectomy was done. Diagnosis was confirmed after histopathological examination and immunohistochemistry of the resected specimen. Patient received adjuvant radiotherapy and there was no recurrence on 6-month follow-up. Owing to the rare occurrence of malignant phyllodes tumour in this age group along with previous operations for complex fibroadenoma, this case is being reported here.

Key Words: *Complex fibroadenoma. Malignant phyllodes tumour. Adolescent female. Mastectomy. Adjuvant radiotherapy.*

INTRODUCTION

The term cystosarcoma phyllodes was coined by Muller in 1838.[1] This term was misleading as the term sarcoma denotes malignancy; however, it is now well known that the disease has both benign and malignant forms.[2] It is a rare fibro-epithelial tumour of the breast which constitutes less than 1% of all breast neoplasias.[3] The age of predilection is usually between 35 - 45 years. The term 'phyllodes' is attributed to the leaf-like extensions of the hypercellular stroma seen on histopathology. We hereby report a case of malignant phyllodes tumour in a 14-year girl who was operated twice with the diagnosis of fibroadenoma at the same site in same breast. Diagnosis of the malignant phyllodes was made upon histopathological examination. Blocks of previous surgeries were also retrieved and reviewed, which confirmed the diagnosis of complex fibroadenoma at that time. In this case, there can be two reasons for a biopsy-proven fibroadenoma recurring as malignant phyllodes tumour. The first one is wrong diagnosis on histopathological examination at the time of previous surgery, which doesn't appear to be true as the blocks of previous surgery were reviewed; and the second one is transformation of a fibroadenoma into malignant phyllodes tumour.

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Kuijper, *et al*. suggested that fibroadenomas may progress in an epithelial direction to carcinoma *in situ* or in a stromal direction to phyllodes tumours.[4] Noguchi, *et al* also reported progression of three cases of fibroadenoma to phyllodes tumour.[5]

CASE REPORT

A 14-year female presented to the surgical Outpatient Department (OPD) of the Hospital with complaints of a rapidly growing lump in her right breast. The patient had been operated twice with the diagnosis of complex fibroadenoma in the last 2 years, but again developed recurrence at the same site.

On examination, the lump was hard and involved almost the entire breast distorting its morphology (Figure 1). The swelling was fixed to the skin but was free from the chest wall. There were several ulcers over the swelling. No axillary lymph nodes were palpable. Core needle biopsy was done, which was suggestive of phyllodes tumour. Sonomammography of the breast revealed benign breast lesion with multiple cysts. Radiograph of

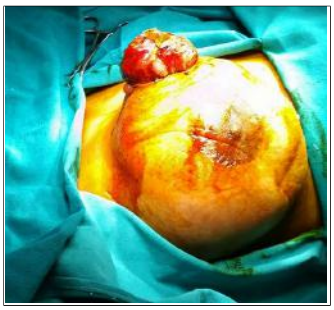

Figure 1(a): Preoperative photograph of the patient showing lump involving whole breast and infiltrating the skin.

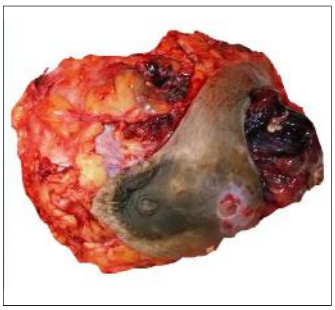

Figure 1(b): Photograph of the resected specimen of right breast showing ulceration, hemorrhagic and cystic areas.

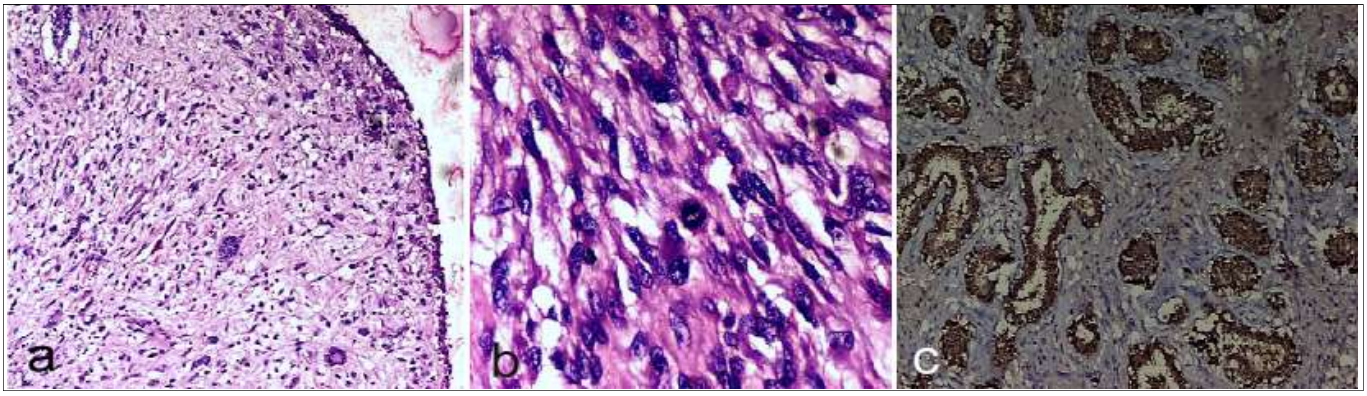

Figure 2: (a) Section showing malignant spindle cells and tumour giant cells on myxoid background (Hematoxylin & eosin X 100). **(b)** High power view of malignant spindle cells having raised nucleo-cytoplasmic ratio, clumped chromatin and prominent nucleoli. Mitoses and apoptotic bodies are also seen (Hematoxylin & eosin X 400). **(c)** S100 Immunohistochemistry showing strong positivity in myoepithelial cells in areas showing glandular adenosis. Stromal cells are negative for the stain (S100 IHC X 400).

the chest and sonography of the abdomen were unremarkable. Owing to the involvement of whole of the breast by the lump, skin fixity, and rapidly increasing size of the lump, total mastectomy was done. Postoperative recovery of the patient was uneventful.

Histopathological examination of the excised specimen showed leaf-like architecture with clefts and stromal overgrowth showing infiltrative margins. The stroma was hypercellular and composed of spindle cells showing marked nuclear atypia. Abnormal mitotic figures were present and mitotic count was >10/10 high power fields at some foci (Figures 2a and 2b). Haemorrhage, cystic areas and giant cells were also seen in the stroma. There was no lympho-vascular invasion and all the margins were negative. Areas of microglandular adenosis and epithelial hyperplasia of usual type were also seen in some areas. Occasional glands showed features resembling atypical hyperplasia. S100 immunohistochemistry for myoepithelial cells was done to rule out accompanying infiltrating ductal carcinoma (Figure 2c). Hence, a final diagnosis of malignant phyllodes tumour was made, which is a rare tumour of the breast and extremely rare in young females. The blocks of previous operations were reviewed in which hematoxylm and eosm (H&E) stained sections showed intracanalicular and pericanalicular types of fibroadenoma with apocrine changes in the ducts, also there was cystic enlargement of some ducts with focal calcification suggestive of complex fibroadenoma.

After the diagnosis of malignant phyllodes tumour was confirmed, she was treated with adjuvant radiotherapy. There was no local recurrence or distant metastasis in the 6 month follow-up of the patient. She has also been advised follow-up every 3 months for next 3 years to look for any recurrence or distant metastasis.

The patient has been planned for reconstruction of the breast in stages. In the first stage, tissue expansion will be done with the help of saline tissue expander; in second stage, expander will be replaced with appropriate sized silicone implant; and in the third stage, nipple areola reconstruction will be done.

DISCUSSION

High-grade malignant phyllodes tumours constitute about 15 - 30% of all phyllodes tumours. The incidence of disease is highest in 35 - 45 years of age. Many studies have been done to understand the exact biological behaviour of these tumours.[6] Malignant phyllodes tumours are fast growing tumours with frequent involvement of almost the entire breast leading to dilated veins, stretched skin, and ulcerations. Although axillary lymph nodes metastasis is very rare, it has been reported in few case studies.[1] Haematogenous spread is reported in 10 - 25% of high-grade malignant phyllodes tumours, most frequently to the lung (70 - 80%), pleura (60 - 70%), and bones (25 - 30%). There are very few case reports of malignant phyllodes tumour in adolescent females.[7] In the present case, the patient was a 14-year young girl who had a large swelling occupying her whole right breast leading to skin ulcerations and destruction of the breast. Even though the biopsy was pointing towards a benign lesion but owing to the involvement of whole of the breast by the lump, skin fixity, and rapidly increasing size of the lump, total mastectomy was done. Histopathology confirmed the diagnosis of malignant phyllodes tumour. The blocks of previous two operations were procured and the diagnosis of complex fibroadenoma was re-confirmed. The reason for the discrepancy in diagnosis could be progression of fibroadenoma to phyllodes tumour. Noguchi, *et al*. suggested that there are two types of fibroadenoma: the commoner polyclonal type and the rarer variety of monoclonal tumour.[5] It is important to differentiate between monoclonal and polyclonal fibroadenomas because monoclonal fibroadenoma should be treated surgically, and polyclonal fibroadenoma can be monitored clinically. They also observed that monoclonal fibroadenomas progress to phyllodes tumour. PCR method can be used on fine needle aspiration sample to differentiate between monoclonal and polyclonal varieties to aid in treatment decisions. Monoclonal fibroadenoma can progress to or recur as phyllodes tumour later; and

hence, should be treated like phyllodes tumour with a wide excision at the onset.

The preferred treatment of phyllodes tumour is local resection of the tumour with healthy borders of around 1 - 2 cm in order to prevent local recurrence or a simple mastectomy, if tumour is large and occupying the whole breast tissue, as it was in our case.[8] Lymphatic involvement is considered rare; and hence, axillary lymph node dissection is not recommended, unless they are clearly involved with tumour.[9] Zeng, *et al*. suggested radiotherapy after wide local excision for high risk lesions (tumour >5 cms, presence of stromal overgrowth, >10 mitoses/10hpf, infiltrative margins), to prevent recurrence, as was done in this case.[10] The role of chemotherapy seems to be limited to the treatment of unresectable local disease with recurrence or metastasis.

Although rare, there is risk of transformation of fibroadenoma to malignant phyllodes tumour, so close follow-up is essential in patients specially in cellular fibroadenoma as these are the ones which have higher chances of progression to malignant phyllodes tumour. If there is recurrence in any operated case of fibroadenoma, it is very important to differentiate it from phyllodes tumour to initiate appropriate treatment; and prevent complications.

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Lung Cancer with Concomitant Double Gene Mutation

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ABSTRACT

A patient presenting with concomitant epidermal growth factor receptor (EGFR) mutation and anaplastic lymphoma kinase (ALK) translocation is very rare. We report a non-small cell lung cancer (NSCLC) patient with concomitant EGFR (exon 19-del) mutation and ALK rearrangement. The positron emission tomography-computed tomography (PET-CT) scan revealed a highly metabolic mass lesion in the left lower lobe, measured 5.0 cm in the largest dimension in the S6 segment. Transbronchial lung biopsy (TBLB) showed the pathological diagnosis of invasive adenocarcinoma. Thus, the patient underwent left lower lobectomy and hilar-mediastina lymph node dissection (pT2N0M0). The tumor harbor an ALK (D5F3 +) rearrangement and EGFR (exon 19-del) mutation. The patient initially received four cycles of chemotherapy (pemetrexed and carboplatin), and achieved partial response (PR).

Key Words: *Lung cancer. Adenocarcinoma. ALK. EGFR. Double gene mutation.*

INTRODUCTION

Lung cancer has the highest incidence and mortality of all cancers, non-small cell lung cancer (NSCLC) accounts for 80-85%. Cancer treatment has entered the era of molecular targeted therapy. Personalized targeted therapy of NSCLC is based on the mutation status of epithelial growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (K-Ras), B-Raf proto-oncogene (BRAF) and anaplastic lymphoma kinase (ALK) fusion gene, with different mutations requiring different treatment. However, previous research indicates that the ALK fusion gene in NSCLC with EGFR and KRAS mutations are mutually exclusive.[1]

Herein, we present a rare case of adenocarcinoma of the lung harboring EGFR- and ALK-activating mutations, simultaneously.

CASE REPORT

A 63-year female non-smoker was admitted to the Hospital in June 2015 because of cough and sputum for three months. Chest CT scan revealed a mass lesion in the left lower lobe, measuring 5.0 cm in the largest dimension in the S6 segment (Figure 1A arrow). Transbronchial lung biopsy (TBLB) showed the pathological diagnosis of invasive adenocarcinoma (cT2N0M0 Stage I b). As there were no surgical contraindications, the patient underwent left lower lobe resection and hilar mediastinal lymph node dissection. The final pathology revealed the nodule in the left S6 segment was invasive adenocarcinoma (80% adherent and 20% papillary growth pattern), and invading the wall of the trachea or bronchus; the bronchial stump was cancer-free and there was no tumor invasion of the lymph nodes (0/22) (pT2N0M0 Stage I b).

Fresh surgical specimen was sent for molecular analysis, the tumor harbor an ALK (D5F3+) rearrangement mutation by reverse transcription-polymerase chain reaction (Figure 1B), EGFR mutation (19-del) was also detected within the tumor (Figure 1C). The patient initially received four chemotherapy regimens of PC (Pemetrexed 75 mg/m^2 D1 + Carboplati AUC=Five D1) from July to October 2015 and achieved partial response (PR), but there were intolerable gastrointestinal reactions. The patient was treated with icotinib (EGFR-TKI) targeted therapy in November 2015 until disease progressed six months later. Finally, she received crizotinib (ALK inhibitor) targeted therapy, but which only worked five months.

DISCUSSION

The case was a female patient with adenocarcinoma of the lung harboring EGFR (19-del) and ALK rearrangement. Personalized targeted therapy of NSCLC is based on the mutation status of EGFR, K-Ras, BRAF and ALK fusion gene, with different mutations requiring different treatment. Therefore, NSCLC treatment should now be further sub-classified by histology and driver mutation, if one is known or present.

Recent research has found that ALK rearrangements and EGFR mutation may coexist in the same tumor, with an incidence rate of 1-6%.[2,3] In addition, clinical studies showed that NSCLC patients with EGFR gene mutations and ALK fusion gene may benefit from EGFR-TKIs and ALK inhibitors, respectively. Sort through current bibliographies and literature reviews in the field,[2-9] six

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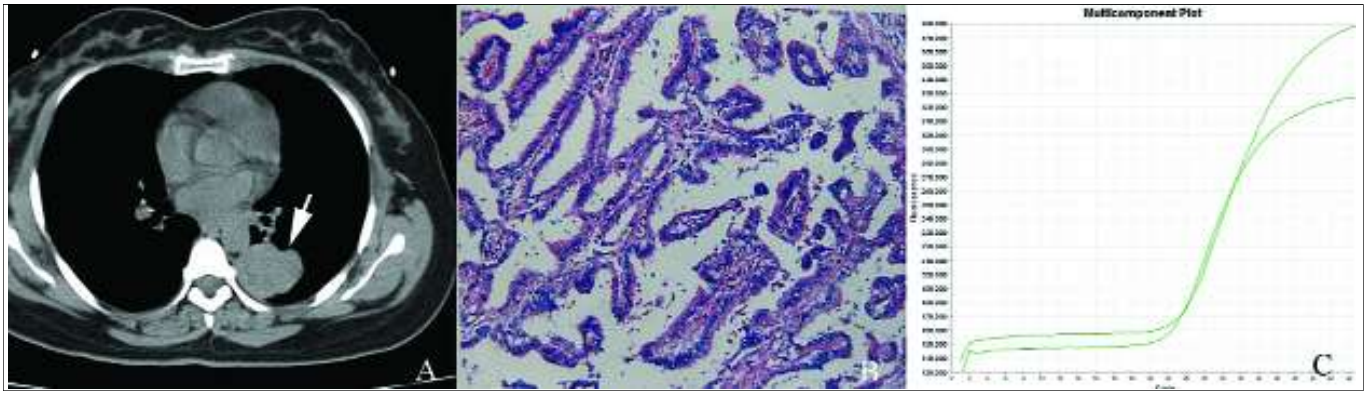

Figure 1: (A) CT scan revealed a mass lesion in the left lower lobe, measuring 5 cm (arrow); Haematoxylin & eosin x 100, and (B) IHC:ALK(D5F3+), (C) Exon 19-del mutations (fluorescence value) .

patients had a response with progression-free survival (PFS) ranging from five to nine months. One patient even obtained a partial response for two years after gefitinib treatment. Five cases experienced progressive disease and showed resistance to EGFR-TKI, which suggest that EGFR-TKI treatment in EGFR mutated patients is better than in patients with co-existing EGFR mutation and ALK rearrangement. However, Lee reported two patients who did not respond to EGFR-TKI, but achieved a durable partial response to ALK inhibitors.[2] In this case, the respond to EGFR and ALK was six and five months, respectively that is not very well. Currently, the sensitivity of patients with coexisting mutations is still not clear. The coexistence of gene mutation may reduce the therapeutic sensitivity, in other words, the more methods the treatment, the worse the prognosis. The complexity of multiple gene mutations need targeted drug combination. Since gene mutations may be changed after antitumor chemotherapy, it is necessary to obtain the genetic testing once more in order to provide guidelines. There is a clear need to further investigate the optimal targeted treatment method of NSCLC harboring EGFR gene mutations coexisting with ALK-positive.

In conclusion, regardless of cancer gene mutation, as long as the clinician adhere to the concept of evidence-based medicine treatment and precision medicine, the patients can benefit from targeted therapy.

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A Rare Complication of Percutaneous Nephrolithotomy: Colonic Lumbar Hernia

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Abstract

Percutaneous nephrolithotomy has a success rate of 90%; however, its overall complication rate is approximately 83%, including urinary extravasation, bleeding necessitating transfusion, and postoperative fever. Septicemia, colonic and pleural injury are rare major complications. Neighboring organ injury, especially colonic injury, is frequently seen during tract dilatation procedure. In this study, we report a case of colonic lumbar hernia, which is a rare complication of percutaneous nephrolithotomy.

Key Words: *Percutaneous nephrolithotomy. Postoperative complication. Lumbar hernia.*

INTRODUCTION

Percutaneous nephrolithotomy (PCNL) is the gold standard treatment for renal stones measuring larger than 2 cm. Nonetheless, it has potential significant complications such as sepsis and death.[1] Incisional colonic hernia has been reported as a complication of conventional open surgery and laparoscopic procedure. Colonic lumbar hernia is a rare complication of PCNL. To the best of our knowledge, this is one of the first cases of colonic lumbar hernia related to PCNL procedure.

In this case, our purpose is to report the case of colonic lumbar hernia as a rare complication of PCNL procedure.

CASE REPORT

A 50-year male was referred to urology clinic for further evaluation of palpable mass at left flank. He had a history of PCNL procedure that was performed two years ago for left kidney stone. The procedure was performed using double access and mechanical dilators. Physical examination revealed two access incision and 3 cm subcutaneous mass enlarging with Valsalva maneuver (Figure 1). Bowel herniation through the tract of PCNL from 2 cm fascial defect was observed on ultrasound examination. Computed tomography (CT) revealed 3 cm subcutaneous hernia that included large bowel segment (Figure 2). The patient refused any surgical intervention and had no complaints during one year folow-up period.

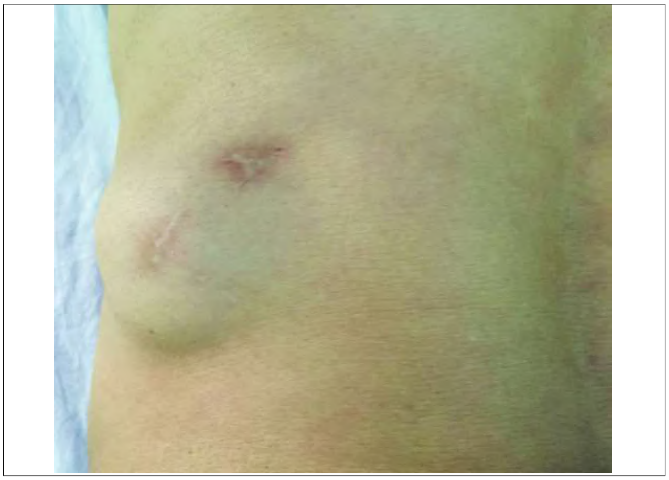

Figure 1: Palpable mass and two acceses in the left flank area.

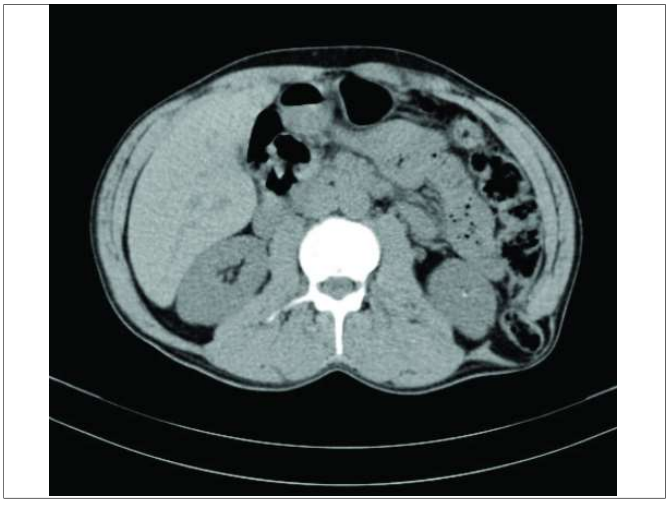

Figure 2: Computed tomography image of the lumbar hernia in the left area.

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DISCUSSION

PCNL has been an effective minimally invasive surgical (MIS) method in treatment of renal stones since 1970.[1] It is the treatment method of choice for kidney stones measuring larger than 2 cm and multiple renal stones. Tefekli, *et al*.[2] described the modified Clavien grading system from Grade 1 to 5; with grade 1 complication defined as "no need for pharmacologic treatment or surgical, endoscopic, and radiologic interventions" and grade 5 complication being "loss of patient due to the procedure". Neighboring organ injury and nephrectomy were described as grade 4 complication. In aforementioned study, they investigated 811 patients and there was no colonic lumbar hernia in their series.[2]

Hernias occurring after laparoscopy are known as trocar site hernias and accepted as incisional hernias in the literature. Mechanical bowel obstruction can also occur in trocar site hernias secondary to laparoscopic interventions. Maio, *et al*. was first to report trocar site hernia and small bowel obstruction in a case that underwent laparoscopic cholecystectomy.[3] Tonouchi, *et al*. reported the incidence of trocar site hernia as 0.65 to 2.8%.[4] In this case, we report that, there were no symptoms to indicate a bowel obstruction.

Variations in colonic anatomy are thought to be induced by factors like mild embryological rotation and fixation anomalies, short transverse mesocolon, and intraperitoneal ascending or descending colon. Additionally, increase in abdominal pressure and varying amount of retroperitoneal fat tissue are also suggested to be involved in the process of colonic malplacement.[5-7] Incidence of partial retro-renal displacement of colon is reported to be 9-10%, while complete retro-renal displacement of colon is reported to be seen in only 1% of cases.[6,7] Colonic injury is a rare severe complication of PCNL. Its incidence is below 1%, and it is mainly seen in cases with retro-renal colonic displacement.[8] We speculate that the patient might have suffered a peri-procedural peritoneal injury in order to develop such a hernia, as there was no history of colonic injury. Additionally, the performance of double access may also have contributed to the hernia development by paving the way for the fascial defect.

In conclusion, the clinicians should bear in mind that colonic lumbar hernia can be seen as a complication of PCNL. If there is no strangulation and bowel obstruction risk, surgical procedure can be postponed.

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A Definition of Family Medicine and General Practice

M. Jawad Hashim

ABSTRACT

Definitions of general practice and family medicine have tended to be difficult to understand. We propose a new definition that is brief and clear. General practice / family medicine is defined as the medical specialty that manages common and long-term illnesses in children and adults, focusing on overall health and well-being. The definition should be followed by local examples of diseases within the scope of primary care such as asthma, diabetes as well as end-of-life care. This definition is based on the concept of common illnesses, reflecting the reality that family physicians manage most health conditions in a community. Unlike 'comprehensive care', common illnesses is a scope of practice that is familiar and credible to laypersons. The new definition focuses on a unique and enduring attribute of the specialty: holistic care. This core characteristic of general practice encompasses patient-centred care and continuity across the family lifecycle. By being sharply focused, the definition can guide the nature of clinical care in general practice as well as the content of undergraduate and postgraduate training in family medicine.

Key Words: *General practice. Family medicine. Primary care. Continuity. Holistic care.*

Previous definitions of family medicine and general practice have been unsatisfactory as they are difficult to understand. Frequently, the principles of family medicine or the desirable attributes of a general practitioner (GP) are listed in these lengthy definitions. A few examples illustrate these limitations.

The American Academy of Family Physicians states:

"Family medicine is the medical specialty which provides continuing and comprehensive healthcare for the individual and family. It is a specialty in breadth that integrates the biological, clinical and behavioral sciences. The scope of family medicine encompasses all ages, both sexes, each organ system and every disease entity."[1]

A 2000 definition of the "general practitioner" by three academics defines the role pedantically:

"The general practitioner is a specialist trained to work in the frontline of a healthcare system and to take the initial steps to provide care for any health problem(s) that patients may have. The general practitioner takes care of individuals in a society, irrespective of the patient's type of disease or other personal and social characteristics, and organises the resources available in the healthcare system to the best advantage of the patients…. [Truncated]."[2]

The 2002 European consensus definition of "General Practice/Family Medicine" asserts:

"General practitioners/family doctors are specialist physicians trained in the principles of the discipline. They are personal doctors, primarily responsible for the provision of comprehensive and continuing care to every individual seeking medical care, irrespective of age, sex and illness. They care for individuals in the context of their family, their community, and their culture – always respecting the autonomy of their patients. They recognise that they will also have a professional responsibility to their community. In negotiating management plans with their patients, they integrate physical, psychological, social, cultural and existential factors, utilising the knowledge and trust engendered by repeated contacts. General practitioners/family physicians exercise their professional role by promoting health, preventing disease and providing cure, care, or palliation. This is done either directly or through the services of others according to health needs and the resources available within the community they serve, assisting patients where necessary in accessing these services…. [Truncated]."[3]

For those outside the field, these definitions are filled with jargon and almost incomprehensible. The definitions were, in most part, written with a priori understanding of general practice. By listing all the desirable attributes of a general practitioner or espousing the principles of family medicine, these definitions lose focus of the field.

There is a need to focus on the core of the discipline and identify a single term that best encapsulates the ethos of the field. Whilst earlier definitions have incorporated 6 to 9 characteristics, we sought the single-most enduring value that general practitioners bring to clinical medicine. Among the possible choices from the literature were the biopsychosocial model, comprehensive scope, holistic care, patient- or person- centred care, continuity of care and family-oriented care. All of these features are

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important to family medicine, however, one characteristic stands out, i.e. holistic care.

Academic leaders in the field have emphasized the core value of holistic care in general practice.[4] Holistic care (increasingly preferred over the more arcane term biopsychosocial model) is an approach that considers biomedical, psychological, environmental, and social factors.[5] As a high-level perspective, holism incorporates person-centred care as well as empathy towards patients' illness and suffering. Medical students and trainees are often drawn to a career in this field, based on holistic care.[6] A holistic approach is the underpinning philosophy of family medicine as an independent discipline. Indeed, GPs strive for a generalist approach focusing on the overall health and well-being of their patients.[7]

An ideal definition should demarcate the boundaries as a concept. A scope that is comprehensive of all medical conditions can be perceived as incredible.[8] Thus, the claim that family physicians treat "every disease entity" appears far-fetched. In reality, general practitioners make a referral in about 5 to 15% of all patient visits.[9] Typically, family physicians treat the most common illnesses in a community. While the mix of common illnesses can vary across communities, it is nevertheless a useful concept in describing the work of general practitioners worldwide. “Common illnesses” is a term that is more likely to resonate with laypersons than abstract principles, such as comprehensive care. In a more fundamental sense, it reflects our commitment to the needs of the community. Common illnesses can include diabetes, eczema, asthma, stroke, domestic violence, drug abuse, tuberculosis, HIV/AIDS, and hospice care depending on the community. In contrast, rare diseases managed by subspecialists, have an incidence of less than 0.05 per 100 persons.[10]

Table I: A definition of family medicine and general practice.

General practice/family medicine is the medical specialty that manages common and long-term illnesses in children and adults focusing on overall health and well-being.

Based on these concepts of scope and a holistic approach, a new definition of general practice can be formulated (Table I). This definition incorporates the values of continuity ("long-term") and caring for patients of all ages ("children and adults").

The target audience for this definition are laypublic and policy-makers as well as the healthcare community. The proposed definition carries a risk of oversimplification, especially if the term “common illnesses” is not expanded with examples that illustrate the complexity of cases managed by GPs. The diversity of conditions encountered in family medicine is difficult to convey and perhaps difficult to comprehend by laypersons. For this reason, the definition should be followed by examples of common illnesses, such as asthma, diabetes, and end-of-life care.

Policy-makers will find the definition useful in making a case for greater support for general practice within the healthcare system; while family medicine educators and practitioners will gain clinical focus and highlight their unique contribution to healthcare. Thus, this definition may guide clinical care and medical education in family medicine by matching its aspirations, while being focused and accountable.

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The Need for Formal Training in the Peer Review Process and Role of Publons Academy

Sir,

We have read with interest ”Reviewers' Role in Research” by Zareen[1], published in your esteemed journal. We would like to offer following comments to improve the peer review process in Pakistan.

1. The current model of peer review has received criticism in the recent years; but currently, this is the best model available to evaluate research submitted for publication. Peer review is of many types including open peer review, single or double blind peer review, collaborative peer review, and post-publication peer review.[2] Each one has its own merits and demerits, which are beyond the scope of this letter. Although most of the journals in Pakistan follow the single/double blind peer review, it is important for the researchers to know the value of a post-publication peer review, which in some cases can surpass even the traditional peer review.[3] Therefore, authors should try to explore and locate journals, which allow this alternate form of peer review.

2. Recent revision of the faculty promotion rules by Pakistan Medical and Dental Council (PMDC) and the paradigm shift in the research culture in the last decade has led to a substantial increase in research publications from Pakistan. Currently, 72 biomedical journals are published in Pakistan.[4] Despite this change in the publication culture, there are few avenues to train the students, residents, and faculties in the art of medical writing. Those who successfully write and submit a manuscript are faced with the challenges of responding to peer review comments which are either vague, non-specific or outright offensive, as correctly pointed out by Zareen.[1]

3. It is important to understand that writing a manuscript for publication and performing peer review of a manuscript submitted for publication, require different set of expertise. In case of Pakistan and most of the developing countries, published authors or senior faculty members are sent manuscripts for review and comments. Many of them just pass on these manuscripts to their juniors and students, who are not qualified enough to comment; and result is a poor peer review, which leaves the authors confused and disheartened.[1] There is evidence that formal training in the peer review process can help learn the art of peer review and enhance the quality of peer review performed.[5,6] Pakistan Association of Medical Editors (PAME) has conducted few courses in the past.[7] We would recommend and discuss a recent initiative to teach the peer review process online via an on-demand course from Publons Academy.[8]

4. This is an online course consisting of ten modules which teaches details of peer review process in the form of 5-10 minutes video lectures (https://publons.com/community/academy/). Topics include identifying common flaws, evaluating methodology, data and results, structuring and effectively communicating the evaluation and understanding ethics of peer review (reviewer biases, conflicts of interest, and misconduct). The course has been designed by experts from different fields having background in publishing, peer review and editorial expertise. This service is available free of cost and any researcher from any part of the globe can enroll. Modules are supplemented by a 1-3 minute video titled “Expert tip”, in which an expert in the editorial and peer review process offers tips and hints to perform better peer review. Some modules are followed by exercises, (including post-publication peer review of articles), which have to be evaluated by a supervisor. Slides of all modules and lectures can be downloaded in PDF format. The peer review resource section of the Publons Academy offers many useful resources for the reviewers and on how to spot a predatory journal.[9] In addition, this section also provides links to external resources on peer review from reputable publishers and experts all around the globe.

We recommend that medical journals in Pakistan should encourage their registered reviewers to complete this online course. PAME, College of Physicians and Surgeons Pakistan (CPSP) and PMDC should collaborate to increase the awareness of a formal training program in peer review like Publons Academy, so that the next generation of researchers and peer reviewers is well prepared to face the challenges to perform a rigorous, un-biased and scientific peer review.

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Posterior Reversible Encephalopathy Syndrome Secondary to Acute Post-Streptococcal Glomerulonephritis in a 12-year-old Girl

Sir,

Posterior Reversible Encephalopathy Syndrome (PRES) is a syndrome characterized by headache, confusion, seizures and visual loss.[1] The major abnormality is cerebral vasogenic edema. Its pathogenesis is still under debate.[2]

It is a reversible condition which usually affects the occipital lobes, but structures of the posterior fossa can also be involved.[3]

PRES has been reported in patients of 4 to 90 years of age with mean age ranging from 39 to 47 years.[4]

Fugate *et al*. found causes of PRES as hypertension (61%), autoimmune disease (45%), cytotoxic drugs (19%), sepsis (7%), preeclampsia (6%), and multiple organ dysfunctions (1%).[5]

Herein, we present a case of PRES secondary to renal involvement in a 12-year-old girl.

A 12-year-old girl presented to emergency department with complaints of abdominal pain, headache and vomiting for 2 days, fits and altered level of consciousness for 1 day. Abdominal pain was sudden, severe and involved flank regions. Vomiting was non-projectile, frequent, containing no blood. Headache was severe, more in frontal region and associated with blurring of vision. There were 2-3 episodes of fits, each lasting for 3-4 seconds, associated with impaired consciousness. There was burning micturition and decreased urine output. She had no other complaints.

She was vitally stable apart from her raised blood pressure (BP), i.e. 150/110 mmHg. She was semi-conscious with anthropometric measurements at 25th centiles for her age. There was no pallor, body rash, lymphadenopathy, and oral ulcers. Her Glassgow coma scale (GCS) was 13/15 with normal muscle tone and power. She had brisk reflexes with negative signs of meningeal irritation and down-going plantars. Her pupils were bilaterally equal and reactive while fundoscopy revealed bilateral papilledema. Abdomen was tender in lumbar regions. Rest of the examination was unremarkable.

She was a case of hypertensive encephalopathy. On the basis of given clinical evaluation, encephalitis, glomerulonephritis, and renal artery stenosis were considered as possible underlying causes leading to hypertension.

Complete Blood Counts (CBC) showed Total Leukocyte Counts (TLC) 12,000/mm^3 with 70% neutrophils, hemoglobin 13 g/dl, erythrocyte sedimentation rate (ESR) 40 mm/1st hour, and C-reactive protein 48 mg/dl. Blood sugar, electrolytes, liver function tests, renal parameters and cerebrospual fluid (CSF) examination were completely normal.

Urine microscopy showed protein ++, blood +++, red blood cells (RBCs) 60-70/HPF, white blood cells (WBCs) 3-4/HPF. Anti-streptolysin O (ASO) titer was positive (400 IU/ml) with low complement C3 levels, 70 mg/dl. On imaging procedures, she had normal ultrasound abdomen with normal Doppler renal scan.

We started initial treatment by maintaining her airway, breathing, circulation; and giving her injectable antibiotics, diuretics, antihypertensives and analgesics.

Two days after hospitalization, patient developed gross hematuria and sudden loss of vision. MRI brain showed abnormal symmetrical MR signals return from bilateral frontal, posterior temporal and occipital regions, which

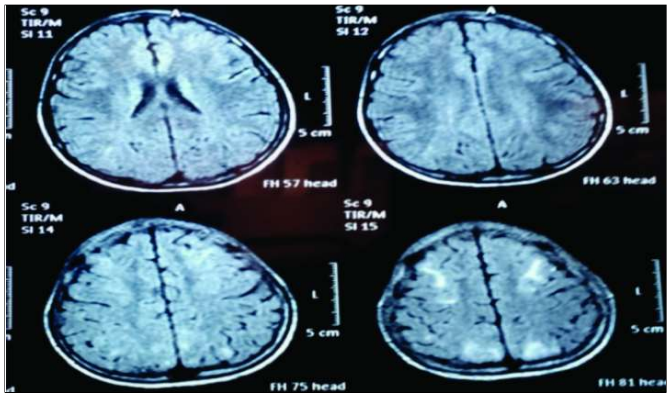

Figure 1: Abnormal symmetrical hyperintense signals return from bilateral frontal, posterior temporal and occipital regions, suggestive of PRES.

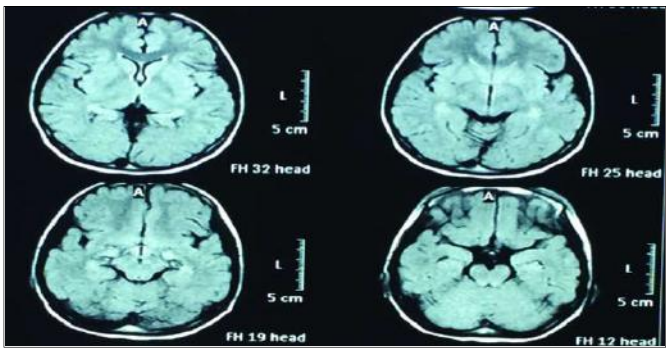

Figure 2: MRI brain on 6 weeks follow-up showing complete resolution of abnormal hyperintense signals.

were iso on T1w, high on T2w; and FLAIR images showed no diffusion restriction (Figure 1).

Final diagnosis was PRES secondary to acute post-streptococcal glomerulonephritis.

We continued same treatment. Her BP was controlled and vision restored after 4 days. She was discharged on antihypertensive medications and advised follow-up. Six weeks after her initial scan, her repeat MR scan showed complete resolution (Figure 2).

Hypertension is an important trigger for PRES. It is of utmost importance to identify the characteristic radiological findings on MRI. PRES should always be kept as a likely possibility in children who present with encephalopathy and seizures in the background of raised BP or kidney disease; because delay in diagnosis and treatment may cause permanent neurological deficit.

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Multiple Lung Cavities in Hodgkin's Lymphoma

A 33-year lady presented with cough, scanty sputum with occasional blood streaks, and on and off fever of 6 months duration. She denied history of shortness of breath, chest pain, weight loss, night sweats or contact with a person with similar symptoms. Her examination was unremarkable apart from few scattered crackles in the chest. Of particular note, there was no lymphadenopathy. Computerized tomography (CT) scan of the chest and fluorodeoxyglucose-positron emission tomography (FDG-PET)/CT images are shown in Figures 1 and 2.

CT scan of chest revealed a large anterior mediastinal mass (Figure 1A, 1C & 2C, white arrows) and multiple cavitary lesions in both lungs (Figures 1A, 1C, 2A, 2C, black arrows). FDG-PET/CT scan showed FDG-avid cavitary consolidation in the left upper lobe (Figure 1B), mainly in the lingular area with SUVmax 4.1. In addition, there were multiple cavitary FDG-avid nodular opacities noted in the left lung (Figure 2B) along with similar mild FDG-avid nodular opacity in the right middle lobe.

Biopsy from the anterior mediastinal mass confirmed the diagnosis of classical Hodgkin's lymphoma (HL), nodular sclerosis type. Because of the presence of multiple lung cavities, tuberculosis (TB) was considered, but 3 sputum samples for acid fast bacilli were negative. A biopsy from the larger cavitary lung lesion showed HL with negative workup for TB and fungal elements.

After a delay of three weeks because of patient's refusal, she was started on ABVD chemotherapy regimen (adriamycin, bleomycin, vinblastine and dacarbazine) with a good response. She was non-compliant, did not complete the planned treatment and disappeared. She presented again after three months with large lymph nodes in the neck and progression of HL was confirmed. After a long delay, she received salvage chemotherapy with good partial response, but refused autologous stem cell transplant and disappeared again. She again came to our institution with disease progression and massive lymph nodes in the neck. She received second line salvage chemotherapy with a partial response and died of progressive disease after few months.

Pulmonary involvement in HL is common and may occur in up to 40% of cases. Although cavitation in HL may occur in large mass lesions due to necrosis post-treatment, cavitary lung lesions on presentation are rare, occurring in less than one percent of cases.[1-4] Most of these lesions are solitary; and only few cases with multiple cavitary lesions have been reported.[2-4]

The exact mechanism for cavity formation in HL is not known but it is thought to be due to rapid tumor growth

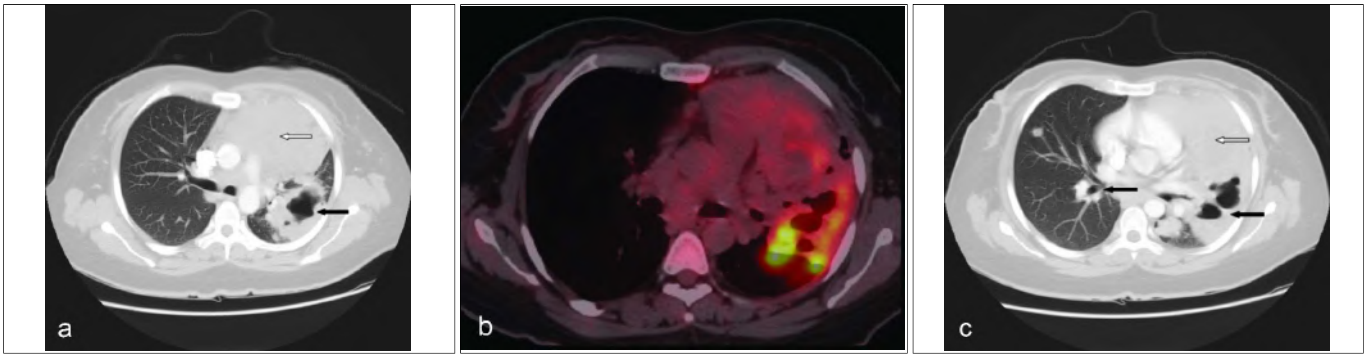

Figure 1 (a,b,c): CT scan of chest showing a large anterior mediastinal mass (1A & 1C, white arrows) and multiple cavitary lesions in both the lungs (1A, 1C, black arrows). FDG-PET/CT scan showing FDG avid cavitary consolidation in the left upper lobe (Figure 1B), mainly in the lingual area with SUVmax 4.1.

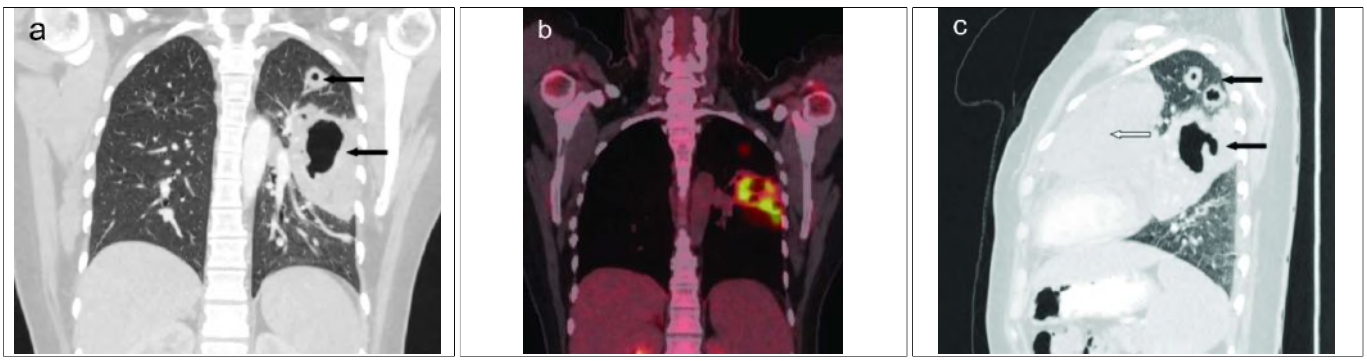

Figure 2 (a,b,c): CT scan of chest, coronal & lateral views showing cavitary lesions in both the lungs (2A & 2C, black arrows). Also, FDG-PET/CT scan showing FDG avid cavitary consolidation in the left upper lobe (2B) and multiple cavitary FDG-avid nodular opacities noted in the left lung (2B).

causing tumor nodules in the lung to cavitate, which subsequently develop as central necrosis from lack of adequate blood supply.[3]

In endemic areas, TB must be ruled out. Other causes of cavitary lung lesions include fungal infections, particularly aspergillosis in immunocompromised individuals, vasculitis and rheumatic diseases, primary and metastatic lung tumors, cystic fibrosis and sarcoidosis. Rarely, TB may co-exist with HL.[5]

This case highlights the importance of proper tissue sampling and use of imaging studies in the diagnosis of atypical presentation of HL in a young female.

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Pleomorphic Adenoma of Submandibular Gland with Extensive Cystic Keratinization

Sir,

Pleomorphic adenoma (PA) is the commonest salivary gland tumor, accounts for 60 - 65% of salivary gland tumors.[1] It chiefly affects the parotid gland, followed by the submandibular and minor salivary glands.[2] Histologically, the tumor shows combination of glandular epithelium and mesenchyme-like tissue.[1] Although, PAs demonstrate sebaceous, mucous, oncocytic, and squamous metaplasia with formation of keratin pearls, but the latter seldom exhibits the formation of extensive keratin, filled cystic areas lined by epithelium.[3]

A 21-year male presented to the Department of Oral Medicine and Radiology (NIMS Dental College, Jaipur, India) with the chief complaint of swelling in the left submandibular region from 6 months. A coronal CT scan revealed a well defined radiolucent mass on the left submandibular gland extending inferiorly to the submandibular region, measuring 6.2 x 7.5 cm in dimension. Based on these features, a provisional diagnosis of PA was given. Patient was referred to the surgical oncology unit. Under general anesthesia, a standard submandibular incision was given and submandibular gland and mass was removed and sent to the Department of Oral and Maxillofacial Pathology for the histopathologial evaluation. The gross specimen was rubbery, mucoid, and glistening with homogenous tan and creamy color areas (Figure 1a).

Microscopical examination revealed ductal and myoepithelial cells arranged in sheets, tubular structures filled with eosinophilic coagulum surrounded by fibrous capsule (Figure 1b), numerous kerain pearl-like structures (Figure 1c), and large cyst like spaces lined by squamous epithelium filled with keratin along with chondroid and myxoid areas (Figure 1d).

Based on microscopical features, a final diagnosis of PA with extensive keratinized cyst formation was rendered. The follow-up period of 2 years was uneventful.

PAs with extensive squamous metaplasia are unusual and can impose diagnostic challenge to pathologists. The microscopical differential diagnoses include squamous cell carcinoma (SCC) and mucoepidermoid carcinoma (MEC).[4] With the absence of dysplasia, cellular atypia, mitotic figures and invasion, a diagnostic possibility of SCC was excluded.[3] Histopathologically, MEC shows numerous cystic areas along with mucous, intermediate, and epidermoid cells. Unlike our case, MEC demonstrates cystic areas lined by mucous cells, MEC lacks fibrous capsule and extensive keratinized areas.[3,4]

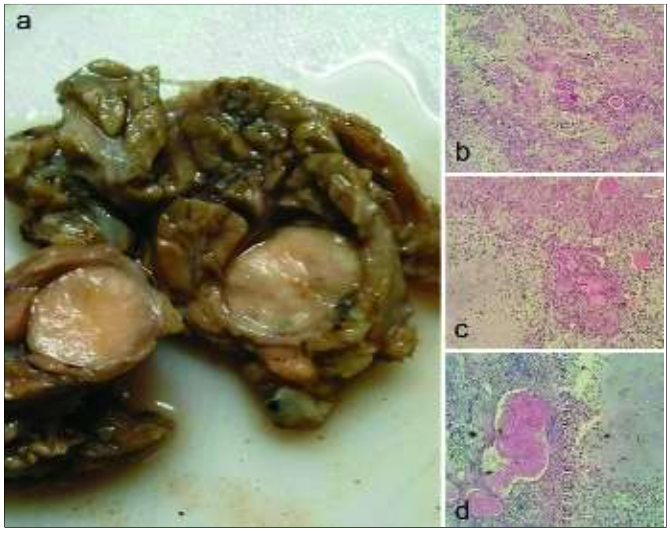

Figure 1: (a) Gross specimen. **(b)** Ductal and myoepithelial cells arranged in cords with fibrous capsule (Hematoxylin and Eosin stain X10). **(c)** Numerous keratin pearl formations with myxoid area (Hematoxylin and Eosin stain X20) **(d)** Large keratin-filled cystic spaces with chondroid area (Hematoxylin and Eosin stain X40).

The case presented here depicts the importance of a differentiation of PA with extensive squamous metaplasia from the malignancies, like SCC and MEC, in order to avoid aggressive treatment modalities.

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Intracortical Lipoma of Tibia in an Adult

Sir,

Intracortical lipoma (IL) is an interesting and rare entity among the osseous neoplasm. The first case report of IL was published in 2014,[1] and only few cases were reported afterwards.

Lipoma in soft tissue, is the most common benign neoplasm of mesenchymal origin and is rarely documented in bones. Histologically, it is a collection of mature adipose tissue without the presence of atypical cells.[2] Osseous lipomas are classified on the basis of their location either as intraosseous (within bone) or juxtacortical (on surface of bone). Both of these entities are further categorized into two subdivisions: intraosseous lipomas into intramedullary (central) or intracortical and juxtacortical lipomas into parosteal and subperiosteal subtypes. Parosteal lipoma is the second commonest after the intramedullary lipoma.[3] The mean age of presentation is noted to be 43 years with almost equal distribution in males and females.[4] As per World Health Organization (WHO) bone tumor book, lipoma of bone is something which is rarely seen and accounts for only 0.1% of primary bone tumor.

A 70-year female had right lower limb pain for a few months. Plain X-ray revealed multiple well defined bony lytic lesion with sclerotic margin and central translucent area in the metaphysis of tibia (Figure 1a). On the basis of radiology, differential diagnoses included synovial osteochondromatosis, giant osteochondroma, giant cell tumor, and intracortical lipoma.

Patient underwent conservative surgery. Multiple bony hard circumscribed nodules with smooth surface were received. Histological evaluation, after careful decalcification, revealed mature adipose tissue without any evidence of hematopoiesis present within the mature cortical bone. No atypia, pleomorphism, mitosis or necrosis was seen in adipocyte histologically (Figure 1b). Thus the diagnosis of intracortical lipoma was made.

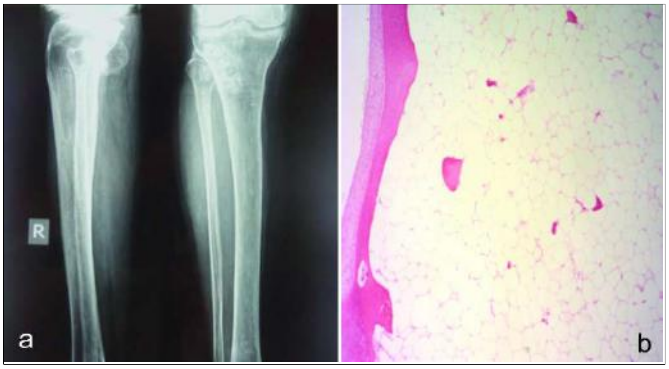

Figure 1: (a) X-ray right tibia and fibula frontal and lateral projection. (b) Photomicrograph. [H and E, 100x].

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Increased Risk of Dental Issues in Type II Diabetics

Sir,

Type 2 diabetes (T2D) is well known to be associated with the changes in the oral bacterial diversity and frequency. It was unknown whether these changes are indicative of the disease or the underlying etiology, until Max Goodson, a dental expert, determined the glucose concentration, bacterial counts, and the relative frequencies of 42 bacterial species in the saliva samples from 8,173 adolescents from Kuwait, using DNA probe analysis.[1] Besides this, the clinical data related to obesity, dental caries, and gingivitis were also gathered. A comparative analysis was carried out between the adolescents with high salivary glucose (HSG) concentration and those with low salivary glucose. The authors concluded that the changes in the salivary glucose concentration affect the salivary microbiome.[1]

In dental perspective, the frequent consumption of glucose has long been associated with dental caries. In addition to that, the above mentioned study supports that hyperglycemia, associated with T2D, triggers the dental decay as HSG makes the saliva more acidic by decreasing the pH. This increased acidity reduces the overall bacterial counts of oral microbiome and alters the prevalence of some bacterial species, particularly the acidophilic bacteria, such as *Streptococcus mutans*, which is the initiator of dental caries. Thus, the investigators predicted that an elevated salivary glucose concentration increases the risk of dental erosion, dental caries, and gingivitis.[1]

In this interesting study, adolescents with HSG were found to show a high percentage (almost double) of carious teeth and a high incidence of gingival redness.[1] However, the new investigations pave the way to a potential strategy for the physicians to emphasize on the importance of the oral hygiene. Therefore, as a part of routine diabetes health check-up, monitoring of oral health should be considered as a basic component. Some patients tend to neglect oral hygiene and consider the T2D as the only medical problem. An early referral to a dentist would prevent the severity of gingivitis and avert T2D complications. At the same time, dentists can help patients by simple chair-side screening for T2D that include finger-stick random capillary glucose and Finnish Diabetes Risk Score (FINDRISC) that assesses age, waist circumference, physical activity, daily consumption of vegetables/fruits, history of hypertension and self-reported body mass index.[2] Henceforth, as the first healthcare personnel to handle the suspected/ affected T2D, we can improve the quality of life of diabetics and should not miss this opportunity of referring them to our colleagues.[3,4]

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INSTRUCTIONS TO AUTHORS

The JCPSP agrees to accept manuscripts prepared in accordance with the "Uniform Requirements submitted to the Biomedical Journals" as approved by the International Committee of Medical Journal Editors (ICMJE) guidelines, published in the British Medical Journal 1991; 302:334-41, printed in the JCPSP, Vol. 3 No. 2, April – June, 1993, updated and reprinted in 2003, 2007, 2008, 2012 and January 2017, Vol. 27(1).

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Authors should submit the manuscript typed in MS Word. Manuscripts should be written in English in British or American style/format (same style should be followed throughout the whole text), in past tense and third person form of address. Sentences should not start with a number or figure. Any illustrations or photographs should also be sent in duplicate. Components of manuscript should be in the following sequence: a title page (containing names of authors, their postal and Email addresses, fax and phone numbers, including mobile phone number of the corresponding author), abstract, key words, text, references, tables (each table, complete with title and footnotes) and legends for illustrations and photographs. Each component should begin on a new page. The manuscript should be typed in double spacing as a single column on A4 (8-1/2" x 11" or 21.5 cm x 28.0 cm), white bond paper with one inch (2.5 cm) margin on one side.

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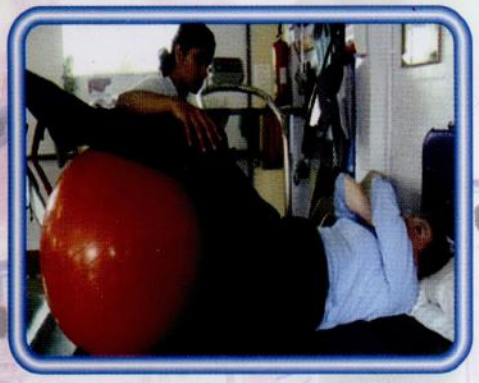

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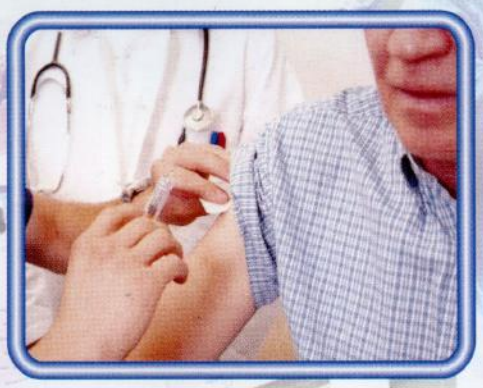

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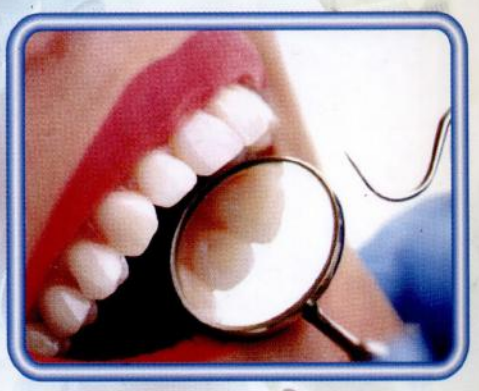

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