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Breast Cancer in Pakistan: A Looming Epidemic

Naseem Begum

The age standardised incidence rate (ASIR) of breast cancer is highest in Pakistan among the Asian countries.[1,2] The results of the first population-based cancer registry in Pakistan, the Karachi Cancer Registry (KCR), with 99% morphologically verified cancers, showed ASIR of 51.7/100,000 in 1995-1997.[1,2] The GLOBOCAN 2012 estimates indicated an ASIR of 50.3/100,000.[3] The population-based Punjab Cancer Registry (PCR), of Lahore district with a population of 15 million, has shown an ASIR of 47.6/100,000 for 2010-2012.[4]

The five-year survival rate for breast cancer vary worldwide. The CONCORD2 study,[5] on Global Surveillance of Survival 1995-2009 analysed the data of 5,486,928 women with breast cancer from 279 population-based cancer registries from 67 countries around the Globe. In women diagnosed with breast cancer during 2005-09, age-standardised five-year net survival was 80% or higher in 34 countries. However, breast cancer survival was lower than 70% in countries like Malaysia (68%), India (60%), and very low in Mongolia (57%) and South Africa (53%). In North America and Oceania, survival from breast cancer was high (84-89%). In Europe, the survival was generally lower than in North America and Oceania. It was difficult to assess the survival in Africa. Likewise, there was no data available on Pakistan as there was/is no national population-based cancer registry system in place.

In Pakistan, like in most low- and middle-income countries (LMIC), there is a lack of follow-up on patients. Therefore, the country has no primary data on mortality due to breast cancer. However, the Age Standardised Mortality Rate (ASMR) for Pakistan (GLOBOCAN 2012 estimates) was 25.2/100,000, highest among South Asian countries.[3] The major reason for high ASMR is the advanced stage of disease at presentation, due to lack of awareness and access to care, delayed clinical evaluation, diagnosis and staging, and absence of timely access to optimum treatment.

A key determinant of breast cancer outcome in any population is the degree to which it is detected at an early stage. In the USA, five-year survival is 99% for localised, 85% for regional, and 27% for distant-stage disease.[6] Screening and early detection has played a major role in reducing the mortality rate in developed countries.

Data on stage-distribution for Pakistan is scarce. In a study carried out on stage-distribution of breast cancer in two tertiary care cancer hospitals in Pakistan (SKMH and INMOL Lahore),[7] including 179 biopsy and stage-proven patients from SKMH and 470 patients from INMOL, 71% and 63% presented in stage III and IV, respectively; 25% patients at INMOL and 36% at SKMH presented in stage IV (metastatic). In a recently published descriptive, retrospective study conducted at the Liaquat National Hospital, Karachi, Pakistan, records of 8,291 breast cancer patients (all biopsy-proven) registered from 1994-2014, were analysed.[8] The number of patients with stage 1 increased from 53 (0.64%) in 1994 to 847 (10.21%) in 2014. Year-wise data showed a slow upward trend for cases diagnosed in stage 1. High ASIR, high ASMR, and advance stages at presentation, all point to an alarming situation in Pakistan.

A comprehensive National Cancer Control Programme (NCCP) is mandatory for any country to confront the growing cancer crisis. Therefore, on the request of Ministry of Health, Pakistan, a multi-disciplined team consisting of experts from two leading international organisations – International Atomic Energy Agency and World Health Organization – conducted an integrated mission under programme of action for cancer treatment (imPACT), in Pakistan in 2013. The mission experts assessed the national capacity with regard to cancer control planning, cancer registries and surveillance, primary prevention, screening and early detection, diagnosis and treatment, and palliative care services. The mission gave its recommendations for actions to be taken by the relevant authorities, and facilitated the identification of priorities and projects. The recommendations included the creation of an active National Steering Committee, drawn from representatives in the Ministry of Health / relevant health centres / professionals, and other relevant national stakeholders. It is required by this experts mission to convene regular meetings with the intent to draw up a strategic national cancer control programme, and to monitor and evaluate the progress of this programme.

Currently, in Pakistan there are fragmented efforts for screening and early detection of breast cancer. There was an initiative on the part of Federal Government of

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Pakistan and the first dedicated breast cancer care centre was established in Islamabad in 2014, followed by the second at Lady Aitchison Hospital, Lahore in 2017. These two centres aim at cost-free community-based mass breast screening programme.

There are some initiatives, in the form of dedicated breast care centres, one stop breast clinic (for triple assessment), and stand alone breast mammography clinics in private sector (both profit and non-profit), where cost is one of the impeding factors. The philanthropic initiatives and the services of a couple of mobile vans to take the mammography at the doorsteps, especially of the women living in the rural areas, also exist. Are these fragmented efforts enough to manage the crisis of cancer in general and breast cancer in particular?

The breast cancer control efforts should not be conducted in isolation, but integrated into an overall framework of a comprehensive strategic NCCP. A comprehensively structured and effective NCCP links and integrates cancer control planning, cancer registration and surveillance, prevention, early detection, diagnosis and treatment, and palliative care. A well-planned cancer control infrastructure should form the basis of a robust national investment, which will benefit cancer patients for decades to come. Government commitment, supported by expertise from PACT's strategic partners, is vital to develop and implement successful cancer control programmes.[9]

A NCCP with all the stakeholders on board has yet to be drafted, developed and implemented in Pakistan. This delay appears to be due to lack of political commitment, complicated system of bureaucracy, involvement of non-willing experts, and an inactive national steering committee for NCCP.

Most of all, a low priority is assigned to health sector as expenditure on health is a meagre 2.6% of GDP. Pakistan is a signatory to the Universal Declaration of Human Rights and the two subsequent international legal instruments namely, the International Covenant on Economic, Social and Cultural Rights (ICESCR) and the International Covenant on Civil and Political Rights (ICCPR), that recognise a citizen's right to health, which the signatory has to respect, fulfill and protect.[10] In the above context and in view of the gravity of situation, there is a dire need to reestablish national health priorities on urgent basis in order to address the rising cancer incidence in general and breast cancer in particular.

Development and implementation of a NCCP with strong political commitment and a well-planned, realistic National Action Plan (NAP) with allocation of sustainable resources, including financial and human resources, is the need of the hour. For successful implementation of the NCCP, the NAP, in addition, should assign tasks to specific institutions / individuals and allocate dedicated funding towards the costs of each activity with a time-frame. The cancer control should be incorporated in all relevant national policies and programmes. There can be life after breast cancer; but the prerequisite is early detection.

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ORIGINAL ARTICLE

Correlation between Serum Amyloid A-Low Density Lipoprotein and Genotoxicity in Smokers

Aneela Jamil[1], Amir Rashid[1], Abdul Khaliq Naveed[2] and Asifa Majeed[1]

Abstract

Objective: To investigate the relation between serum amyloid A-low density lipoprotein (SAA-LDL) and genotoxicity in smokers.

Study Design: An experimental study.

Place and Duration of Study: Army Medical College, Rawalpindi and National Institute of Health (NIH), Islamabad, from June 2014 to February 2015.

Methodology: Seventy healthy Sprague Dawley rats were purchased from NIH and exposed to cigarette smoke in smoke chamber for three months. Blood samples were drawn from each rat at the end of the study period. SAA-LDL was determined by enzyme-linked immunosorbent assay (ELISA). Genotoxicity was assessed by cytokinesis block micronucleus (CBMN) assay. Pearson correlation was used to find correlation between SAA-LDL and genotoxicity.

Results: Strong positive correlation was found between SAA-LDL and micronuclei frequency in smoke-exposed rats ($r=0.799$, $N=70$, $p<0.01$).

Conclusion: Statistically significant strong positive correlation between SAA-LDL and genotoxicity in smoke-exposed rats shows that changes in one is associated with changes in other and *vice versa*.

Key Words: *Cigarette smoking. SAA-LDL. DNA damage.*

INTRODUCTION

Cigarette smoking and tobacco chewing are common modes of consuming tobacco all over the world. Cigarrete smoking contributes to about 6 million deaths annually. Smoking is the major risk factor of various health disorders;[1] therefore mechanisms involved in these disorders need to be assessed. Genotoxicity is produced by environmental exposure to genotoxins and lifestyle factors, like alcohol, smoking, drug and stress. Genotoxicity is thought to be one of the mechanisms by which cigarette smoke initiates disease.[2]

Cigarette smoke contains many carcinogens and has been implicated in the development of several cancers. Cigarette smoke also contains many oxidants and free radicals that induce oxidative damage to DNA.[3] Cigarette smoke causes oxidative damage in DNA, either directly or through generation of reactive oxygen species.[4]

Micronucleus assay has been found to be an excellent tool to serve as a genotoxicological biomarker. Micronuclei (MN) are also termed as Howell-Jolly bodies. They are tiny nuclei present in the cytoplasm of dividing cells produced as a result of chromosomal damage. Fragments from chromosomal damage are not integrated into the nucleus during mitosis.[5] Assessment of MN in peripheral blood lymphocytes and in epithelial cells is a most widely used method for measuring genotoxicity in human populations.[5]

Oxidative stress has been shown to be involved as a major factor in the development of chronic diseases including atherosclerosis. Oxidative stress arises when the balance between the production of reactive oxygen species and their detoxification by antioxidants is disturbed, leading to cell damage and finally chronic disease.[6]

Smoking leads to oxidative stress as cigarrete smoke not only contains reactive oxygen and reactive nitrogen species but also increases the production of free radicals by inflammatory cells. Free radicals have deleterious effects on membrane lipids, DNA, and proteins, leading to tissue damage; and they also exert negative influence on cell antioxidant defence system. Oxidative stress biomarkers may be used as early predictors of smoke exposure.[7]

This study, therefore, aimed to determine the association between SAA-LDL, an oxidative stress marker, and DNA damage in smoke-exposed rats.

METHODOLOGY

The experiment was conducted in the Army Medical College, Rawalpindi and NIH, Islamabad, after approval by Ethical Committee of Army Medical College. It was an

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animal experimental study on male Sprague Dawley rats provided by NIH.

The study was conducted on 70 healthy male Sprague Dawley rats, purchased from NIH, Islamabad. Adult male healthy Sprague Dawley rats, 90-120 days old, weighing 200 ±50 grams, were included in the study. Diseased rats with any abnormality were excluded from the study. Animals were given regular diet and water *at libitum* throughout the experiment. Animal diet was prepared at animal house, NIH.

Animals were placed in smoke chamber that was properly ventilated with daily photoperiod of 12 hours light and 12 hours dark. It was ensured to maintain chamber temperature at 23 ±2°C by central temperature regulating system. Cigarette smoke was given to rats in smoke chamber for five days a week for 12 weeks.

At the end of 12 weeks, rats were taken to the laboratory of the animal house NIH, where they were anesthetized and blood samples were drawn by intracardiac puncture. 6-7 ml blood was collected from each rat. 3ml was poured into the vacuum tubes containing clot activator. The blood was centrifuged for 10 minutes at the speed of 3000 rpm. When centrifugation was complete, serum was pipetted out by means of micro-pippete and transferred into the polypropylene eppendorf storage tubes. These tubes were stored at a temperature of -80°C in the research laboratory of Army Medical College for estimation of SAA-LDL. SAA-LDL level was determined using commercially available quantitative sandwich enzyme immunoassay kit from Bioassay Technology Laboratory. Remaining 3 ml blood was collected into the heparinized tubes to assess genotoxicity.

Genotoxicity was assessed by cytokinesis block micronucleus (CBMN) assay. Lymphocytes cultures were done, slides prepared, and were examined under the microscope for the evaluation of MN frequency. The MN frequency was determined by analyzing 1,000 BN cells per sample, according to established criteria for identification of BN cells (e.g., two main nuclei, even partially overlapping, of approximately equal size, staining pattern and staining intensity) and scoring of MN (e.g., small nuclei with diameter at least one-third of main nuclei, separated from or marginally overlapping one main nucleus, with similar morphology and staining as the main nuclei). The MN frequency for each sample was expressed as MN/1,000 BN cells.[8]

Data were entered into statistical package for social sciences (SPSS) version 19. Mean and standard deviation (SD) was calculated for SAA-LDL and MN assay. Pearson correlation test was used to find correlation between SAA-LDL and genotoxicity. Significant p-value was < 0.05.

RESULTS

The correlation between mean of SAA-LDL and genotoxicity, assessed by micronucleus assay, in smoke exposed rats, is shown in Table I. The mean SAA-LDL and micronucleus frequency was 2.99 ±0.59 and 6.77 ±0.73, respectively. There was a strong positive correlation between SAA-LDL and micronuclei frequency (r = 0.799, N = 70, p <0.01).

Table I: Results of correlation between micronuclei frequency and SAA-LDL in smokers.

Variables	Mean ± SD	P-value	*r-value
Micronuclei frequency	6.77 ± 0.73	< 0.01	0.799
SAA-LDL	2.99 ± 0.59		

** r value is correlation coefficient or Pearson r.*

DISCUSSION

Many endogenous and exogenous agents lead to DNA damage. Endogenous agents are mainly reactive free radicals produced in the oxidative metabolism of cells. Cigarette smoking is an important exogenous agent along with chemicals and radiations.[9] Cigarette smoke, a major public health hazard, contains many well-established carcinogens, which may induce carcinogenesis by their mutagenic and genotoxic effects. Cigarette smoke changes the structure of DNA leading to genotoxicity that contributes to several cancers including mouth, stomach, lungs and urinary bladder.[10]

Hirsch *et al*. conducted an experimental study on male Sprague Dawley rats. He showed significant rise in DNA damage in type II alveolar cells of rats after smoke exposure. DNA damage in alveolar cells was homogeneous with alveolar cell apoptosis. They also concluded that genotoxicity and cell apoptosis may be due to increased neutrophil count and oxidant-antioxidant imbalance in the airways of smoke exposed rats.[11]

Damasceno *et al*. assigned female obese rats into two groups: control and obese rats exposed to cigarette smoke for a period of two months. He documented that obese rats exposed to smoke had significantly increased DNA damage and serum triglycerides and cholesterol versus that of control. Moreover, superoxide dismutase level was significantly low in smoke exposed rats compared to that of control rats. Based on these results, he concluded that obesity and smoking together had aggravated the genotoxicity and deranged the lipid profile.[12] Increased DNA double-strand breaks compared to non-smokers, were shown by Paschalaki *et al*.[13]

A study was conducted in India by Sundararajan *et al*., in which he included 60 females. Twenty-three out of 60 cases had no lesion in the cervix and were included in group A; while 37 were in group B, diagnosed with cervical cancer. Frequency of MN was calculated. The MN frequency was statistically significant in Group B as compared to that in group A. A significant linear association was observed between the frequency of MN and cervical cancer stage.[14]

Uma *et al*. carried out his study on rural population of South India. He recruited 30 smokers and 30 non-smokers in his study. He measured the frequency of satellite associations in peripheral blood lymphocytes by cytogenetic assay as a biomarker for chromosomal damage. The results of this study illustrate that the cigarette smoke has genotoxic effect, and cigarette smoke induced-genotoxicity increases with intensity of smoking.[15]

Rana *et al*. also reported similar results. Sixty-seven male subjects participated in the study. Micronucleus assay was performed on buccal mucosal cells of the study population, and 8-hydroxyl-2-deoxyguanosine was estimated in their urine samples. MN count was statistically increased in tobacco chewers followed by smokers and alcoholics ($p < 0.05$). Similarly, 8-hydroxyl-2-deoxyguanosine was raised in tobacco chewers followed by smokers and alcoholics ($p < 0.05$). Cigarette smoke induces oxidative stress that leads to genotoxicity.[16]

Consistent results were illustrated by Gordana Kamceva *et al*. His research on human subjects showed significant rise in oxidative stress marker, malondialdehyde, in active smokers compared to that of non-smokers. He also analysed antioxidant enzymes: superoxide dismutase and glutathione peroxidase. Results showed no significant difference of glutathione peroxidase between two groups while superoxide dismutase was significantly increased in active smokers versus non-smokers. He, therefore, concluded that smokers had high oxidant stress and oxidative tissue damage and low antioxidant defence activity than that of non-smokers.[17]

Conflicting results were documented by Lykkesfeldt *et al*. and Block *et al*.[18,19] They showed no significant change in the antioxidant status of smokers versus non-smokers.

Sarker *et al*. suggested that sidestream smoke exposure increased oxidative stress as shown by increase in reactive oxygen species in cultured human pulmonary fibroblasts exposed to sidestream cigarette smoke; and also increased levels of oxidative DNA damage significantly as assessed by the quantitative PCR assay in the human gene, hypoxanthine phosphoribosyl transferase 1, in sidestream smoke exposed human cells.[20]

Passive inhalation of cigarette smoke caused significant oxidative stress in a study by Vasconcelos *et al*.[21] Sugiura *et al*. conducted his study on male smokers and non-smokers. He measured serum levels of reactive oxygen metabolites in the study subjects. Results showed significant differences in the levels of reactive oxygen metabolites among the two groups.[22] Zhang *et al*. in his study evaluated the effect of whole smoke exposure on oxidative stress biomarkers in bronchial epithelial cells. Increased levels of oxidative stress biomarkers were observed in these cells following whole smoke exposure.[23]

Isik *et al*. in his study evaluated the effect of smoking on oxidative stress in a healthy population using malondialdehyde as an oxidative stress biomarker. Thirty smokers and 30 non-smokers were recruited in the study. Results showed significant increase in the serum levels of malondialdehyde in smokers compared to non-smokers.[24]

Isabel *et al*. investigated oxidative stress and genotoxicity in human peripheral blood lymphocytes exposed to smoke. 8-hydroxy-2'-deoxyguanosine was used as an oxidative stress biomarker. He showed significant increase in DNA strand breaks and the level of 8-hydroxy-2'-deoxyguanosine in smoke exposed cells as compared to that of the control.[25]

CONCLUSION

Genotoxicity and oxidative stress are correlated in smokers as shown in the results by the statistically significant strong positive correlation between genotoxicity and SAA-LDL in smoke-exposed rats.

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ORIGINAL ARTICLE

Frequency of Macroprolactin in Hyperprolactinemia

Noreen Abbas Sherazi[1], Mirza Zain Baig[2] and Aysha Habib Khan[3]

ABSTRACT

Objective: To determine the frequency of Macroprolactin (MaPRL) in patients with increased total prolactin and its clinical and financial impact.

Study Design: Cross-sectional study.

Place and Duration of Study: Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital, Karachi, from March to May 2015.

Methodology: Patients with high total prolactin were screened by polyethylene glycol (PEG) precipitation for determination of MaPRL. Clinical history, imaging work-ups, and cost incurred in further investigations were collected by telephonic interview after verbal consent. Patients were stratified into true hyperprolactinemia and macroprolactinemia after PEG treatment, based on monomeric prolactin levels. Medical records of cases registered with AKUH were reviewed to confirm the diagnosis.

Results: Two hundred and thirty-nine patients were identified with high prolactin levels. Macroprolactinemia was identified in 145 (60.7%) and true hyperprolactinemia in 94 (39.3%) patients. Galactorrhea was significantly more in true hyperprolactinemic females (p=0.022), followed by visual disturbances (p=0.01) and headache (p=0.006). Moreover, as majority of population were females, the clinical features in the macroprolactinemia group as compared to true hyperprolactinemic group were mostly related to non-pituitary causes like drug intake [42.5% (54) vs. 37% (30)], heat intolerance due to thyroidal illness [41.7% (53) vs. 38.3% (31)] and surgery [26.8% (34) vs 22.2% (18)] in females. Further radiological workup (MRI, CT) were conducted in 35 (37.2%) patients with true hyperprolactinemia. Twenty-one (60%) of the patients were confirmed to have pituitary adenomas. In eight (5.5%) patients with MaPRL, only one had pituitary micro-adenoma on radiological workup. Total cost impact on the basis of investigations, was significantly higher in the group undergone imaging, despite 7 out of 8 individuals found to have normal imaging results. The median total cost in true hyperprolactinemic group undergone imaging was Rs. 4370 (IQR=2412.5, 22850) as compared to macroprolactinemic groups; Rs. 3,250 (IQR=2150, 4278). There was significant difference in the cost burden of both the groups (p <0.001).

Conclusion: High frequency of MaPRL was identified in patients with hyperprolactinemia. Screening with PEG precipitation in hyperprolactinemic sera is simple and cost-effective.

Key Words: *MaPRL. PEG precipitation. True hyperprolactinemia. Oligomenorrhea.*

INTRODUCTION

Prolactin (PRL) occurs in three isoforms, i.e. a monomeric PRL {MW~23 kDa}, a big PRL {MW~50 kDa} and a complex of monomeric prolactin and IgG known as macroprolactin (MaPRL) or as "big, big PRL" {MW <100 kDa}.[1] Circulating total prolactin hormone in normal and patients with increased prolactin levels mainly comprise of monomeric PRL (<85%) and MaPRL (less than 2%). MaPRL is biologically inert, as it is impermeable to the capillary blood barrier due to its large molecular size but is measured in the prolactin assay leading to falsely elevated prolactin levels.[2,3] However, in few cases of hyperprolactinemia; MaPRL becomes the dominant form, as it has been reported from 10% to 45% in hyperprolactinemic patients.[4-6]

The polythylene glycol (PEG) precipitation is used widely in clinical laboratories performing prolactin assay to screen for MaPRL.[2,3] PEG precipitation distinguishes patients with true hyperprolactinemia, which is due to increase of bioactive monomeric PRL, from those with MaPRL, in which monomeric prolactin is normal in concentrations. Inability of laboratories to perform PEG precipitation leads to overreporting of hyper-prolactinemia and consequent over investigation of the patient by treating physicians.[4,5]

PEG precipitation and analysis for MaPRL and monomeric prolactin of all samples with hyper-prolactinemia was started at the laboratory as a quality improvement initiative. This study was conducted to determine the frequency of MaPRL in patients with increased total prolactin and its impact on clinical and financial outcome.

METHODOLOGY

A retrospective cross-sectional study was carried out at Section of Clinical Chemistry, Department of Pathology

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and Laboratory Medicine, The Aga Khan University, Karachi. The study was approved by the Ethical Review Committee of The Aga Khan University Hospital. Patients' sera having high total prolactin levels (>25-200 ng/ml in females and >15-200 ng/ml in males) were screened by PEG precipitation for MaPRL determination. Patients were contacted by telephone and those who gave verbal consent, were interviewed about clinical history, imaging workups and cost incurred in further investigations. Medical records of cases registered at AKUH were reviewed to confirm the diagnosis.

Serum samples with increase prolactin concentration were mixed with an equal volume of 25% PEG in saline, and incubated for 10 mins at room temperature. The monomeric PRL level in the supernatant was quantified by enzyme-amplified chemiluminescent immunoassay (ECLIA) on Immulite 2000, from Siemens, Germany.

Analytical sensitivity of the assay was 0.5 ng/mL. An Intra-assay coefficient of variation (CV) at the PRL concentration of 11.9 ng/mL was 4.8% and inter-assay CV at the concentration of 22.3 ng/mL was 4.0%. Reference ranges used in the laboratory were 1.9-25.0 ng/mL for women and 2.5-17.0 ng/mL for men.

The reproducibility of the PEG precipitation procedure was monitored by inclusion of control sera in each assay. Absolute levels of monomeric prolactin in sera after PEG precipitation were used for reference range i.e. 3.6-12.4 ng/ml in males and 4-18.5 ng/ml in females.[7]

The data was analyzed on SPSS (version 21.0). Macroprolactinemia accounts for up to 15% to 30% in frequency so for sample size calculation, taking 95% confidence interval with 5% type 1 error, 196 number of patients were required to achieve the target population. Two hundred and thirty-nine patients were recruited for better spread of data. Frequencies and percentages for categorical variable, mean and standard deviation (SD) for discrete or continuous variables and for non-parametric data, median with interquartile range were calculated. Patients were stratified under macro-prolactinemia group and true hyperprolactinemia group, according to monomeric reference range after PEG treatment. Cost comparison between both true hyperprolactinemia and those with MaPRL were performed by mean expenditure in performing imaging studies. The Chi-square test used for categorical variables; in case sample size or frequency found less than <5 then Fishers' exact test was employed for continuous variables. Additionally, for non-parametric data, Mann-Whitney and Kolmogorov-Smirnov tests were used to check normality of data. P-value <0.05 was taken as level of significance in analysis.

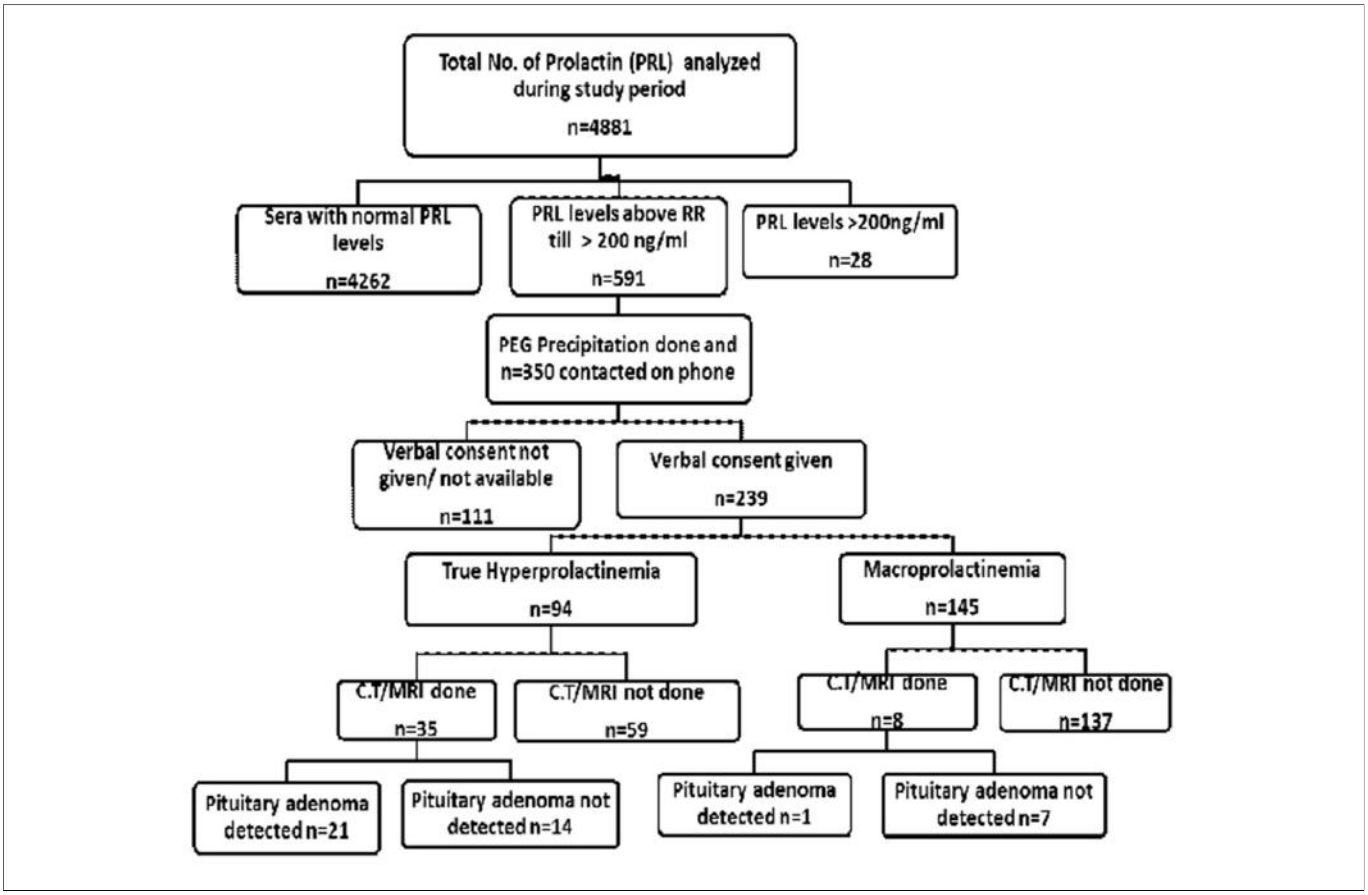

Figure 1: Consort diagram of study participants. The flow of patients enrolled, screened and imaged is shown in accordance to the CONSORT statement.

RESULTS

Figure 1 shows the total number of patients tested for serum prolactin at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine during the study period, with breakup of those screened and enrolled in the study.

Three hundred and fifty patients, out of 591 with PRL levels between normal range and till 200 ng/ml, were screened for MaPRL by PEG precipitation. Out of these, 239 gave informed consent and provided the clinical details. Median age of the patients was 28 years, (IQR=24, 35) and male/female ratio was 31/208, with female preponderance. MaPRL was present in 60.7% (n=145) of hyperprolactinemic patients. The monomeric PRL levels are significantly different (p <0.001) from 26.7 ng/ml (IQR=21.2, 44.9) to 12.9 ng/ml (IQR=9.8, 15.1) in true hyperprolactinemia and macroprolactin after PEG precipitation.

Table I compares the demographic and biochemical details of patients with true hyperprolactinemia and macroprolactinemia. Total prolactin was significantly higher (p <0.001) in patients with true hyperprolactinemia as compared to patients with MaPRL and this difference was maintained after treatment with PEG. There was also significant difference in the cost burden of both the groups (p <0.001). The median total cost in true hyperprolactinemic group undergone imaging was Rs. 4,370 (IQR=2412.5, 22850) as compared to macroprolactinemic group was Rs. 3,250 (IQR=2150, 4278).

Table I: Comparison of demographic, biochemical and expenses in patients with true hyperprolactinemia due to MaPRL screened at Aga Khan University Hospital Clinical Laboratories (n=239).

Variables	True Hyperprolactinemia (n=94)	MacroPRL (n=145)	p-value
Age (years)	28 (IQR=24, 35)	28 (IQR=24, 35)	0.846
Gender			
Male	13 (13.8%)	81 (86.2%)	0.750
Female	18 (12.4%)	127 (87.6%)	
Marital Status			
Married	68 (72.3%)	118 (81.4%)	0.101
Single	26 (27.7%)	27 (18.6%)	
Total Prolactin (ng/ml)	49.3 (IQR=35.4,81.9)	27 (IQR=24,32)	<0.001**
Monomeric Prolactin (ng/ml)	26.7 (IQR= 21.2,44.9)	12.9 (IQR= 9.8,15.1)	<0.001**
Radiological (MRI+CT)	35 (37.2%)	8 (5.5%)	
Adenoma detected	21	1	<0.001**
Adenoma not detected	14	7	
Total cost (PKR)	4370 (IQR=2412.5, 22850)	3250 (IQR=2150, 4278)	<0.001**

***Highly significant; *Significant.*

The indications for testing of prolactin were diverse and varied between males and females (Figures 2a and 2b). Overall in females, predominantly menstrual disturbances, infertility, oligomenorrhea were the main indications for testing followed by heat intolerance, obesity, headache, cold intolerance, visual disturbance and galactorrhea.

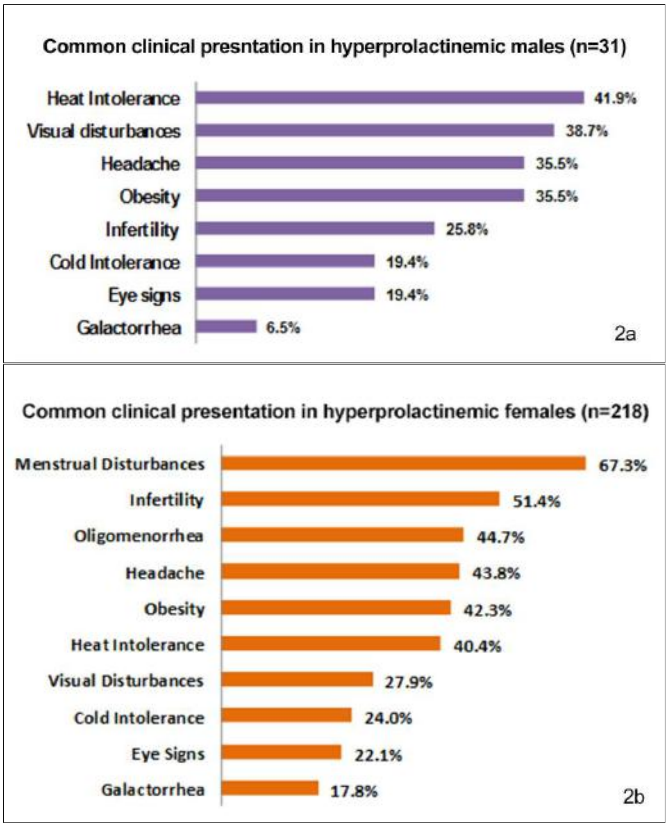

Figure 2: Indications for screening of patients for hyperprolactinemia and frequency comparison between true hyperprolactinemia and patients with MaPRL.

While in males, indications for testing were predominantly heat intolerance, visual disturbance, infertility and obesity followed by headache, cold intolerance and galactorrhea. Monomeric and MaPRL levels in patients with hyperprolactinemia did not differ when compared with clinical presentation in either males or females. Upon stratification, Galactorrhea was significantly more in true hyperprolactinemic females (p=0.022), followed by visual disturbances (p=0.01) and headache (p=0.006). Moreover, as majority of population were females, the clinical features in the macroprolactinemia group as compared to true hyperprolactinemic group were mostly related to non-pituitary causes like drug intake [42.5% (54) vs. 37% (30)], heat intolerance due to thyroidal illness [41.7% (53) vs. 38.3% (31)] and surgery [26.8% (34) vs. 22.2% (18)] in females.

Imaging studies (MRI, CT) were conducted in 35 (37.2%) patients with true hyperprolactinemia. However, only 8 (5.5%) patients with MaPRL were directed for further imaging. Statistically significant difference (p <0.001) in financial impact was seen between the two groups. Among the 35 patients, who underwent MRI/ CT scan, medical records showed that 21 (60%) of the patients were confirmed to have pituitary adenomas. Whereas, in the group of patients with hyperprolactinemia due to MaPRL, only one patient was identified with pituitary microadenoma as shown in Table I.

DISCUSSION

A high frequency of patients with hyperprolactinemia was identified due to MaPRL in our patient population. Out of the 145 patients diagnosed with MaPRL, 1 out of 8 patients who underwent MRI or CT scan were identified with microadenoma on CT scan. Previous reports also described minor CT or MRI scan abnormalities consistent with the presence of a microadenoma in macroprolactinemic patient. Consistent with this observation, abnormal pituitary CT scans 21% vs. 75% are reported in macroprolactinemic and true hyperprolactinemic patients, respectively. Such patients need follow-up scans and monitoring of pituitary microadenoma, as surgical intervention is needed in growing microadenomas/macroadenomas.[10-12]

MaPRL is the complex of monomeric prolactin attached to IgG, which results in increased size of prolactin molecule and hinders its renal clearance leading to increased levels of total PRL. As shown in this study, it is difficult to differentiate between true hyperprolactinemia and that due to MaPRL by clinical judgment alone; as most of the patients with MaPRL were also symptomatic. The absence of MaPRL screening by laboratories performing prolactin assay leads to over investigating patients with imaging studies and; hence, increase cost of management. High frequency of MaPRL is identified in this study and as reported by other studies also, menstrual disturbances, infertility and galactorrhea remains the most common clinical findings as mentioned in Table II.

Table II: Comparison of frequency of main clinical findings in women with Macroprolactinemia in our study and other major publications.

Symptoms	Vallette-Kasic et al.[7] (n=96)	Gibney et al.[19] (n=32)	Isik et al.[13] (n=84)	Present study (n=127)
Infertility	32%	22%	4.9%	54.3% (69)
Menstrual disturbances	39%	59%	38.9%	66.9% (85)
Galactorrhea	46%	22%	39.2%	13.4% (17)

Cost-effectiveness of macroprolactin screening is already established in literature and mean cost was higher in normal macroprolactin individuals' undergone imaging, which was cost burden due to unnecessary investigations.[13-15] As seen in our study, the mean expenditure was much less in MaPRL group, who did not need to go for imaging after PEG screening.

It is important that clinical laboratories performing prolactin testing should screen for MaPRL in all hyperprolactinemic sera. It is equally important that clinicians involved in managing these patients should be aware of this potential diagnostic pitfall and insist on macroprolactin screening. However, the results should be evaluated in detailed clinical context as few patients with MaPRL can have microadenomas, which are identified on MRI or CT scan and need careful monitoring and follow-up.

PEG precipitation is a simple, economical and a rapid method for the detection of MaPRL. Screening for MaPRL in all prolactin assays above reference range is a recommended good laboratory practice by most organizations. Method specific reference intervals are better than percent recovery method.[7,8,10]

CONCLUSION

High frequency of MaPRL was identified in patients with hyperprolactinemia. Screening with PEG precipitation in hyperprolactinemic sera is simple and cost-effective.

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ORIGINAL ARTICLE

Factors Affecting Dermatological Manifestations in Patients with End Stage Renal Disease

Muhammad Anees[1], Ghazala Butt[2], Shaista Gull[1], Asif Nazeer[1], Ijaz Hussain[2] and Muhammad Ibrahim[3]

Abstract

Objective: To determine skin changes in patients of End Stage Renal Disease (ESRD) on maintenance hemodialysis (MHD) and factors affecting these changes.

Study Design: Cross-sectional observational study.

Place and Duration of Study: Nephrology Department, Mayo Hospital, Lahore in collaboration with Dermatology Department, King Edward Medical University, Lahore, from October 2015 to January 2016.

Methodology: Two hundred patients who were undergoing MHD for more than three months were included in the study. Patients' demographic data, laboratory reports and dialysis records were noted in a predesigned questionnaire. Skin examination was carried out by consultant dermatologist after patient's permission.

Results: Among 200 patients included in study, 105 were males and rest of them were females. Major causes of ESRD were Diabetes Mellitus (n=83, 41.5%, followed by Hypertension (n=80, 40%), Nephrolithiasis (n=15, 7.5%) and Chronic glomerulonephritis (n=5, 2.5%). At least one cutaneous finding was present in every patient. Common skin findings observed were pigmentation (86%), xerosis (83%), pallor (79%), pruritus (69%), acquired ichthyosis (50.5%), and bacterial skin infections (18.5%). Among them, nail manifestations were half-and-half nails (52%), onychomycosis (30.5%), onycholysis (20.5%), subungual hyperkeratosis (23.5%), and Mee's lines (7.5). Among hair changes were sparse scalp hair (38.5%), brittle and lustreless hair (28%). The factors contributing to skin changes were patient's age, cause of ESRD, anti HCV positivity, high urea and creatinine levels, duration and frequency of hemodialysis, hemoglobin levels, calcium phosphate product and socioeconomic status. Some skin manifestations were interrelated with each other like xerosis with pruritus ($p<0.001$), pruritus with bacterial infection ($p<0.022$), acquired Ichthyosis ($p=0.008$) and hair changes ($p=0.035$).

Conclusion: ESRD patients on hemodialysis develop various skin changes during the course of disease process, which contribute to increased morbidity. Different factors affecting skin changes were the cause of ESRD, adequacy and duration of dialysis, employment, financial status, anti HCV positivity, and metabolic factors.

Key Words: *Chronic kidney disease. Hemodialysis. Skin manifestations. Pruritus. Xerosis. Pallor.*

INTRODUCTION

Chronic Kidney Disease (CKD) is defined when Glomerular Filtration Rate (GFR) is less than 60 ml/minute/1.73 m^2 for more than three months.[1,2] End Stage Renal Disease (ESRD) is the stage of CKD when life cannot be maintained without renal replacement therapy or renal transplant. Skin being the largest organ of the body shows manifestation of CKD very early. Skin changes can occur before dialysis and even after initiation of dialysis and transplantation they persist. As skin is the most easily accessible organ of the body, it can be used as diagnostic window to internal uremic milieu. Although these skin changes are benign but have negative impact on quality of life. CKD patients develop number of skin problems like xerosis, pruritus, hyperpigmentation, infections, ichthyosis, half-and-half nails, onychomycosis, onycholysis, subungual hyperkeratosis, brittle hair and sparse body hair.[2-4] In Pakistan, work has been done on the skin manifestations in these patients,[5-8] but there is paucity of the data on the factors affecting these skin problems. By identifying factors, we might be able to reduce skin changes in ESRD patients and improve their quality of life and disease outcome. So this study was conducted to determine the factors affecting skin changes in patients on hemodialysis.

METHODOLOGY

This observational cross-sectional study was conducted at the Department of Nephrology, Shalamar Hospital and Mayo Hospital in collaboration with Dermatology Department, Mayo Hospital, Lahore, from October 2015 to January 2016. All patients who were on MHD for more than three months were included in the study. The patients who were undergoing hemodialysis for less than three months and those with renal transplant dysfunction were excluded from the study. Approval was obtained from the Ethics Committee of King Edward Medical University, Lahore prior to initiating the study.

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The protocols used conformed to the ethical guidelines of the 1975 Helsinki Declaration. Patients' demographic and hemodialysis data were collected on a predesigned form.

Patients' blood samples were drawn and sent to laboratory for hemoglobin (Hb), biochemical (blood urea, serum creatinine, iron studies, serum albumin, serum calcium, phosphorus, intact parathyroid hormone, serum electrolytes) and viral markers (HbsAg and Anti HCV). Skin, nails and hair examinations were carried out by consultant dermatologist of Dermatology Department of the Mayo Hospital after patients' permission. All the skin manifestations were noted in a predesigned form. Necessary investigations like potassium hydroxide mount, Gram's stain, fungal culture, and culture and sensitivity for bacterial infections were done where indicated.

The data was entered using software SPSS version 21. The descriptive analysis was done using mean and standard deviation for continuous data and frequency was used for categorical data. Binary logistic regression analysis was done using Backward stepwise (likelihood ratio) to determine the significance of the variables affecting the skin manifestations. A p-value of less than or equal to 0.05 was considered as statistically significant value.

RESULTS

Almost half of the patients (n=105, 52.5%) were males and rest of them were females (n=95, 47.5%). The mean age of the patients was 48.15 ±13.74 years and most of the patients (n=107, 53.5%) were in the middle age (44-64 years) group. Major causes of ESRD were Diabetes Mellitus (n=83, 41.5%), followed by Hypertension (n=80, 40%), Nephrolithiasis (n=15, 7.5%), Chronic glomerulonephritis (n=5, 2.5%), Connective Tissue Disorders (n=5, 2.5%), Autosomal Dominant Polycystic Kidney Disease (ADPKD) (n=3, 1.5%) and unknown (n=9, 4.5%). Most of the patients were getting twice weekly hemodialysis sessions (n=155, 77.5%). Mean hemoglobin and serum albumin levels were 10.17 ±1.69 gm/dl and 3.59 ±0.42 g/dl, respectively. Majority of the patients were having low hemoglobin and serum albumin levels (n=137, 68.5%) and (n=161, 80.5%), respectively showing malnutrition. Serum calcium and calcium phosphorus product were normal in (n=186, 93%) and 175 (87.5%) patients, respectively. Half of the patients (55%) were having iPTH level >300 pg/ml. Patients were HbsAg and Anti HCV negative (n=169, 84.5%) and (n=122, 61%), respectively. At least one skin manifestation was present in every patient. Skin manifestations found among dialysis patients were pigmentation (n=172, 86%), xerosis (n=166, 83%), pallor (n=158, 79%), pruritus (n=138, 69%), and acquired ichthyosis (n=101, 50.5%). Among infections of the skin, bacterial infections were found to be most common (n=37, 18.5%). Among nail and hair manifestations were half-and-half nails (n=104, 52%), onychomycosis (n=61, 30.5%), onycholysis (n=41, 20.5%), Mees' lines (n=15, 7.5%), koilonychia (n=12, 6%), subungal hyperkeratosis (n=47, 23.5%), Beaus lines (n=3, 1.5%), sparse scalp hair (n=77, 38.5%), and brittle and lustreless hair (n=56, 28%). None of the patients had calciphylaxis. Different factors affecting skin manifestation are shown in Table I.

In this study, different skin factors were statistically correlated with each other. Xeosis is related with pruritus ($p<0.001$) and onychomyciosis ($p=0.05$). Pruritus is related with bacterial infection ($p=0.022$), acquired ichthyosis ($p=0.008$), sparse body and scalp hair ($p=0.035$), sparse scalp hair ($p=0.022$), and brittle and lusterless hair ($p<0.047$). Bacterial infections are not related with onychomycosis ($p=0.097$).

DISCUSSION

In this study, there is very high prevalence of dermatological manifestation as compared to studies conducted in the world like Tusinia, Egypt and India as shown in Table II.[9-11] There are different reasons of such a high prevalence in these patients. Due to under-development of the specialty and shortage of training institutes in Nephrology, there is severe shortage of nephrologists. This shortage of nephrologists causes unawareness of the kidney diseases not only among doctors,[12] but even the patients are not well aware of the early symptoms of kidney diseases. CKD patients have strong negative thoughts about the need of dialysis and 70% of the patients refuse dialysis when offered to them. These patients present for dialysis at a very late stage to nephrologist,[13] when they have already developed advanced complications including skin manifestations. Even the patients who are getting dialysis are not optimally managed.[14] As seen in this study, majority of the patients get inadequate dialysis (twice-weekly dialysis); whereas, international guidelines recommend thrice weekly dialysis.

Pigmentation is highly variable in different studies from 40% to 80%.[8-10] As evaluation of the skin color depends on the accuracy of the examiner's vision and the type and intensity of the environmental light, therefore, examination by a more accurate method such as a color meter reflectance is recommended.[15] Pigmentation was the most common skin lesion in this study. Moreover, in this study, cause of ESRD, low hemoglobin level and employment have statistically significant relationship with pigmentation. A brown-to-slate-gray discoloration may occur as a result of hemosiderin deposition in association with iron overload from excessive transfusions. In this study, most of the patients are anemic 137(68.5%) but iron is adequate in majority of the patients {(TSAT>20) in 85% and serum ferritin

Table I: Factors effecting dermatological manifestations.

Dermatological manifestations	Factors			P-value
Skin manifestations				
1. Pigmentation 172 (86%)	Cause of ESRD			
		DM	Non DM	
	Yes	15	13	0.017*
	No	68	104	
	Hb (Gm/dl)			
		<11	≥ 11	
	Yes	13	15	0.001*
	No	124	48	
	Employment			
		Yes	No	Housewife
	Yes	3	11	14
	No	56	45	71
2. Xerosis 166 (83%)	Duration of hemodialysis			0.059
		<18months	>18months	
	Yes	95	71	0.015*
	No	13	21	
	Blood Urea (mg/dl)			
		>200mg/dl	<200mg/dl	
	Yes	119	17	0.033*
	No	26	8	
3. Pallor 158 (79%)	Hemoglobin (Gm/dl)			
		≥11	<11	
	Yes	46	112	0.001*
	No	17	25	
	Frequency of hemodialysis			
		Twice/week	Thrice/week	
	Yes	127	31	0.049*
	No	28	14	
4. Pruritus 138 (69%)	Anti HCV			
		Negative	positive	
	Yes	75	63	0.003*
	No	47	15	
5. Bacterial infection 37 (18.7%)	Blood Urea (mg/dl)			
		>200mg/dl	<200mg/dl	0.025*
	Yes	32	5	
	No	143	20	
	Employment			
		Yes	No	Housewife
	Yes	16	9	12
	No	43	47	73
	Calcium Phosphorus Product			0.028*
		<55	>55	
	Yes	35	2	0.052
	No	140	23	
Nail manifestations				
1. Half-and-Half Nails 104 (52%)	Blood Creatinine (mg/dl)			
		>8	<8	
	Yes	45	59	0.09
	No	26	70	
2. Onychomycosis 61 (30.5%)	Anti HCV			
		Negative	Positive	
	Yes	28	33	0.016*
	No	94	45	
	Blood Urea (mg/dl)			
		>200mg/dl	<200mg/dl	
	Yes	59	2	0.01*
	No	116	23	
3. Onycholysis 41 (20.5%)	Anti HCV			
		Negative	Positive	
	Yes	18	23	0.013*
	No	104	55	
4. Koilonychia 12 (6%)	Corrected Calcium (mg/dl)			
		<8.5	8.5-9.5	>9.5
	Yes	4	6	2
	No	134	42	12
	Monthly Income in Rupees			0.004*
		<10,000	10,000-30,000	>30,000
	Yes	6	6	0
	No	60	106	22
				0.037*
Hair Manifestations				
1. Brittle and lusterless hair sparse scalp hair 56 (28%)	Age (years)			
		16-44	45-64	65-78
	Yes	18	32	6
	No	56	75	13
	Cause of ESRD			0.028*
		DM	Non DM	
	Yes	28	28	0.017*
	No	55	89	
	Frequency of HD			
		Twice/week	Thrice/week	
	Yes	51	5	0.001*
	No	104	40	

**Statistically significant value.*

>500ng/dl in 113 (60%) patients}. This iron and low hemoglobin may be the probable reason of the slate gray pigmentation. In this study, patients who were employed have more pigmentation on the sun exposed areas than unemployed patients. Basically, the employed patients have more sun exposure with sun causing an increase in deposition of melanin in the basal layer and superficial dermis and accumulation of poorly dialyzable beta-melanocyte stimulating hormone. In this study, cause of ESRD, like DM as compared to non-diabetics, also affected pigmentation as seen in another study.[4]

Xerosis was the second most common skin manifestation in dialysis patients in this study. Similar incidence was observed by other local and international studies.[16,17] Exact cause of the xerosis is not known. There are different probable mechanisms of xerosis like decreased water content in epidermis, decreased sweat gland, atrophy of the sebaceous glands and hypervitaminosis A. In this study, duration of dialysis and urea level in the serum affected xerosis. Similar observation was made by Beheshti *et al*.[4] The raised urea level is a sign of inadequate dialysis.

Pallor and pruritus was present in 79% and 69% patients, respectively. Similar prevalence of pruritus was observed by local,[4,6] and international studies.[9-11] In this

Table II: Showing comparison of dermatological manifestations in different studies.

Skin manifestations (sample size)	Present study (200)	Masmaudi *et al.* (Tunisia) (458)	Sultan *et al.* (Egypt) (100)	Pk. Kolla *et al.* (India) (143)
Pigmentation	86%	38.4%	54%	39.4%
Xerosis	83%	52.8%	54%	57%
Pallor	79%	60.7%	45%	-
Pruritus	69%	56.6%	55.%	56.6%
Acquired Ichthyosis	51%	-	-	-
Bacterial infection	18.5%	14.6%	5%	14.6%
Half-and-half nail	52%	46.3%	28%	9.09%
Koilonychias	6%		39%	18%
Onychomycosis	20.5%		6%	8.88%
Onycholysis	30.0%	20.9%	3%	2.19%
Subungual Hyperkeratosis	23.5%	-	10%	-
Brittle and lusterless hair	28%	-	46%	21.7%

study, majority of the patients were anemic, which was manifested in the form of pale color of the skin. Pallor of skin had statistically significant relationship with low hemoglobin and frequency of dialysis. There are different causes of anemia in patients with CKD like nutritional deficiencies, reduced RBCs life span, erythropoietin (EPO) deficiency, blood loss due to coagulation abnormalities. Instead of adequate iron store, this low hemoglobin is due to under-dose of the erythropoietin.[18] In this study, patients with twice weekly dialysis had pallor skin as compared to thrice weekly dialysis. Again, it supports that adequacy of dialysis may improve the anemia and decrease pale skin.

Pruritus was also common skin manifestation in patients with dialysis affecting 50-90%; about one-third of the patients prior to dialysis and two-thirds after dialysis experience itching.[19] Pruritus may be localized and generalized, episodic and constant, and mild to severe.

Exact mechanism of the uremia induced pruritus is not known, but it is assumed to be due to metabolic disequilibrium. In this study, pruritus was not related with age, gender, hypercalcemia, calcium phosphorus product, inadequate dialysis but it was associated with anti HCV positive patients. No study has highlighted this issue in ESRD patients but similar observation is made by another study in patients without CKD.[20] According to this study, patients with antibodies for HCV were having more pruritus either they were HCV PCR positive or negative. However, there is need to do more studies to explore this issue in HCV +ve patients with and without dialysis. In this study, xerosis and pruritus has statistically significant relationship (p=0.000). Similar observation was made by Onelmis *et al*.[21] It means some skin manifestations are interrelated with each other and further augment the skin problems.

In this study, bacterial infection was the most common skin infection. There was very low frequency (1%) of fungal infection in this study as compared to another local study where it was 52%.[9] The low incidence of fungal infection may be due to the season, because this study was conducted in winter with minimum humidity which prevents fungal infections. Hemodialysis procedure *per se* as well as disturbances in both innate and adaptive immunity make hemodialysis patients susceptible to infections.[22] In this study, high urea, employment and calcium phosphorus product were statistically related to bacterial skin infections. High urea level is one of the markers of the inadequate dialysis that favors uremic milieu in the body leading to impaired immunity. Employed patients are more exposed to environment containing dust, smoke and poor hygiene causing increased skin infections. Pruritus and bacterial infections had strong statistically significant association with each other. Actually pruritus leads to skin excoriations with repeated scratching causing break-down of skin.

Acquired Ichthyosis (AI) is a nonhereditary condition associated with internal disease. It is a marker of systemic disease and attributed to certain medications also.[3] Xerosis and acquired ichthyosis are common problems that can be associated with hyperpigmentation in the setting of kidney disease. In this study, almost 50% of the patients on haemodialysis were having acquired ichthyosis which has not been reported in previous studies.[4-7] In this study, AI is related with pruritus (p=0.008) and with xerosis. There is a need to find the probable cause of such a high prevalence of AI in our population.

Common hair changes in these patients are: diffuse hair loss of scalp, sparse and lusterless body hair, discoloration and dryness of the hair. These hair changes are related to xerosis, pruritis, severity of illness and due to medication like heparin, lipid lowering and anti-hypertensives used in these patients. In this study, hair changes were present in 28-30% of the patients. Similar frequency was noted in another local study.[6] In this study, age, cause of ESRD, and frequency of hemodialysis affect hair changes in these patients. In this study, hair change their texture like lusterless, sparse body and scalp hair, which may be the physiological change with advancing age. In this study, the frequency of dialysis, i.e. thrice weekly, affects hair changes. Frequency of dialysis is one of the parameters of adequate dialysis like twice weekly or thrice weekly. Inadequate dialysis affects not only morbidity and mortality of dialysis patients but it also affects quality of the hairs of the patients. This may be the probable reason for the high prevalence of hair problems in these patients as inadequate dialysis will lead to accumulation of uremic toxins in the body, causing hair changes.

Nail changes are much higher in CKD patients as observed in another study.[23] In half-and-half nail,

Lindsay's Nail, the proximal half of the nail becomes white while the distal portion becomes reddish-brown to brown due to nail bed edema associated with a dilated capillary. The exact etiology of this nail change remains unknown. In this study, serum creatinine level had a statistically significant association with Lindsay's Nail. It may be a sign of inadequate dialysis reverting on increasing adequacy of dialysis,[24] but it sometimes improves with renal transplantation.[25] In this study, patients who were anti-HCV positive had more nail changes as compared to patients who were anti-HCV negative. In this study, monthly income has statistically significant association with koilonychia and onychomycosis (p=0.004), (p=0.047), respectively. As already discussed, most of the patients belonged to low socioeconomic group which leads to malnutrition and poor hygiene, causing nail changes.

CONCLUSION

ESRD patients on hemodialysis develop various skin changes during the course of disease process, which contribute to increased morbidity. Different factors affecting skin changes were cause of ESRD, adequacy and duration of dialysis, employment, financial status of the patients, anti-HCV positive patients and metabolic factors. By reversing these factors, we can improve the quality of life of these patients.

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ORIGINAL ARTICLE

Utility of Diffusion Weighted Magnetic Resonance Imaging with Multiple B Values in Evaluation of Pancreatic Malignant and Benign Lesions and Pancreatitis

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Abstract

Objective: To determine the feasibility of diffusion-weighted imaging in evaluation of pancreatic lesions and in differentiation of benign from malignant lesions.

Study Design: Descriptive study.

Place and Duration of Study: Baskent University Adana Teaching and Research Center, Adana, Turkey, between September 2013 and May 2015.

Methodology: Forty-three lesions [pancreas adenocarcinoma (n=25)], pancreatitis (n=10), benign lesion (n=8)] were utilized with diffusion-weighted magnetic resonance imaging with multiple b-values. Different ADC maps of diffusion weighted images by using b-values were acquired.

Results: The median ADC at all b values for malignant lesions was significantly different from that for benign lesions ($p<0.001$). When ADCs at all b values were compared between benign lesions/normal parenchyma and malignant lesions/normal parenchyma, there was a significant statistical difference in all b values between benign and malignant lesions except at b 50 and b 200 ($p<0.05$). The lesion/normal parenchyma ADC ratio for b 600 value (AUC=0.804) was more effective than the lesion ADC for b 600 value (AUC=0.766) in differentiation of benign and malignant lesions. The specificity and sensitivity of the lesion/normal parenchyma ADC ratio were higher than those of ADC values of lesions. When the ADC was compared between benign lesions and pancreatitis, a significant difference was found at all b values ($p<0.001$). There was not a statistically significant difference between the ADC for pancreatitis and that for malignant lesions at any b value combinations ($p>0.05$).

Conclusion: Diffusion-weighted magnetic resonance images can be helpful in differentiation of pancreatic carcinoma and benign lesions. Lesion ADC / normal parenchyma ADC ratios are more important than lesion ADC values in assessment of pancreatic lesions.

Key Words: *Benign. Diffusion-weighted imaging. Magnetic resonance imaging. Pancreas carcinoma. Pancreatitis.*

INTRODUCTION

Pancreatic carcinoma has a high mortality; it is the fourth leading cause of death from cancer in both genders,[1] owing to atypical symptoms and late diagnosis. Five-year-survival is lower than 5%. Approximately 95% of pancreatic cancers are adenocarcinomas. When the tumour is localized and small, it is possible to cure with surgery.[2]

When pancreatic cancers are diagnosed, the tumours usually exceed the border of the organ, invade tissues nearby and extend to the perineural distance in more than 85% of the patients. Resection may provide cure only in 15-20% of the patients.[3,4]

The most frequently used radiological modalities in diagnosis of pancreatic diseases are ultrasonography, computed tomography, magnetic resonance imaging (MRI) and endoscopic retrograde cholangiography. However, sometimes, these modalities may not distinguish pancreatic carcinoma from other pancreatic diseases.[5] Especially, recently, advanced MRI systems using diffusion-weighted imaging (DWI) enable evaluation of abdominal organs and characterization of several diseases. DWI is based on Brownian motion (i.e. the microscopic movement) of water molecules in biological tissues. Apparent diffusion coefficients (ADCs) obtained with DWI using diffusion gradients are the best radiological method to quantify united effects of water diffusion and capillary perfusion in biological tissues. DWI offers information about biophysical contents as microcirculation, density and cell organization. ADCs are a function of the ratio of components inside and outside the cell. Lower ADCs result from increased cell concentrations and cell densities. High ADCs in benign pathologies with lower cell density are normal. Malignant pathologies with a higher cell density and a higher cell diameter compared to normal tissues have lower ADCs than surrounding normal tissues. However, this finding depends on restriction of water diffusion.[6]

The advantage of this technique is that there is no need for contrast material. Previously, the applications of this

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technique were restricted to neuroimaging; because, DWI was affected by heart, breath and peristaltic motions. Fast sequences, such as echo-planar imaging (EPI), are restricted by artefacts due to heart, lung and peristaltic motions, abdominal wall motions and adjacent bowel gas. Recently, there has been some research about DWI utility in the diagnosis of pancreatic cancers, pancreatic cystic lesions and the exocrine function of the pancreas in chronic pancreatitis.[7-9]

The purpose of this study was to evaluate the feasibility of DWI in detection of lesions in the pancreas and the periampullary region; and in the analysis of benign and malignant lesions and pancreatitis.

METHODOLOGY

This retrospective study was approved by Baskent University Institutional Review Board (Project No. KA17/14) and supported by Baskent University Research Fund.The study population consisted of patients, who underwent MRI for evaluation of suspected pancreatobiliary diseases between September 2013 and May 2015. Malignant and benign neoplastic lesions were pathologically proven. The clinical follow-up and laboratory investigations were used to establish the diagnosis of pancreatitis. The exclusion criteria were prior diagnostic interventions, operation, chemotherapy or radiotherapy.

All patients were evaluated with a 1.5-Tesla MR machine (Magnetom Avanto™) with a 33-mT/m maximum gradient strength, 75-mT/m/ms peak slew rate using a torso phased array and body coils (12 elements selected). The routine abdominal MRI parameters were as follows: a 3- to 5-mm slice thickness, 0.6 to 1-mm (20%) intersection gap, and 160 - 190 × 256 matrix, with a sensitivity-encoding factor of two and bandwidth of 260 Hz/px. The sequences and parameters of routine abdominal MRI were as follows: axial and coronal T2-weighted turbo-spin echo (TSE); axial and fat-saturated (FS) T2-weighted TSE (TR/TE, 1900/80 ms; FA, 150°); and axial and coronal T1-weighted TSE before and after intravenous contrast material with fat saturation (TR/TE, 1900 ms/80 ms).

The screening duration was roughly 4 minutes. Axial abdominal diffusion-weighted images (DWIs) with a free-breathing single-shot echo-planar-imaging (SSH-EPI) sequence were obtained in the same plane. Sequence parameters of DWIs were TR/TE: 4200 ms/87 ms; field of view: 350-400 mm; section thickness: 5 mm; intersection gap: 20% (1 mm); sections: 24-38; matrix: 132 × 192; echo train length (EPI factor): 156, sensitivity encoding factor: 2 and two excitations. Parallel imaging generalised autocalibrating partially parallel acquisition (GRAPPA) with modified sensitivity encoding (mSENSE) and the motion-probing gradient pulses were used. Spectral presaturation was applied to decrease chemical shift artefacts. DWIs with seven different b-values (0, 50, 200, 400, 600, 800 and 1000 s/mm^2) were obtained. The mean values from three-set abdominal magnetic resonance images were measured, then isotropic DWIs with different b-values were obtained.

Eight ADC maps were performed at a commercial workstation with standard software (Leonardo console software ver. 2.0 Germany). Automatically generated ADC maps of DWIs by using all b-values (0, 50, 200, 400, 600, 800 and 1000 s/mm^2) and seven different ADC maps created by using EPIs with b=0 s/mm^2 and six different b-values (i.e. 0 and 50 s/mm^2, 0 and 200 s/mm^2, etc.), and with four b-values (0, 600, 800 and 1000 s/mm^2) (referred to various ADC in the rest of the text) were obtained. The combined mean ADCs of the detected lesions were calculated directly from these ADC maps.

Image analyses were performed by two radiologists (E.K and G.E. with 9 and 11 years of experience in abdominal radiology) at the Advantage Workstation 4.4 (GE Healthcare, Milwaukee, WI, USA). The radiologists were unaware of clinical history, MRI and histopathological results, operation findings. The researchers evaluated the DWIs and the conventional MR images and arrived at a consensus for detecting the lesions measured at auto-ADC maps.

For homogeneous and solid lesions, ROIs were drawn as large as possible to fit the lesion size. An average of three measurements per lesion was calculated. Measurements of signal intensities for heterogeneous and necrotic lesions and ADCs for focal lesions were determined by drawing so that they eccentrically covered the visualised low-ADC area and hyperintense area at high b-values (b=800 or b=1000 s/mm^2). A freehand region of interest (ROI) was drawn on the enhancing solid portion of the solid pancreatic tumor on DW images and ADC maps to avoid vessels and necrosis. For cystic lesions, a freehand ROI was drawn to encompass as much of the lesion as possible. Each ROI was copied to ensure that they were of the same size and location as on DWIs obtained by using different b-values in the same lesion. The lesions were analysed quantitatively for assessment of benignity/malignity by measuring ADCs. The ADCs were measured by placing manually defined ROIs in the lesions on nine ADC maps. The ROI was carefully placed and copied and pasted as described above for measuring signal intensities. The ROI size varied with the lesion size and, depending on the lesion size, they were outlined on up to three sections in each mass. The ADCs of the lesions are expressed as the median (min-max).

Statistical analysis was performed by Statistical Package Program for Social Sciences 17.0 (SPSS Inc.). For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro-Wilk tests and histograms. Comparisons between the groups were made by using one-way ANOVA test for normally distributed data and Kruskal Wallis test was used for the data not normally distributed. Since analysis of variance was significant, comparisons were made by using the

Post Hoc test and Mann-Whitney U-test. Receiver operating characteristic curves (ROC curves) were constructed and the areas under curve (AUC) as well as sensitivity and specificity were calculated. P <0.05 was considered significant.

RESULTS

Forty-three patients aged 59.4 ±12.81 years (range: 28-83 years) were included in the study. Out of 43 patients, 25 (58.1%) were males and 18 (41.8%) were females. Of all the patients, 25 (58.1%) had malignant pancreatic lesion, 10 (23.2%) had pancreatitis, and 8 (18.6%) benign pancreatic lesions. All malignant lesions were pancreatic ductal adenocarcinoma, and benign lesions were pancreatic serous cystadenoma and cysts.

The mean size of malignant lesions was 37.9 ±17.5 mm (range:15-97 mm) for the longest diameter and 30.5 ±13.6 mm (range: 12-75 mm) for the shortest diameter. The mean size of benign lesions was 37.1 ±18.4 mm (range: 14-65) for the longest diameter and 31.4 ±19.6 mm (10-60 mm) for the shortest diameter. The ADCs of benign lesions, malignant lesions and pancreatitis on DWIs at different b values are summarized in Table I.

There was statistically a significant difference between ADCs of three groups at any b value combinations (Kruskal Wallis test). There was statistically a significant difference between the lesion/normal parenchyma ADCs of three groups except at b 50 (Kruskal Wallis test). The median (max-min) ADCs at all b values for malignant lesions were significantly different from those of benign diseases (Mann-Whitney U test) p values were shown in Table I. The median (max-min) ADCs at all b values for benign lesions were significantly different from those of pancreatitis (Mann-Whitney U test) p values were shown in Table I.

There was not statistically a significant difference between the ADCs of pancreatitis and those of malignant lesions at any b value combinations. p values were shown in Table I.

Table I: Comparisons of lesion ADCs (×10^{-3} mm^2/s) of malignant and benign pancreas lesions and pancreatitis at different b-value combinations.

b values (s/mm^2)	50	200	400	600	800	1000	various	auto
Benign lesions								
Max.	8.9000	4.2700	3.5000	3.3800	3.3100	3.3200	2.9100	2.8600
Min.	2.2000	2.9000	1.9900	2.5000	2.6000	2.2000	2.3000	2.2600
Median	4.7300	3.3500	3.2750	2.7350	2.8100	2.4250	2.5350	2.5500
Malignant								
Max.	4.0500	3.3200	3.5000	2.4900	2.4900	2.0800	2.1900	2.0900
Min.	1.5000	1.3300	1.2200	1.1000	1.1000	0.9000	1.0300	0.9800
Median	2.8600	1.8900	1.7000	1.6500	1.4000	1.4300	1.4200	1.4000
Pancreatitis								
Max.	6.3400	3.1400	2.9600	2.4000	2.0900	1.8300	1.9400	1.8000
Min.	1.9500	1.3900	1.0500	0.8800	0.7900	0.8400	0.4200	0.7300
Median	2.8950	2.3300	1.9200	1.7750	1.4150	1.3850	1.3800	1.2950
p1 (Kruskal-Wallis)	0.010	0.010	0.001	0.001	0.001	0.001	0.001	0.0001
p2 (Benign vs. Malignant)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
p3 (Benign vs. Pancreatitis)	0.001	0.001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
p4 (Malignant vs. Pancreatit)	0.213	0.270	0.255	0.529	0.928	0.287	0.377	0.113

various: ADC generated using 600-800-1000 b values
auto: ADC generated automatically using all b-values (0, 50, 200, 400, 600, 800 and 1000 s/mm^2).
p2,p3,p4 tests were analysed by Mann-Whitney U-test.

Table II: Comparisons of lesion/normal parenchyma ADCs (×10^{-3} mm^2/s) of malignant and benign pancreas lesions and pancreatitis at different b-value combinations.

b values (s/mm^2)	50	200	400	600	800	1000	various	auto
Benign lesions								
Max.	1.5100	1.7700	2.0100	1.7710	1.9741	2.0360	1.9801	2.0151
Min.	0.7300	0.7200	1.2700	0.2880	0.3933	0.4515	0.4183	0.1275
Median	1.0446	1.3901	1.6032	1.7050	1.8639	1.9783	1.8467	1.9773
Malignant								
Max.	2.2700	2.3000	2.2000	2.0900	2.3100	2.2100	2.2300	2.1500
Min.	0.2500	0.2900	0.4300	0.4000	0.4400	0.5200	0.5000	0.5100
Median	0.9707	0.9134	0.9552	0.9086	0.9636	1.0189	1,0000	1.0060
Pancreatitis								
Max.	1.8300	2.4200	1.9700	2.2900	2.4300	2.4100	2.4600	2.5400
Min.	0.5200	0.7900	0.6300	0.6900	0.5700	0.8300	0.7700	0.6900
Median	0.9945	1.0826	1.1314	1.1847	1.0051	1.1508	1.1026	1.1482
p1 (Kruskal-Wallis)	0.505	0.006	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
p2 (Benign vs. Malignant)	0.374	0.173	0.027	0.009	0.002	0.004	0.003	0.003
p3 (Benign vs. Pancreatitis)	0.633	0.173	0.027	0.009	0.002	0.004	0.003	0.003
p4 (Malignant vs. Pancreatit)	0.397	0.070	0.240	0.131	0.843	0.460	0.679	0.815

various: ADC generated using 600-800-1000 b values
auto: ADC generated automatically using all b-values (0, 50, 200, 400, 600, 800 and 1000 s/mm^2).
p2,p3,p4 tests were analysed by Mann-Whitney U-test.

Table III: ROC analysis of lesion ADCs and lesion/normal ADCs* ratio in differentiation of benign and malignant lesions and pancreatitis for b-200 and 400, 600 values.

b values (s/mm²)	AUC#	Cutoff (×10⁻³ mm²/s)	Sensitivity (%)	Specificity (%)	p-values
b 200	0.777	2.23	81.3%	77.3%	0.004
b 200*	0.781	1.019	75%	77.7%	0.003
b 400	0.768	1.96	75%	86.4%	0.005
b 400*	0.787	1.071	75%	72.7%	0.003
b 600	0.766	1.81	72.2%	76%	0.006
b 600*	0.804	1.26	75%	90.9%	0.002

AUC#, Area Under the Curve;
b 200, b 400*, b 600* related with lesion/normal ADC ratio.*

The lesions/normal parenchyma ADCs at all b values for malignant lesions were significantly different from those of benign lesions except at b 50, 200 (Mann-Whitney U test); p-values are shown in Table II.

The lesions/normal parenchyma ADCs at b 400, 600, 800, 1000 and various and auto for benign lesions were significantly different from those of pancreatitis (Mann-Whitney U test); p values were shown in Table II. There was no statistically significant difference in the lesion/ normal parenchyma ADCs between pancreatitis and malignant lesions at any b value combinations. p values shown in Table II.

The results of ROC analysis for comparision of ADCs between benign and malignant lesions and pancreatitis are shown in Table III. In the ROC curves for differential diagnosis of malignant and benign pancreatic lesions, and pancreatitis, the AUCs for b 200 and b 400 values were found to be higher than those for other b values. The AUCs for the ADCs with b 200 and b 400 s/mm² were 0.777 and 0.768 respectively (Table III).

The threshold value for b 200 was 2.23 (×10⁻³ mm²/s) and permitted distinction with a sensitivity of 81% and specificity of 77% at ADC for b 200. The threshold value for b 400 was 1.96 (×10⁻³ mm²/s) and permitted distinction with a sensitivity of 75% and specificity of 86% at ADC for b 400.

In the ROC curves for differential diagnosis of malignant and benign pancreas lesions, and pancreatitis, the AUCs for b 400 and b 600 values were found to be higher than for other b values. The AUCs for the ADCs with b 600 and b 400 s/mm² were 0.766 and 0.768 respectively (Table III).

The threshold value for b 600 of the lesion/normal parenchyma ADC values was 1.26 and permitted distinction with a sensitivity of 75% and specificity of 91% at ADC for b600.

Using normal parenchyma ADC values as phantom markers, the lesion/normal parenchyma ADC ratio for b 600 value (AUC=0.804) was more effective than the lesion ADC for b600 value (AUC=0.766) in differentiation of benign and malignant lesions and pancreatitis (Table III). In addition, specificity and sensitivity of the lesion/normal parenchyma ADC ratio was higher than those of ADCs of lesions.

Figure 1 and Figure 2 show distribution of lesion ADCs and lesion ADC/normal parenchyma ADC ratios of benign and malignant pancreatic lesions and pancreatitis according to b-values.

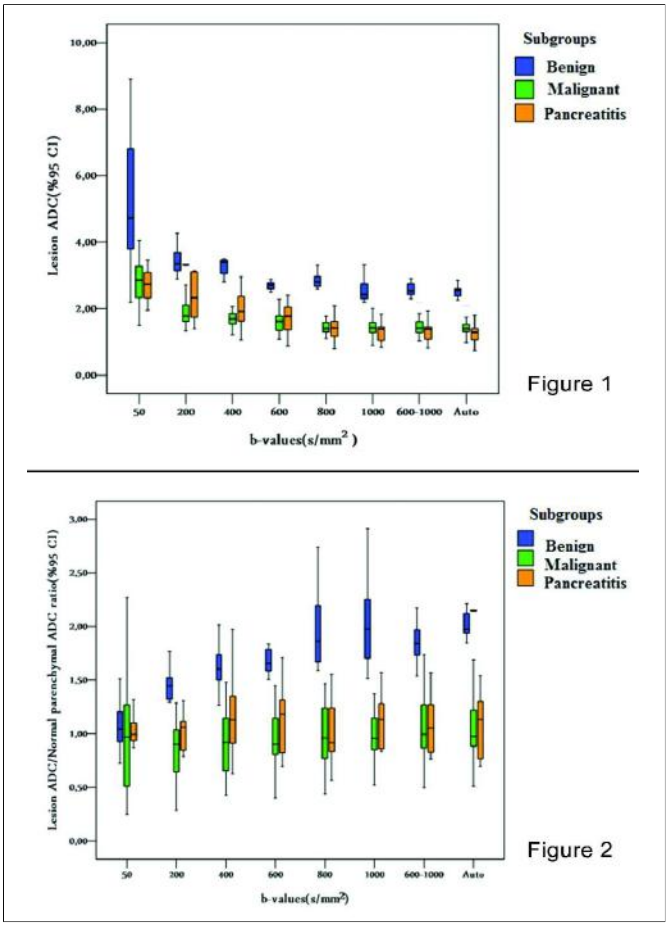

Figure 1 and 2: Distribution of lesion ADCs and lesion ADC/normal parenchyma ADC ratios of benign and malignant pancreatic lesions and pancreatitis according to b-values. Error bars show 95% confidence intervals (CIs) of the mean ± standard deviation (SD) of lesion ADC values or ADC ratios. (*) Auto-ADCs calculated by using all b-values (0, 50, 200, 400, 600, 800 and 1000 s/mm²). Various-ADCs calculated by using b-values of 0, 600, 800 and 1000 s/mm².

DISCUSSION

In the present study, DW-MRI and ADCs, ADC and imaging quality of DWI, lesion detection and success in characterization of lesions were analysed in patients with pancreatic and periampullary tumors. ADC values of the lesions were compared with those of benign lesions to investigate efficacy of ADCs at different b values and DWI.

The ADC values at all b values for malignant lesions significantly differed from those of benign diseases. The ADCs of benign lesions also significantly differed from those of pancreatitis at all b values. However, there was no statistically significant difference between the ADC values of pancreatitis and those of malignant lesions at any b value combinations.

In evaluation of the lesion/normal parenchyma ADC ratios, b 600 s/mm^2 value was found to have higher specificity and sensitivity than b 200 and b 400 values. It is obvious that the lesion ADC/normal parenchyma ADC ratios were more effective than the lesion ADC values for differentiation of benign and malignant lesions. As a result, the b 600 value was the most useful b value for the lesion ADC/normal parenchyma ADC ratios. The optimum cut-off value for b 600 of the lesion ADC/ normal parenchyma ADC ratios was 1.26. As a reference for differentiation of benign and malignant lesions, this value had a sensitivity and specificity of 75% and 91% respectively. Erbay *et al*. showed that the lesion/normal parenchyma ADC ratio for renal lesions had a lesser degree of overlap between the malignant and benign lesions than the lesion ADC.[10] Similarly, this study showed that the lesion/normal parenchyma ADC was more important for pancreas lesions.

There are some reports about the efficiency of DWI for evaluation of the pancreas exocrin function in patients with chronic pancreatitis, about characterization of pancreas cystic lesions and detection of pancreas cancer through pancreas DWI.[7-9]

The ADC values of pancreas cancer are affected by cell density and amounts of necrosis and fibrosis and mucin. Muraoka *et al*. showed that the ADC of pancreas cancer was lower than that of normal pancreas parenchyma.[11] In the present study, mean ADCs were found to be 1.27 ±0.52x10^{-3} mm^2/s in pancreas tumors and 1.90 ±0.41x10^{-3} mm^2/s in the normal pancreas parenchyma. Muraoka *et al*. reported that ADCs (ADC: 1.88 ±0.39x10^{-3} mm^2/ s) in cancers with a less amount of fibrosis are higher than those (ADC: 1.01 ±0.29x10^{-3} mm^2/ s) in pancreas cancers with dense fibrosis.[11] In the study by Muraoka *et al*., only b 0 and b 500 values were used and there was a significant correlation between the amount of collagen fibers and ADCs (r= -0.87, p<0.05) at b 500 value.[11] There are some reports yielding similar results to this study.[9,12] In a study by Lee *et al*.,[12] the ADC of normal pancreas was found to be higher than that of pancreas cancer and mass-forming pancreatitis. In fact, ADC 500 was 2.06 ±0.42 in the normal pancreas, 1.46 ±0.20 in pancreas cancer, 1.23 ±0.22 in mass-forming pancreatitis, 1.43 ±0.29 in solid pseudopapiller tumor and 1.62 ±0.60 10-3 mm^2/ s in neuroendocrine tumor; ADC1000 was 1.62 ±0.34 in normal pancreas, 1.23 ±0.18 in pancreatic cancer, 1.04 ±0.18 in mass-forming pancreatitis, 1.16 ±0.36 in solid pseudopapiller tumor and 1.30 ±0.413 mm^2/ s in neuroendocrine tumor.[12] In addition, similar to the present study, in Matsuki *et al*. study including 8 patients with pancreas adenocarcinoma, the patients had low ADC values relative to normal pancreas paranchyma.[9] In the current study, the mean ADCs of malignant pancreas lesions were significantly lower than those of normal pancreas for the b 200, 400,600 and 800 values, which are consistent with the literature.

In the present study, a total of 25 pancreas adeno-carcinomas were evaluated. The median (min-max) ADC of these cases was 1.40 (0.98-2.09) x10^{-3} mm^2/s for the auto-ADC and 1.43 (0.90-2.08) x10^{-3} mm^2/s for b 1000 value, which are consistent with those of Lee's and Matsuki's studies.[9,12]

Unlike their studies, in the present study, a total of 8 different ADCs at b 50, 200, 400, 600, 800, 1000, 600-1000 and automatic ADC by using different b values were calculated.

Restricted diffusion occurs due to fibrosis. The amount of fibrosis (desmoplasia) is an important factor affecting ADC values in pancreas cancers. However, in this study, correlations between the amount of fibrosis and ADCs were not evaluated in pancreas cancers. Necrosis is one of the most substantial pathological changes in pancreas cancers, and it may cause increased diffusion. However, lower amounts of necrosis do not significantly affect ADCs.[11]

Mucin may cause restriction of diffusion confront with other fluids similar to water. Yamashita *et al*. showed that musin-producing tumour of the pancreas had restricted diffusion because of its viscous content compared to other cystic lesions.[8] However, there have been reports revealing that mucin might cause increased diffusion in solid tumors and is not the main cause of restricted diffusion in pancreas cancers.[11]

The results of this study suggested that massive cell density in pancreas adenocarcinoma might cause restricted diffusion and low ADCs compared with normal pancreas parenchyma. The most valuable b value was b 200 in discrimination of benign and malignant pancreas lesions. Although discrimiantion of maliganant lesions from benign lesions could be made at all b values, the most useful one was b 200. This indicates that low b values are useful for lesion ADC. However, the most valuable one is b 600 for lesion/normal parenchyma ADC ratio. For discrimination of benign from malignant lesions, we found that b 50 and b 200 values for lesion / normal parenchyma were not helpful.

For cystic lesions of the pancreas, differentiation with radiologic modalities may be obscure. Approximately 5% of serous cystadenomas have similar features to musinous cystic tumors. Sometimes, pseudocysts may be indiscriminable from musinous cystic tumors with imaging methods. Musin-producing cystic carcinomas, adenocarcinomas, sarcomas and metastatic cystic pancreas lesions must be considered in the differential diagnosis of cystic pancreas lesions.[13] Compared to

surrounding normal pancreas parenchyma, cystic pancreas lesions have been reported to have high ADC values and high signal intensities. In one study including 32 patients by Yamashita *et al*., ADCs were 2.7 ±0.9x 10^{-3} mm^2/s in musin-producing tumors, 3.2 ±1.0x 10^{-3} mm^2/s in pseudocysts and 5.8 ±2.0x10^{-3} mm^2/s in serous cysts.[8]

Inan *et al*. have reported that ADCs of abscesses, neoplastic and hydatid cysts were considerably lower than those of simple and pseudo cysts.[14] In the present study, 8 benign lesions were evaluated. The median (min-max) ADCs in these lesions was 2.55 (2.26-2.86)x10^{-3} mm^2/s for auto-ADCs and 2.42 (2.20-3.32)x10^{-3} mm^2/s for b1000. The ADCs of these lesions were higher than those of pancreatitis and pancreas carcinoma. The ADCs found in the present study were similar to previously reported results for serous cystadenomas.

Changes in ADCs in cystic lesions result from cyst content. Hydatid cysts, abscesses, serous and musinous cystadenomas, musinous cystadenocarcinomas have low ADCs due to their viscous content. Serous cystadenomas are multiseptated, multiloculated lesions containing glycogen, proteinaceous or hemorrhage. The contents of mucinous cystadenomas and mucinous cystadenocarcinomas are mucin, protein and hemorrhage. Because of less viscous content, ADCs of simple cysts and pseudocysts are higher than those of previously reported lesions.[14-16]

Focal pancreatitis may develop as acute or chronic pancreatitis. It may be confused with pancreatic carcinoma because of similar symptoms such as obstructive jaundice and weight loss. Radiological and clinical findings overlap. CA 19-9 levels may be high in the focal and/ or diffuse pancreatitis.[17,18] Differentiation of focal pancreatitis and pancreas cancers is significant to avoid redundant surgery. The region of focal pancreatitis is hypo-isointense on T1-weighted images and hypo-hyperintense on T2-weighted images compared to normal pancreas parenchyma. However, these findings are not sufficient to differentiate focal pancreatitis from pancreas carcinomas. The early contrast peak of cases with focal pancreatitis is known, whereas pancreas carcinomas show late contrast enhancement. However, one study by Momtahen *et al*. has revealed gradual enhancement of focal pancreatitis. Therefore, it may not differentiate from pancreas carcinoma displaying later contrast enhancement.[19] A normal pancreatic canal diameter confirms focal pancreatitis. A sudden termination of the main pancreatic ductus with distal dilatation is a sign of pancreatic cancer. In the study by Momtahen *et al*., using DWI with b 0 and 600 for evaluation of patients with focal pancreatitis, ADCs were found to be 2.09 ±0.18x10^{-3} mm^2/s in the area of focal pancreatitis and 2.03 ±0.2x10^{-3} mm^2/s in the remaining normal pancreatic tissue, without a significant difference.[19] Their study is similar to the present study in that they used b 0 and 600 values and the present study evaluated multiple b values. It was proven in the current study that the lesion ADC/normal parenchyma ADC ratios were more effective than the lesion ADCs for discrimination of benign and malignant lesions. As a result, the b 600 value was the most useful b value for the lesion ADC/ normal parenchyma ADC ratios. The optimum cut-off value for b 600 of the lesion ADC/normal parenchyma ADC ratios was 1.26×10^{-3} mm^2/s.

This indicated that DW-MRI was an important and non-invasive method in differentiation of focal pancreatitis and pancreas carcinomas. However, in several studies, ADCs were 1.68x10^{-3} mm^2/s for mild chronic pancreatitis, 1.57x10^{-3} mm^2/s for severe chronic pancreatitis and 1.94x10^{-3} mm^2/s for cases without pancreatitis.[9,11]

DWI indicates changes in microscopic diffusion in water. In chronic pancreatitis, normal pancreatic tissue is destroyed and destructed and fibrosis and chronic inflammation occur. It is possible that fibrous tissue and impaired exocrine and endocrine functions may reduce water diffusion and result in a decreased ADC.[20] In the present study, 10 patients with pancreatitis were evaluated, and the ADCs of pancreatitis were low, which is similar to pancreas carcinoma. The median ADCs in these lesions was 1.29x10^{-3} mm^2/s for auto-ADC and 1,38x10^{-3} mm^2/s for b1000.

In the 10 cases of pancreatitis in the present study, although ADCs in the pancreatitis regions were lower than those in the normal pancreas parenchyma for some b values, there was not a statistically significant difference. This shows that efficacy of ADCs is low in differentiation of normal pancreas parenchyma and area of pancreatitis. In addition, lesion ADCs and lesion/ normal parenchyma ADC ratio were not useful in differentiation. However, it has been reported that ADCs in pancreas carcinomas were lower than in normal pancreas. In a study by Muraoka *et al*.,[11] the mean ADCs±SD was found to be 1.27 ±0.52x10^{-3} mm^2/s for pancreas carcinomas and 1.90 ±0.41x10^{-3} mm^2/s for normal pancreas parenchyma. Other studies reported that the mean ADCs±SD of pancreatic adenocarcinoma was 1.31 ±0.24x10^{-3} mm^2/s and 1.570 ±0.24x10^{-3} mm^{-2}/s by using multiple b values.[21,22]

The present study has some restrictions. One limitation is that the number of patients and the variety of pancreas pathologies are low. Computurized tomography is usually preferred in the management of pancreas masses; especially, in the evaluation of vascular invasion. Therefore, the number of MRI administered in order to assess the pancreatic lesions during the study period was relatively low. Second, the present study is retrospective in nature. Third, if comparisons had been made according to pathological grades or fibrosis content of pancreas lesions, more detailed information about effects of fibrosis on ADCs could have been obtained.

Recently, studies using intravoxel incoherent motion (IVIM) imaging to investigate pancreas lesions have been reported.[21,23-25] They showed that IVIM may be

helpful for differentiating pancreatic adenocarcinomas from normal pancreas parenchyma, changes of chronic pancreatitis, and neuroendocrine tumor. IVIM shows perfusion and diffusion of tissue. They found that perfusion fraction (f) and incoherent microcirculation (Dfast) showed superior diagnostic capability than the ADC values in characterizing pancreatic diseases.[24,25] However, pancreatic lesions with IVIM parameters were not evaluated because the MR machine in the clinic had no IVIM sequence. If IVIM sequences had been used for evaluations, pancreatitis could have been differentiated from pancreas adenocarcinoma.

CONCLUSION

DWIs can be beneficial in differentiation and characterization of pancreatic carcinoma and benign lesions. Especially, lesion ADC / normal parenchyma ADC ratios are more important than lesion ADCs in differentiation of benign and malignant pancreas lesions and in differentiation of benign lesions and pancreatitis. Because the ADCs of pancreatitis and pancreas carcinomas are similar, use of DW MRI is not recommended only to discriminate between cancer and mass-forming pancreatitis. For differentiation of these diseases, additional modalities and new sequences as diffusion tensor imaging and intravoxel incoherent motion diffusion-weighted MR imaging are necessary.

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ORIGINAL ARTICLE

Evaluation of Diagnostic Scores for Acute Appendicitis

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ABSTRACT

Objective: To assess the sensitivity of the Alvarado score (AS), modified Alvarado score (MAS), Fenyo-Lindberg score (FS), Lintula score (LS), Eskelinen score (ES), Teicher score (TS), and Christian score (CS) [seven scorings] in patients with acute appendicitis (AA).

Study Design: Analytical study.

Place and Duration of Study: The First Affiliated Hospital of Chongqing Medical University, China, from January 2012 to June 2015.

Methodology: Patients with diagnosis of AA were evaluated retrospectively to compare the scoring systems. The diagnostic sensitivity (the correct number of diagnoses divided by the total number) was compared. Data were analyzed using SPSS software.

Results: One hundred and seventy-nine patients were studied. The sensitivity of AS was 92.7%; It outperformed each of the other scores. The sensitivity of FS, LS, and TS in women was lower than that in men ($p=0.016$, $p<0.001$, and $p<0.001$, respectively). The sensitivity of the FS, ES, TS, and CS in patients with a duration of illness greater than 48 hours was lower than that in patients with a duration of illness less than 48 hours ($p<0.001$ for all).

Conclusion: AS is the most useful and sensitive diagnostic tool for AA. FS, LS, and TS had a lower diagnostic sensitivity in women; and FS, ES, TS, and CS had a low sensitivity in patients with a duration of illness greater than 48 hours.

Key Words: *Acute appendicitis. Diagnosis. Alvarado score. Sensitivity.*

INTRODUCTION

Acute appendicitis (AA) is the commonest cause of abdominal pain, which require surgery. Symptoms of AA overlap with various conditions, making it difficult to diagnose.[1] The diagnosis in the young, elderly, and female is more difficult to make than in others, because numerous other diseases may behave like AA in these patients.[2] The inability to make an early diagnosis is a critical reason for morbidity and mortality. Based on various reports, the negative appendectomy rate is about 13-40%.[3-5] Therefore, it is necessary to improve the methods for early diagnosis and intervention.

In recent years, ultrasonography and computerized tomography have been used to make a rapid and accurate diagnosis of AA. Subsequently, the negative laparotomy rate decreased to about 10%.[6,7] However, time laging, high expenses, and variable accessibility of imaging methods are the reasons owing to which the current diagnostic techniques do not adequately address the condition, particularly in developing countries where imaging is not widely used.[8] In such situations, the diagnosis of AA relies upon symptoms, physical signs, and laboratory tests.

The purpose of the clinical decision rules is to assist doctors in the evaluation of patients.[9] In 1986, based on symptoms, signs, and the results of diagnostic tests of patients with suspected AA, Alvarado constructed a 10-point clinical scoring system, called the Alvarado score, to assist in the diagnosis of AA.[10] Other scorings have also been developed, such as the modified Alvarado score (MAS), Fenyo-Lindberg score (FS), Lintula score (LS), Eskelinen score (ES), Teicher score (TS), and Christian score (CS).

Some scorings for the diagnosis of AA have been compared, but most of the analyses only focus on comparing between/among two or three scoring systems. However, to the authors' knowledge, the application and usefulness of seven scorings in the diagnosis of AA has not been evaluated in China. The objective of this study was to assess the sensitivity of the seven scorings in the diagnosis of AA.

METHODOLOGY

The present study involved the selection of consecutive patients with AA, who were undergoing appendectomy. Pathologists confirmed appendicitis by histopathological examination in the Department of General Surgery at the First Affiliated Hospital of Chongqing Medical University from January 2012 to June 2015. Information on the disease history, clinical findings, and results of laboratory tests were recorded. Patients in whom histopathological findings were negative, were excluded from the study. The Principal Committee of the First Affiliated Hospital of Chongqing Medical University authorized this research. The seven scoring systems are based on different variables, with different points assigned to each variable according to previous

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reports.[10-16] For example, the following five criteria were used in the diagnosis of AA for the CS: i. Abdominal pain: defined for the study as abdominal pain (not right iliac fossa alone) occurring within 48 hours of presentation, ii. Vomiting: one or more episodes, iii. Right lower quadrant tenderness, iv. Low grade fever: defined as fever ≤38.8ºC, and v. Polymorphonuclear leukocytosis: defined as a total count ≥10,000, with polymorphs ≥75%. Table I and Table II show the criteria that were used in the diagnosis of AA for the other scores.

Table I: Characteristics of the Alvarado score and the Modified Alvarado score, and the distribution of data for individual variables in each scoring system.

Feature	Alvarado score	The distribution of data for individual variables (n=179)	Modified Alvarado score	The distribution of data for individual variables (n=179)
Nausea-vomiting	1	37.99% (n=68)	1	37.99% (n=68)
Anorexia-acetone (in the urine)	1	92.74% (n=166)	1	92.74% (n=166)
Tenderness in right lower quadrant	2	100% (n=179)	2	100% (n=179)
Migration	1	94.97% (n=170)	1	94.97% (n=170)
Rebound pain	1	95.53% (n=171)	1	95.53% (n=171)
Elevation of temperature ≥37.3°C	1	45.25% (n=81)	1	45.25% (n=81)
Leukocytosis >10.0×10^9/L	2	88.27% (n=158)	2	88.27% (n=158)
Shift to the left (>75% neutrophils)	1	94.97% (n=170)		
The cut-off to predict acute appendicitis	≥7		≥7	

Table II: Characteristics of the Fenyo-Lindberg score and the Lintula score, and the distribution of data for individual variables in each scoring system.

Feature	Fenyo-Lindberg score	The distribution of data for individual variables (n=179)	Lintula score	The distribution of data for individual variables (n=179)
Sex				
Male	8	51.40% (n=92)	2	51.40% (n=92)
Female	-8	48.60% (n=87)	0	48.60% (n=87)
Pain duration				
<24h	3	39.66% (n=71)		
24h-48h	0	38.55% (n=69)		
>48h	-12	21.79% (n=39)		
Progression of pain				
Yes	3	98.32% (n=176)		
No	-4	1.68% (n=3)		
Severe pain			2	84.36% (n=151)
Relocation of pain				
Yes	7	94.97% (n=170)	4	94.97% (n=170)
No	-9	5.03% (n=9)		
Vomiting				
Yes	7	37.99% (n=68)	2	37.99% (n=68)
No	-5	62.01% (n=111)		
Aggravation with cough				
Yes	4	100% (n=179)		
No	-11	0% (n=0)		
Body temperature ≥37.5ºC			3	43.58% (n=78)
Pain in RLQ				
Yes	4	100% (n=179)	4	100% (n=179)
No	-6	0% (n=0)		
Rebound tenderness				
Yes	5	95.53% (n=171)	7	95.53% (n=171)
No	-10	4.47% (n=8)		
Rigidity				
Yes	15	17.32% (n=31)	4	17.32% (n=31)
No	-4	82.68% (n=148)		
Bowel sounds change			4	44.70% (n=80)
WBC				
<8.9×10^9/L	-15	10.61% (n=19)		
9-13.9×10^9/L	2	46.93% (n=84)		
>14×109/L	10	42.46% (n=76)		
The cut-off to predict acute appendicitis		Total score -10 ≥-2		≥21

RLQ = Right lower quadrant; WBC = White blood cell.

Statistical analysis was performed using SPSS version 21 for Windows. Ages were presented as mean ± standard deviation (SD). The ratio of male, female, and duration of illness was obtained by dividing the number of male, female, or duration of illness by the total number. The diagnostic sensitivity was defined as the correct number of diagnoses divided by the total number. The diagnostic sensitivity of each score was compared using the Chi-square test. All analyses were two-sided, with statistical significance set at $p<0.05$.

RESULTS

One hundred and seventy-nine patients were enrolled in this study. Their ages ranged from 13 to 87 years (mean 44.13 ±19.52 years). There were 92 (51.4%) males and 87 (48.6%) females (male:female=1.1:1). The variables of scoring systems and the distribution of data for individual variables in each scoring system was given in Tables I, II and III. Table IV summarizes the results of this study. Overall, AS had a sensitivity of 92.7%, while the sensitivity value of the MAS was 88.3%; FS, 87.7%; LS,

Table III: Characteristics of the Eskelinen score and the Teicher score, and the distribution of data for individual variables in each scoring system.

Feature	Eskelinen score	The distribution of data for individual variables (n=179)	Teicher score	The distribution of data for individual variables (n=179)
Sex				
Male			2	51.40% (n=92)
Female			-1	48.60% (n=87)
Age				
20 years			-1	37.43% (n=67)
-39 years				
>50 years			3	32.96% (n=59)
Pain at presentation				
RLQ	7.02	97.21% (n=174)		
No in RLQ	3.51	2.79% (n=5)		
Pain duration				
<48h	4.26	78.21% (n=140)		
≥48h	2.13	21.79% (n=39)		
Duration 1½ days			2	74.30% (n=133)
Duration 2 days			1	3.91% (n=7)
Duration 3 days			-3	21.79% (n=39)
GU symptoms			-3	6.15% (n=11)
Pain in RLQ				
Yes	22.81	100% (n=179)		
No	11.41	0% (n=0)		
Rebound tenderness				
Yes	8.5	95.53% (n=171)		
No	4.25	4.47% (n=8)		
Rigidity				
Yes	13.24	16.76% (n=30)	3	16.76% (n=30)
No	6.62	83.24% (n=149)	-3	83.24% (n=149)
Right-sided rectal mass			-3	1.12% (n=2)
WBC				
$<10\times10^9$/L	5.88	13.41% (n=24)	-3	13.41% (n=24)
$\geq10\times10^9$/L	11.76	86.59% (n=155)		
$>13\times10^9$/L			2	47.49% (n=85)
The cut-off to predict acute appendicitis	≥55		≥-3	

GU = Gastric ulcer; RLQ = Right lower quadrant; WBC = White blood cell.

Table IV: Discriminating capacity of the scores.

	Number	AS	MAS	FS	LS	ES	TS	CS
Overall (sensitivity)	179	166 (92.7%)	158 (88.3%)	157 (87.7%)	142 (79.3%)	164 (91.6%)	157 (87.7%)	139 (77.7%)
Gender								
Men (sensitivity)	92	86 (93.5%)	78 (84.8%)	86 (93.5%)	83 (90.2%)	88 (95.7%)	89 (96.7%)	66 (71.7%)
Women (sensitivity)	87	80 (92.0%)	80 (92.0%)	71 (81.6%)	59 (67.8%)	76 (87.4%)	68 (78.2%)	73 (83.9%)
P		P=0.695	P=0.136	P=0.016	P<0.001	P=0.059	P<0.001	P=0.051
Times								
<48h (sensitivity)	132	125 (94.7%)	119 (90.2%)	124 (93.9%)	104 (78.8%)	127 (96.2%)	128 (97.0%)	118 (89.4%)
≥48h (sensitivity)	47	41 (87.2%)	39 (83.0%)	33 (70.2%)	38 (80.9%)	37 (78.7%)	29 (61.7%)	21 (44.7%)
P		P=0.090	P=0.189	P<0.001	P=0.764	P<0.001	P<0.001	P<0.001

AS = Alvarado score; MAS = Modified Alvarado score; FS = Fenyo-Lindberg score; LS = Lintula score; ES = Eskelinen score; TS = Teicher score; CS = Christian score.

79.3%; ES, 91.6%; TS, 87.7%; and CS, 77.7%. There was a statistically significant difference observed when AS, MAS, FS, ES, and TS were compared with LS ($p<0.001$, $p=0.022$, $p=0.033$, $p=0.001$, and $p=0.033$, respectively) and CS ($p<0.001$, $p=0.008$, $p=0.012$, $p<0.001$, and $p=0.012$, respectively).

For men, the sensitivities of the scores evaluated in this study were 93.5% (AS), 84.8% (MAS), 93.5% (FS), 90.2% (LS), 95.7% (ES), 96.7% (TS), and 71.7% (CS). There was a statistically significant difference between MAS and ES ($p=0.013$), TS ($p=0.005$). And there was a statistically significant difference between CS and the other scores. For women, sensitivities of the scores evaluated in this study were 92.0% (AS), 92.0% (MAS), 81.6% (FS), 67.8% (LS), 87.4% (ES), 78.2% (TS), and 83.9% (CS). There was a statistically significant difference between the LS and the following scores: the AS ($p<0.001$), MAS ($p<0.001$), ES ($p=0.002$), CS ($p=0.013$), and FS ($p=0.036$). Additionally, there was a statistically significant difference between the FS and the AS ($p=0.044$), MAS ($p=0.044$), between the TS and AS ($p=0.011$), MAS ($p=0.011$). The accuracy of the FS, LS, and TS in women was lower than it was in men ($p=0.016$, $p<0.001$, and $p<0.001$, respectively).

For patients with a duration of illness less than 48 hours, the highest sensitivity was 97.0% (TS). There was a statistically significant difference between the MAS and TS ($p=0.024$), between CS and ES ($p=0.032$), TS ($p=0.015$). Additionally, there was a statistically significant difference between LS and the other scores. For patients with a duration of illness greater than 48 hours, the highest sensitivity was 87.2% (AS). As the results differed significantly between FS and AS ($P=0.044$), there was significant difference between the TS and the following other scores: the AS ($p=0.005$), MAS ($p=0.021$), LS ($p=0.040$). Additionally, there was a statistically significant difference between the CS and the other scores, except the TS. The sensitivity of FS, ES, TS, and CS in patients with a duration of illness greater than 48 hours was lower than that in patients with a duration of illness less than 48 hours ($p<0.001$ for all).

DISCUSSION

Even today, this common condition continues to be difficult to diagnose. Scoring systems have been developed to help evaluate patients with AA in the clinical setting. In this study, we sought to assess the sensitivity of the seven scoring systems applied to patients with AA at the First Affiliated Hospital of Chongqing Medical University.

In all the 179 patients with AA, the sensitivity of AS, MAS, FS, ES, and TS was higher than that of the LS and CS. This result partially shows similarity with those of other studies. Ohmann *et al*. measured the performance of 10 scores using one database in a large multicenter trial, using standardized criteria and contrasted the outcomes with published data. Re-examination of the published data showed that AS exceeded each of the other scores, whereas the FS and CS followed.[17] In this study, AS demonstrated the highest sensitivity (92.7%), while LS and CS had low sensitivity. LS was initially created for the pediatric patients; therefore, this may be the reason for the lower accuracy observed in the adults. The aim of CS was to decrease the negative appendectomy rate. This origin may lead to some missed diagnoses of AA.[16] By contrast, if three out of five criteria were present in CS, the process of active observation was initiated for the patient. In this process, no antibiotics were given. If the fourth criterion was met, surgery was performed immediately. However, in this retrospective review, it was difficult to ensure that active observation was performed carefully in such a way that no antibiotics were given. These factors may have affected the accuracy of CS.

Some studies have found that the sensitivity of a scoring system for appendicitis is influenced by gender, so the data was analysed according to gender. FS, LS, and TS exhibited better performance in terms of sensitivity in the subgroup analysis of men than in women. Female patients with tenderness in the right lower quadrant can potentially have a variety of conditions, including pelvic infectious disease and other gynecological pathologies. Therefore, AA may be misdiagnosed in female patients. Depending on the gender of patients, FS and LS have different numerical values for the same symptoms. Gender is also one of the factors considered in TS. However, in this study, these considerations did not improve the sensitivity of these scoring systems in women, which is consistent with other reports.[17]

In this study, it was observed that the longer the duration of illness, the poorer the sensitivity of FS, ES, TS, and CS. There are a number of other reasons for this. FS, ES, and TS include the duration of pain as one of their components, and have different numerical values for symptoms depending on the length of time. The longer course was associated with lower scores. In addition, in cases involving a longer course of the disease, patients are more likely to be treated with analgesics and antibiotics that may mask the symptoms. These factors may have affected the accuracy of the scores.

Many scoring systems for AA have been created. AS is the most widely used system, and demonstrates best performance in validation studies. AS is the only scoring system presented in the document published by the American College of Emergency Physicians to guide decision-making for AA. Professionals within this organisation believe that combining different symptoms and signs into a scoring system may be more valuable in the prediction of appendicitis.[18] Some scholars think that the scoring weights of AS may be biased for patients

who are suspected of having appendicitis, because the system was created retrospectively, involving patients who had all been operated upon, with the suspicion of appendicitis.[19] In this study, the sensitivity of AS was up to 92.7% and it outperformed the other scores. A systematic review found that the AS is a useful diagnostic method for all patient groups when a cut-off score of 5 is used. Using this cut-off score, only two people missed being diagnosed in this study. However, Gwynn found that about 8.4% of patients with appendicitis had an AS below 5 in a Class III study. Therefore, the cut-off score of AS requires further optimisation in the future.[20]

Today, AA continues to be a troublesome disease. Scoring systems are intended to help the clinical diagnosis of patients with AA. To the authors' knowledge, this is the first time that seven scoring systems for appendicitis have been evaluated and compared in China. However, the sample population needs to be expanded, and the specificity of each score should be analysed. A prospective randomised controlled trial is to be conducted to evaluate the effect of those scores in China in the near future.

CONCLUSION

FS, LS, and TS have a low diagnostic sensitivity in women, while FS, ES, TS, and CS have a low sensitivity in patients with a duration of illness greater than 48 hours. Alvarado score remained the most sensitive test in all comparisons.

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ORIGINAL ARTICLE

Is Lactulose Plus Rifaximin Better than Lactulose Alone in the Management of Hepatic Encephalopathy?

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Abstract

Objective: To compare the efficacy of lactulose plus rifaximin with efficacy of lactulose alone in the treatment of hepatic encephalopathy.

Study Design: A randomized controlled trial.

Place and Duration of Study: Department of Medicine, Jinnah Hospital, Lahore, from December 2014 to June 2015.

Methodology: All patients who presented with hepatic encephalopathy due to decompensated chronic liver disease were randomly divided into two groups of 65 patients each. One group was given 30 ml thrice daily lactulose alone and the other lactulose plus rifaximin 550 mg twice daily for 10 days. Informed consents were taken from the participants' attendants. Grades II-IV hepatic encephalopathy was noted according to West-Haven Classification. All subjects were followed until 10 days after admission.

Results: The mean age of patients was 56.06 ±11.2 years, among which 46.9% were females and 53.1% were males. After ten days of follow-up, reversal was seen in 58.46% in lactulose alone group and 67.69% in lactulose plus rifaximin group (Chi-square p=0.276).

Conclusion: There was no difference in effectiveness of lactulose plus rifaximin and lactulose alone in treatment of hepatic encephalopathy.

Key Words: *Chronic liver disease. Hepatic encephalopathy. Lactulose. Rifaximin.*

INTRODUCTION

Hepatic encephalopathy is a serious life-threatening condition, ranging from mild motor and cognitive dysfunction to coma, and is potentially reversible if adequately treated.[1] The incidence of hepatic encephalopathy is higher in patients suffering from both liver cirrhosis and acute liver failure.[2] Bacteria residing in the intestine produce ammonia, which plays a role in the pathogenesis of hepatic encephalopathy.[3] Hepatic encephalopathy can be treated by reducing the production and absorption of this gut derived ammonia. Non-absorbable disaccharides are currently the main stay of the therapy.[4] A survival rate of 42% was reported by Bustamante *et al*. in patients with cirrhosis with first episode of hepatic encephalopathy at follow-up after a year and 23% at 3 years.[5]

Lactulose is a non-absorbable disaccharide. Systemic review of literature emphasized that effectiveness of lactulose was more than placebo to improve hepatic encephalopathy on short-term basis; however, there was no effect on mortality in the long run.[6] Diarrhea and abdominal pain may complicate treatment with lactulose.[7] Rifaximin is a semi-synthetic derivative of rifamycin and is virtually unabsorbed after oral administration.[8] It exhibits broad-spectrum antimicrobial activity against both aerobic and anaerobic gram-positive and gram-negative bacteria within the gastrointestinal tract.[9,10] In patients with liver dysfunction or renal insufficiency, adjustment of dosage is not required.[11,12] The therapeutic responses to anti-viral therapies can be affected by ethnicity, which is also reported to affect long-term prognosis of patients with advanced liver disease.[13,14]

A number of studies regarding efficacy of lactulose and rifaximin in patients of hepatic encephalopathy have been carried out, but with conflicting results. Decompensated liver cirrhosis, leading to hepatic encephalopathy, represents a major chunk of disease burden in Pakistan, leading to in-hospital stay and financial strain. No study has been published yet regarding Pakistani population.

The objective of this study was to compare the efficacy of lactulose plus rifaximin with lactulose alone in hepatic encephalopathy patients due to decompensated liver cirrhosis (CLD) in addition to usual treatment.

METHODOLOGY

This study was a randomized controlled trial conducted in the Department of Medicine, Jinnah Hospital, Lahore, from December 2014 to June 2015. All patients who presented with hepatic encephalopathy due to

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decompensated chronic liver disease (characterized by presence of coarse echotexture of liver on abdominal ultrasound) were included in the study. Patients having hepatic encephalopathy due to causes other than chronic liver disease, and patients allergic to the administered agents were excluded from the study.

Data was collected after approval from Medical Ethics Committee of AIMC/JHL. Selection of sample was from patients admitted in Medical Department, Jinnah Hospital Lahore. Informed consents were taken from the participants' attendants. Grades II-IV hepatic encephalopathy was noted according to West-Haven Classification. Demographic information, e.g. age, gender, address etc. were obtained. Single-blind was applied to reduce bias. The participants were divided into two groups, by lottery method. Group A was given lactulose 30 ml thrice daily. Group B was given lactulose 30 ml thrice daily plus rifaximin 550 mg twice daily.

All subjects were followed until 10 days after admission; final outcome (efficacy in terms of recovery from hepatic encephalopathy) was recorded in predesigned performa. The usual treatment was given to all patients as per hospital protocol.

Data was entered in SPSS version 20. Mean, standard deviation, and median (with IQR for not normally distributed variable) were calculated for quantitative variables (age of patients, duration of disease). Frequency and percentage was calculated for qualitative variables (gender of patients, socioeconomic status, grade of hepatic encephalopathy). Effect modifiers and confounders (age, gender, grade of hepatic encephalopathy, duration of disease) were controlled through stratification. Chi-square test was applied by taking p $\leq$0.05 to compare efficacy of lactulose plus rifaximin and lactulose alone.

RESULTS

A total of 130 patients fulfilling the inclusion criteria were enrolled in the study. The mean age of patients was 56.06 $\pm$11.2 years. The median age was 55.73 years with interquartile range value of 14. There were 46.9% females (61 patients) and 53.1% males (69 patients). Eighteen (13.8%) patients belonged to high socioeconomic status, 36.9% (48 patients) belonged to middle socioeconomic status; whereas, 49.2% (64 patients) belonged to low socioeconomic status.

At the start of the study out of 65 patients in group A, grade II encephalopathy was present in 26.15% (17 patients), grade III encephalopathy in 38.46% (25 patients) and grade IV encephalopathy in 35.38% (23 patients). After ten days of follow-up, reversal of hepatic encephalopathy was seen in 58.46% (38 patients) in group A.

In Group B, out of the 65 patients at the start of study, 40.0% (26 patients) had grade II encephalopathy, 36.92% (24 patients) had grade III encephalopathy and 23.07% (15 patients) had grade IV encephalopathy. After ten days of follow-up, reversal of hepatic encephalopathy was seen in 67.69% (44 patients) in group B (Table I).

Stratification for outcome was done with regard to age (p=0.256), gender (p=0.579), socioeconomic status (p=0.690), duration of disease (p=0.498), grade of encephalopathy at start of study (p <0.001, significant) and treatment given (p=0.276).

Table I: Stratification for outcome among patients with regard to grade of hepatic encephalopathy at the start of study (n=130).

Grade of hepatic encephalopathy at start	Outcome		Total
	No reversal	Reversal	
II	5 (3.84%)	38 (29.23%)	43
III	13 (10.0%)	36 (27.69%)	49
IV	30 (23.07%)	8 (6.15%)	38
Total	48	82	130

Pearson Chi-square value: 42.901, p-value < 0.001 (significant).

DISCUSSION

Hepatic encephalopathy is a serious life-threatening, but reversible condition which can be manifested in acute and chronic disease with ammonia being the major contributor.[1-3] As a result, the treatment of hepatic encephalopathy revolves around reducing production of ammonia and its absorption in the gut and also by improving the excretion of ammonia by modification of diet or drug therapy.[15]

At the moment, the most commonly used therapies for treating hepatic encephalopathy are lactulose and non-absorbable antibiotics. Sixteen lactulose, recommended as the first-line pharmacological treatment; however, is associated with abdominal cramps, severe diarrhea, nausea, flatulence, and dehydration.[17,7]

Antibiotics may be used as a therapeutic alternative to non-absorbable disaccharides for treating of hepatic encephalopathy.[18] Neomycin, non-absorbable aminoglycoside, prescribed for hepatic encephalopathy in the past, but its use is limited by ototoxicity and nephrotoxicity. Another antibiotics, metronidazole, which differs from neomycin in terms of bacterial spectrum, improves hepatic encephalopathy; however, the common use is limited by its potentially severe neurotoxicity in patients with cirrhosis.

Rifaximin, a semi-synthetic derivative of rifamycin having broad-spectrum antimicrobial activity, is not absorbed in the gut.[8] Its active form remains in high concentrations in gut and gets excreted in feces without causing significant systemic side effects such as nephrotoxicity.[9,10] Furthermore, previous studies have shown that it is effective at reducing hepatic encephalopathy in cirrhotics.

A randomized controlled trial conducted in India by Sharma *et al*. showed complete recovery from hepatic encephalopathy in 76% of patients treated with lactulose

plus rifaximin as compared to 50.8% of patients treated with lactulose alone.[12] The sample size was 120 patients, mean age of the patients was 39.4±9.6 years, while the male/female ratio was 89:31. A significant reduction in mortality was seen after treatment with lactulose plus rifaximin (23.8%) versus lactulose alone (49.1%). A meta-analysis was conducted in 2012 by Eltawil *et al*. in which 12 randomized controlled trials met the inclusion criteria, a total of 565 patients were a part of the study.[13] Clinical effectiveness of rifaximin was found to be equivalent to disaccharides or other oral antibiotics having odds ratio (OR) 0.96; 95% CI: 0.94-4.08 but with a better safety profile (OR 0.27; 95% CI: 0.12-0.59).[13] Another meta-analysis conducted in 2012 by Jiang *et al*. in which 5 trials met the inclusion criteria on a total of 264 patients demonstrated no significant difference between rifaximin plus lactulose and lactulose alone on improvement in patients of hepatic encephalopathy but rifaximin was better tolerated.[14]

The present study was a randomized study which was conducted to compare efficacy of lactulose plus rifaximin with that of lactulose alone for the treatment of hepatic encephalopathy. No relevant study and data were available on ethics background affect on the effectiveness of rifaximin for the treatment of hepatic encephalopathy.[15]

This study does not confirm that lactulose plus rifaximin is more effective in treatment of hepatic encephalopathy as compared to lactulose alone. Further, no significant difference between lactulose plus rifaxaimin and lactulose alone was found in terms of their efficacies. The authors believe more studies, involing a large sample size/number of patients, are required for identification of the factors determining responsiveness to rifaximin in hepatic encephalopathy.

All hepatic encephalopathy therapeutic trials can be criticized from the perspective of evidence-based medicine which includes definitions of study endpoints, control groups treatment, and the proper quantification of therapeutic effects.[1] Sanaka *et al*. described the difficulties of designing a good hepatic encephalopathy treatment trials.[16] The mental status evaluation system using the portal systemic encephalopathy (PSE) index developed by Conn *et al*., is currently widely used.[19] However, the Food and Drug Administration (FDA) strongly objected to the use of this system and favoured the adoption of a detailed mental status evaluation system for hepatic encephalopathy.[1] Further, clinical trials using a new mental state evaluation system, which satisfies the FDA's requirements, is required to confirm the efficacy of rifaximin for the treatment of hepatic encephalopathy.

CONCLUSION

The study did not confirm improved efficacy of lactulose plus rifaximin as compared to lactulose alone in treating hepatic encephalopathy.

Disclosure: It is a dissertation-based article.

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ORIGINAL ARTICLE

Remission Rate of Acute Lymphoblastic Leukemia (ALL) in Adolescents and Young Adults (AYA)

Anita Vallacha, Ghulam Haider, Wiky Raja and Dinesh Kumar

Abstract

Objective: To determine the remission rate in adolescent and young adult (AYA) patients with acute lymphoblastic leukemia (ALL).

Study Design: Descriptive study.

Place and Duration of Study: Department of Oncology, Jinnah Postgraduate Medical Centre (JPMC), Karachi from January, 2016 to March, 2017.

Methodology: Adolescent and young adult (AYA) patients aged 15-39 years, newly diagnosed with acute lymphoblastic leukemia from January, 2016 to March, 2017. Diagnosis was confirmed by bone marrow trephine biopsy and immuno-phenotyping. All the patients were treated with daunorubicin, vincristine, prednisone, and L-asparaginase in the induction phase. The response evaluation was done on day 35 of the induction phase and the remission rate was assessed by the bone marrow examination.

Results: Of the total 50 AYA patients diagnosed with ALL, 41 patients could complete induction phase and 9 patients died during the first week of induction, therefore excluded from the study. Forty (97.8%) patients were <35years of age, 28 (68.3%) were male, of female 10 (24.4%) were housewives, 33 (80.5%) patients belonged to Sindh, 28 (68.3%) presented with fever and body ache, 17 (41.5%) patients had precursor B cell type ALL, with 7 (17.1%) patients had hemoglobin of <7 g/dL,11 (26.8%) patients had white cell count of >30x10^9/L, platelet count of <20x103/µL in 6 (14.6%) patients and complete morphological remission was reported in 29 (70.7%) patients.

Conclusion: The remission induction rate was 70.7% in the adolescents and young adults with acute lymphoblastic leukemia at the study centre.

Key Words: *Remission. Acute lymphoblastic leukemia. Adolescents. Young adults.*

INTRODUCTION

Acute lymphoblastic leukemia is a heterogeneous group of hematological disorder and the most common pediatric cancer that is characterized by the abnormal proliferation of immature cells of lymphoid lineage in the bone marrow, peripheral blood, and other organs.[1] The peak incidence of acute lymphoblastic leukemia is seen at 2-10 years of age. The annual incidence of this cancer in Pakistan is unknown. The annual incidence reported from other countries is 30-40 per million children of less than 18 years of age.[2] Acute lymphoblastic leukemia represents 20% of all leukemias among the adults. Its annual incidence is about 1.58 per 100,000 individuals per year in the age adjusted adults in United States with 6,590 new cases and 1,430 deaths in 2016.[3]

There are no uniform age subgroups of acute lymphoblastic leukemia defined. Age of the patients with pediatric ALL ranges from 0-14 years, adolescents from 15-19 years, young adults from 20-39 years, adults from 40-60 years, and older adults and elderly patients >65 years.[4] Thus, adolescent and young adult (AYA) patients include the age group of 15-39 years. AYA are defined as a vulnerable population by National Cancer Institute.[5] Several clinical trials have shown disappointing results in adults as compared to children with cure rate of 90% and 30-40%, respectively.

Various socioeconomic factors, such as lack of clinical trials, lower compliance rates, and long delays in the initiation of the treatment, may result in the poor outcomes in the AYA population.[6] Children with ALL are treated successfully, but prognosis deteriorates markedly with the onset of adolescence to adulthood. This can be estimated by higher relapse rates and shorter survival in AYA population.[7] There are three subtypes of acute lymphoblastic leukemia with variation in the prevalence, which depends on the environmental, geographical, socio-economic, ethnic and racial factors. These include B cell lineage (precursor B cell ALL, and mature B cell ALL) and T cell ALL with prevalence of 75% and 25%, respectively. Several prognostic factors predict the complete morphological remission rate. These include clinical and laboratory factors like age, sex, white cell count, and good cytogenetics.[8] There is no standard protocol defined in AYA patients with ALL. These patients are treated either with pediatric protocol or with an adult protocol, depending on the treating physician's choice. Clinical trial is recommended in the first line of treatment.[3]

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In Pakistan, the demographics and the outcome of AYA with ALL is not known. This study aimed to determine the remission rate after induction chemotherapy in AYA patients with ALL. This study will hopefully make platform for further multicenter studies and help in improving treatment options, and thus the outcome.

METHODOLOGY

Patients of age 15-39 years (AYA) with diagnosis of ALL presented to the Oncology Department, Jinnah Postgraduate Medical Centre (JPMC), Karachi, from January 2016 to March 2017 were included in the study. The data was prospectively collected with a sample of 50 patients. Patients with newly diagnosed ALL, Eastern Cooperative Oncology Group (ECOG) performance status 0-2, either gender and ejection fraction >55%, were included; patients with central nervous system (CNS) infiltration, ECOG performance status >2 and Philadelphia positive on cytogenetic studies, were excluded. Enrollment of the patients started after the ethical review committee approval from JPMC. Written informed consent was taken. A proforma was structured for this study.

Peripheral blood and bone marrow samples were taken from all the patients before the treatment. Diagnosis of ALL was made on the basis of >20% lymphoblast in bone marrow on morphological and immunohisto-chemical studies, immunophenotyping were performed where morphology and immunohistochemistry was inconclusive. Also cytogenetic studies were done to rule out Philadelphia positive ALL. The clinical variables observed were age at diagnosis, gender, clinical features at presentation, and the laboratory variables were subtype of ALL, hemoglobin, white blood count and platelet count. Echocardiography of all patients was done before starting the treatment.

The treatment protocol was based on DVP-Asp (Linkers regimen) with some modifications due to logistic reasons.[9] The chemotherapy administered includes daunorubicin, vincristine, prednisone and L-asparaginase (Table I). We could not assess initial treatment response on day 14 as given in protocol, because of lack of facilities of bone marrow trephine biopsy in our hospital setup and sickness of patients due to febrile neutropenia as these factors could not allow patients to move to other hospital laboratories for bone marrow trephine biopsy for initial response evaluation. Therefore, treatment response was evaluated by bone marrow analysis on day 35 of the induction, as almost all patients had become stable by that time. Complete morphological remission (CR) was defined as <5% blasts in the bone marrow, no leukemic cells in the peripheral blood, absolute neutrophil count of at least $1.0x10^9$/L, and platelet count of at least 100x103/μL, and the absence of extramedullary leukemic blasts.

Table I: Remission induction therapy.

Daunorubicin	50mg/m2 IV, days 1-3
Vincristine	2mg IV, days 1, 8, 15, 22
Prednisone	60mg/m2 PO, days 1-28
L-Asparginase	6000 u/m2 IM, days 17-28

IV = Intravenous; PO = Per oral; IM = Intramuscular

Statistical analysis was done by using SPSS version 17.0. For patients' characteristics, descriptive statistics were used. Frequencies with percentages were calculated for categorical data.

RESULTS

A total of 50 patients with Philadelphia negative ALL were included in the study. Of them, 41 patients had completed induction phase. Nine patients died during the first week of induction because of infection and progressive disease, and could not complete induction phase of treatment. Therefore, they were excluded from the study and remission rate of 41 eligible patients were reported, who had successfully completed the induction phase of treatment.

All the patients (n=41) were between 15-39 years of age, from which 21 (51.2%) were 15-20 years, 8 (19.5%) were 21-25 years, 5 (12.2%) were 26-30 years, 6 (14.6%) were 31-35 years, and 1 (2.4%) was 36-39 years. There were 28 (68.3%) males and 13 (31.7%) females. Among the female patients, 10 (24.4%) were housewives. Out of the total population, 11 (26.8%) were students, 8 (19.5%) were farmers, and 12 (29.3%) belonged to other professions. Thirty-three (80.5%) participants belonged to province of Sindh, seven (17.1%) to Balochistan, and 1 (2.4%) to Khyber Pakhtunkhwa (KPK). Fever and body ache (n=28; 68.3%) were the most common presentation followed by fever and weight loss in 7 (17.1%) patients. Four (9.8%) patients had presented with fever and bleeding, one patient (2.4%) with bleeding and one (2.4%) with weight loss alone. Immunophenotyping results at diagnosis were available for 27 (65.9%) patients. Of them, 17 (41.5%) cases were classified as having Precursor B-cell ALL, 6 (14.6%) as B-cell ALL, and 4 (9.8%) as T-cell ALL. Fourteen patients (34.1%) were with only diagnosis of acute lymphoblastic leukemia with no subtype defined.

Thirty (73.2%) patients had white cell count of $<30x10^9$/L, while 11 (26.8%) patients had $>30x10^9$/L. Of them, 6 (14.6%) patients had white cell count $>100x10^9$/L. Nineteen patients (46.3%) had hemoglobin between 7-10g/dL, while 15 (36.6%) patients had above 10g/dL, and 7 (17.1%) patients had below 7g/dL. In majority of patients (n=17, 41.5%), platelet count were in the range of 20-$50x10^3$/μL, while 6 (14.6%) had below $20x10^3$/μL, and 8 (19.5%) had above $50x10^3$/μL. Ten patients (24.4%) had platelet count of $>100x10^3$/μL. All the patients were given four drugs as per Linker's ALL

protocol (Table-I). Bone marrow analysis for response evaluation was done on day 35 of the induction. Complete remission rate was achieved in 29 (70.7%) patients, while 12 (29.3%) patients had not achieved morphological remission.

DISCUSSION

Due to lack of population based tumor registry in Pakistan, the exact incidence of ALL in AYA population is not known. The incidence rate (age adjusted) of ALL in U.S population is 1.7 per 100,000 individuals per year,[10] with approximately 5,970 new cases and 1,440 deaths estimated in 2017.[11] The median age at diagnosis is 15 years with 56.1% patients diagnosed below 20 years of age.[12] Little or none has been published on the remission rate of ALL in AYA population in Pakistan. The National Cancer Institute has defined population of 15-39 years of age as AYA population. These patients have different characteristics as compared to children and older adults. These characteristics could be disease-related (biology and clinical presentation) or patient related (treatment tolerance and psychosocial aspects). In comparison to children (1-9 years), AYA patients with acute lymphoblastic leukemia have higher relapse rates and shorter survival.[13]

This study was done to look for the complete remission rate of AYA patients with ALL at the study centre, i.e. Jinnah Postgraduate Medical Centre, Karachi. Good clinical prognostic factors for remission induction are age <35 years, male gender, low white cell count $<30x10^9$/dL and good cytogenetic.[14] In this study, majority of patients (n=40, 97.8%) were <35 years of age. In this study, male predominance (68.3%) was reported. In adult patients with ALL, about 75% of cases comprised of B-cell lineage subtype and 25% of T-cell lineage.[15,16] In 65.9% patients of this study, ALL subtype was defined, which includes 56.1% of B-cell lineage and 9.8% of T-cell lineage. In 34.1% patients, subtype of ALL was not defined. This may be due to loss of differentiation on immunophenotyping because of advanced disease, but further studies are required for this different behavior of leukemic cell. In 11 (26.8%) patients, high white cell count $>30x10^9$/dL was documented at the time of presentation. High white cell count ($>30x10^9$/dL) remained an unfavorable prognostic marker and associated with decreased remission rate.[14,17] These patients required aggressive supportive management because of higher risk for developing tumor lysis syndrome and other complications. In 14.6% patients, platelet count were found <20x103/µL, but no life-threatening bleeding was reported.

For AYA patients with ALL, there is no single standard treatment regimen developed. They are either treated with pediatric protocol or with an adult protocol. Treatment protocols given are based on physician's preference and institutional practice. Multiple clinical trials had reported disappointing results in adults with cure rates of 30-40%; whereas in children, with cure rate of 90%. Five-year event-free survival rate for AYA patients with ALL, treated with adult and pediatric protocol, ranges from 34% to 69% and 63% to 74%, respectively.[18-20] The National Comprehensive Cancer Network guidelines currently recommends a clinical trial in newly diagnosed ALL in AYA patients instead of any standard regimen as first line treatment. The primary goal of induction is to achieve complete clearance of blasts and normalization of peripheral blood count. With either of pediatric or adult protocol, the complete remission rate reported is approximately 90%. Some multi-center trials had reported CR rates as 93.5% for GRA ALL 2003, 94% for DFCI consortium, 90% for CCG 1882, and 98% for PETHEMA ALL-96.[19,21-23]

In this study, the complete remission rate CR achieved was 70.7% at the end of the induction. Other multicentre trials had also reported lower CR rates: 64% for the ECOG trial3, 68% for the SWOG trial, 74% for the GMALL-01 (German ALL trail), and 75% for the GMALL-02 trial.[24] Therefore, we need more aggressive and modified regimen to improve the complete remission rate and thus the overall survival.

This is a single-center study with a small number of patients as compared to others. The estimated complete remission rate in this study is inferior as compared to other studies. Therefore, more multicentre studies are needed to identify other risk factors behind the poor outcome. The authors have reported the initial response to therapy, but further follow-up studies are required to evaluate the overall survival. This is the only study so far published from Pakistan. During the induction phase, high rates of toxic deaths were noted secondary to infections. Therefore, aggressive management of febrile neutropenia is needed. This study emphasizes designing of more aggressive treatment for AYA patients with ALL.

CONCLUSION

Remission induction rate was 70.7% at the study centre in the adolescents and young adults with acute lymphoblastic leukemia.

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ORIGINAL ARTICLE

Role of Weight-Bearing Exercises in the Treatment of Post-Menopausal Osteoporosis

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ABSTRACT

Objective: To determine the change in T-score of post-menopausal osteoporosis patients with weight-bearing exercises.
Study Design: A quasi-experimental study.
Place and Duration of Study: Physiotherapy Department and Orthopedic Unit I, Mayo Hospital, Lahore, from May to October 2014.
Methodology: Two hundred and seventy-four patients were randomly divided into two groups according to inclusion and exclusion criteria using non-probability purposive sampling technique. The group 1 was treated by medication and weight-bearing exercises and group 2 was given medication alone. The dual energy X-ray absorptiometry (DEXA) scan was used to find the T-score before and after treatment and improvement was compared. A p-value less than 0.05 was taken as significant.
Results: The results showed that improvement was occurred in both groups after treatment. The DEXA scan median values after treatment were changed to 3.00 (0) for group 1 (exercises and medication) and 2.00 (1) for group 2 (medication).
Conclusion: The physical activity along with medication play vital role in the treatment of post-menopausal osteoporosis than medication alone.

Key Words: *Osteoporosis. T-score. Medication. Weight-bearing exercise. DEXA scan. Post-menopause.*

INTRODUCTION

World Health Organization (WHO) defined osteoporosis as a progressive systemic skeletal disorder in which bone mineral density becomes decreased and architectural structure of bone becomes changed at micro level and these fragile bones lead to fracture. Osteoporosis can be confirmed when the density is less than -2.5 standard deviations of adult age-similar value.[1,2] In females after menopause, this chronic skeletal problem is increasing throughout the world.[3,4]

The rate of occurrence of osteoporosis worldwide is 200 million. According to statistics, only in United States of America about 14 million people are presented with osteoporosis.[5,6] Nagi stated that among the 200 million people having osteoporosis, 9.9 million belong to Pakistan. Among them, 7.2 million are women, particularly post-menopausal women. The higher rate of prevalence of osteoporosis in females is associated with post-menopausal changes, decreased bone mineral density (BMD), reduced bone size, and longer life as compared to men.[7,8]

The bone consists of 30% matrix and 70% mineral salts. The porosity of bone is due to decreased organic part of bone matrix because bone formation activity becomes less active than bone deterioration activities. The bone mineral density is high in males than females, because it reduces rapidly in females after menopause.[9] Osteoporosis leads to fractures that is linked with increased mortality, morbidity, and low quality of life.[10] Osteoporosis can be prevented by maintaining the bone mineral density through: exercise therapy, medication, lifestyle modification by balanced diet, sufficient calcium and vitamin D usage, cigarette cessation, stopping alcohol intake, fall prevention and change in dietary habits.[11]

For optimal strength and power of musculoskeletal system, exercises, especially weight-bearing and progressive resistive exercises are mandatory. During rehabilitation process of fractures, physical therapy plays an important role.[12] The protocol of exercise to achieve optimum goals should be three to four times per week.[13] Weight-bearing exercise and training consist of physical exercises to increase bone growth and bone density. During exercises, muscles and bones are subjected to work under resistance and against gravity. Bones absorb more calcium from blood when force is added upon them.[14] Exercises influence the strength and bone mineral density.[15] Among these, weight-lifting, push-ups, jumping, brisk walk, aerobic training, and bicycling can be included along with daily activities of living like snow shoveling, working in gardens, vacuum cleaning etc.[16]

The purpose of this study was to determine the effects of physical exercises with medication on bone mineral density in post-menopausal osteoporotic patients.

METHODOLOGY

It was a quasi-experimental study conducted in Physiotherapy Department and Orthopedic Ward Unit 1,

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Mayo Hospital, Lahore, from May to October 2014. The sample size of 274 patients (137 patients in each group) was calculated by using 80% confidence level, 7% absolute precision, and by taking expected percentage of change in muscle strength in patients who were treated with weight-bearing exercises and weight-bearing exercise plus hormone replacement therapy (HRT) as 75% and 67%, respectively.[17]

The patients were selected through non-probability purposive sampling and divided into group 1 (experimental group) and group 2 (control group). The inclusion criteria were patients diagnosed as post-menopausal osteoporosis on DEXA scan with T score <2.5,[18] recruited from the study place. The exclusion criteria were the patients with other systemic diseases (cardiovascular diseases), patients aged below 40 years, all male patients, obese patients, i.e. body mass index (BMI) beyond 30, surgical history (major surgery during last six months), patients not willing to do exercise, patients with known psychological or neurological disorder, and patients who were restricted to medical and therapeutic protocol who could not warm up or stand for weight-bearing.

The patients were grouped by using randomization table. The subjects in each group were evaluated through physical examination and DEXA scan.[19] Detailed history of medication was asked. Group 1 subjects were treated with medications prescribed by doctors and weight-bearing exercises by physiotherapist for three months with three sessions per week. Weight-bearing exercises were performed in the sequence of 5-10 minutes of warm-up exercise, 20 minutes of progressive weight-bearing exercise, 15 minutes of resistance exercise with large muscle group, 5 minutes of stretching and balance.[20] The second group also consisted of 137 patients and was treated with medication alone for same duration. Then DEXA scan was repeated to see the difference. The difference in improvement was noted and compared. The data were collected on predefined proforma.

Using SPSS 20.0, data were managed and analyzed. The quantitative data were presented in the form of mean ±SD. The qualitative data were presented in the form of percentage, proportion, and pie-charts. The p-value less than 0.05 was taken as significant. As the data was skewed (not normally distributed), the most appropriate statistical test was Wilcoxon signed-rank test. It indicated whether the medians were significantly different before and after the treatment of post-menopausal osteoporotic females in both groups.

RESULTS

A total of 274 osteoporotic women who met the inclusion criteria with ages ranged from 40-94 years, with a mean age of 55.49 ±12.86 years were included in the present study. All participants were female above 40 years of age. The past medical history showed that 147 (53.6%) subjects receiving hormone replacement therapy. However, remaining were taking other medicines. Among 274 subjects, 75 (27.4%) women were housewives, 113 (41.2%) working women and 86 (31.4%) were retired.

The DEXA scan values of median in group 1 were changed from 1.00(0) to 3.00(0) and in group 2 were from 1.00(0) to 2.00(1) after treatment (Table I). The manual muscle strength values of median in group 1 were changed from 3.00(1) to 5.00(0) and in group 2 from 4.00(0) to 5.00(0) (Table II). The p-values for DEXA scan and manual muscle strength were less than 0.05 after the application of treatment.

DISCUSSION

This study reveals the role of weight-bearing exercises to osteoporosis in post-menopausal age group.

Table I: Descriptive statistics for DEXA scan of patients before and after treatment.

Treatment plan of the patient	N	Percentiles			P-value
		25th	50th (Median)	75th	
Weight-bearing exercises + medications					
DEXA scan before treatment	137 (50%)	1.00	1.00	1.00	<0.0001
DEXA scan after treatment	137 (50%)	3.00	3.00	3.00	<0.0001
Medication alone					
DEXA scan before treatment	137 (50%)	1.00	1.00	1.00	<0.0001
DEXA scan after treatment	137 (50%)	2.00	2.00	3.00	<0.0001

Table II: Descriptive statistics for manual muscle strength of patients before and after treatment.

Treatment plan of the patient	N	Percentiles			P-value
		25th	50th (Median)	75th	
Weight-bearing exercises + medications					
Manual muscle strength before treatment	137 (50%)	3.00	3.00	4.00	<0.0001
Manual muscle strength after treatment	137 (50%)	5.00	5.00	5.00	<0.0001
Medication alone					
Manual muscle strength before treatment	137 (50%)	4.00	4.00	4.00	<0.0001
Manual muscle strength after treatment	137 (50%)	5.00	5.00	5.00	<0.0001

Osteoporosis is a condition that is gaining its attention as worldwide public health issue causing significant mortality and morbidity. In osteoporosis, bones become fragile and mineral density decreases than normal. It refers to a group of diseases in which bone absorption outpaces bone deposition. Bone becomes incredibly fragile that something as simple as hearty sneeze or stepping off a curb can cause them to break. Though it affects the whole skeleton, but the spongy bones of vertebra, wrist and femur neck are more vulnerable.[15]

The average age of menopause was 55 years which may be earlier. In the present study, women having early menopause developed low bone mineral density at an earlier age. The results of this study correlate with earlier studies that the rate of bone mineral density (BMD) decreases as age passes. This rate rapidly increases after menopause, portentous a window of prospect for preventive measures in the third decade of life to check the conversion of osteopenia to osteoporosis.

Most of the women, professional or non-professional, were doing only routine household work having no walk or gymnasium exercises. The present conducted study is correlated with a past study which reported that the lack of physical activity or weight-bearing exercises is one of the most considered risk factors for osteoporosis.[20] It is suggested that non-pharmacological strategies can be beneficial for post-menopausal women to reduce the risk of fractures, including a balanced diet and regular exercise. Increased weight generates greater force during a fall, twist or turn, resulting in a greater probability of fracture.[21]

In Pakistan, the life expectancy at birth has increased from 41 years in 1950 to 61.9 years in 1998 and is expected to be 72.4 years in 2023. The proportion of elderly and post-menopausal women is on the rise. One of the previous studies indicated a high prevalence of risk factors associated with osteoporosis in the community.[22] In the present series, past 147 subjects were under hormone replacement therapy; however, remaining were taking other medicines. The present study provides some interesting data on the comparison of DEXA scan before and after treatment sores at two different occasions. The resistive training and weight-bearing exercise are linked with a small but significant increase in BMD in women. Besides that, the risks of hip fractures decreases with the help of these exercises.

None of the subjects before treatment had a higher scores than after treatment in group 1. All of them had a higher 135 score after treatment in group 1 and two of them saw no change in their score. In group 2, where treatment option was medication alone, 12 out of 137 showed no change in their score. In both groups (weight-bearing exercises along with medication and medication alone), mean values show that there is improvement in living house situation before and after treatment. The resistance exercise training increases 1.8% and 2.4% bone mineral density for hip and spine in post-menopausal women. It can lead to fall reduction and fracture prevention.[23,24]

In this study, exercise reduced the risk of fall by increasing balance, coordination, and muscle strength. At least 30 minutes of physical activity, such as brisk walk, jogging, cycling, running etc. four days a week is advised for the prevention of osteoporosis. Diagnosis of osteoporosis rests on the measurement of BMD by ultrasound or by DEXA scan. BMD values are converted into T-scores by comparing them with values of normal adult population.[12]

A great majority of Pakistani women do not exercise in normal routine life. Most of them are housewives and sedentary lifestyle makes them exposed to increased risk of heart disease, stroke, diabetes and obesity. Thus, conditioning exercises that work against gravity help the bones to become strong.

CONCLUSION

Physical activity along with medication plays vital role in the treatment of post-menopausal osteoporosis than medication alone.

Disclosure: This is a thesis-based article.

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ORIGINAL ARTICLE

Extended Islanded Reverse Sural Artery Flap for Staged Reconstruction of Foot Defects Proximal to Toes

Muhammad Amin Yousaf, Zain-ul-Abidin, Kamran Khalid, Ata-ul-Haq, Farrukh Aslam Khalid, Faraz Ahmad Tarar, Muhammad Umar Asif and Moazzam Nazeer Tarar

Abstract

Objective: To assess the outcome of extended delayed reverse sural artery flap for reconstruction of foot defects proximal to toes in terms of flap survival, complication and extended area.

Study Design: Case series.

Place and Duration of Study: Jinnah Burn and Reconstructive Surgery Centre, Lahore, from February 2015 to April 2017.

Methodology: Cases who underwent delayed sural artery flap were inducted. Preoperative hand-held doppler was done to confirm the location of perforator. Two suitable perforators were chosen to raise the extended flap by crossing the proximal limit in all cases. The pedicle was kept minimum 3 cm wide and perfusion was assessed. Flap was delayed for one week and vaccum-assisted closure (VAC) dressing was applied over wound. The second surgery was performed after one week. Proximal perforator was clamped and ligated after checking adequate perfusion of flap. Flap was insetted into defect.

Results: Thirty-two patients were reconstructed with delayed reverse sural artery flap. The mean age of the patients was 26.5 $\pm$12.2 years. Twenty-four (75%) patients were males and 8 (25%) were females. Twenty-two (68.7%) cases were degloving wounds after road traffic accidents (RTA), 6 (18.7%) were diabetic foot wounds, 4 (12.5%) sustained injury after falling from height and 7 (21.8%) patients had fracture of metatarsals. Twenty-eight flaps were transferred after one week delay, and only in 4 cases, flap were transferred after two weeks. All flaps survived completely. Complications of infection noted in 3 (9.3%) flaps, 3 (9.3%) flaps showed tip necrosis, 2 (6.2%) flaps undergone epidermolysis and only 2 (6.2%) showed venous congestion.

Conclusion: Delayed islanded reverse sural artery perforator flap is a reliable and versatile option for resurfacing soft tissue defects of lower limb proximal to the toes with lesser complications and extended coverage area.

Key Words: *Sural artery flap. Delayed islanded flap. Epidermolysis. Tip necrosis. Perforator.*

INTRODUCTION

Soft tissue defects of foot, ankle, and lower third of leg have always been a problematic and challenging area for reconstructive surgeons, because of limited mobility and unavailability of skin, relatively poor blood circulation, and unique weight bearing requirements of lower limb.[1] A durable flap with good skin texture, reliable vascularity, good arc of rotation, ease of dissection and minimum donor site morbidity is the most desired option for coverage of such defects.[2]

Although many consider free tissue transfer as gold standard for coverage of large defects with high success rate,[1,3,4] reverse sural artery flap has become workhorse flap for coverage of heel pad and foot defects.[1]

But foot defects, extending beyond ankle joint upto the toes, are difficult to cover with standard reverse sural artery flap. To cover such defects, proximal limit has to be crossed, which ultimately leads to complication of partial or total flap necrosis of 25%.[5] Various sural flap delay procedures have been described by several authors in order to redirect the flow of blood, improve the rate of venous congestion, and minimize the risk of flap necrosis.

Erdmann elevated the flap along with fascia and neurovascular bundle then placed powder-free gloves between fascia and gastrocnemius muscle but without excising the 50% of distal margin of skin. He then transferred the flap after two weeks.[6] In the technique described by Kneser *et al*., the flap was completely raised and was fixed back to its donor site with running sutures for seven to fifteen days. Later, flap was transferred to the defect as a second procedure. This technique allows the chocked vessels to get open and let the flap to become viable on its distal vascular pedicle before transferring the flap.[7,8]

The aim of this study was to determine the technique of flap delay as a modification of the technique described by Kneser *et al*.

METHODOLOGY

This prospectively descriptive study was conducted at Jinnah Burn and Reconstructive Surgery Centre (JB and

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RSC), from February 2015 to April 2017 after approval of Hospital Ethical Review Board. Informed consent was taken from all the patients. All patients presenting in our emergency department with wounds distal to ankle joint and proximal to toes having age between 5 to 55 years, regarding of gender, were included in this study. Patient sustaining simultaneous injury to leg or around the ankle with age group below 5 and above 55 years were excluded.

All wounds were assessed by a senior consultant. Preoperatively the age, gender, comorbidities, mode of injury, timings and extent of injury were noted. X-ray of foot and ankle was obtained to see the underlying fracture of tarsal and metatarsal bones. Preoperative hand-held doppler was performed in all case to mark the perforator. Initial debridement was performed under tourniquet control by consultant plastic surgeon, and defect geometry was evaluated. Extended reverse sural artery was raised at the same time of debridement. Flap size was kept 1cm larger than the defect by anticipating flap edema and swelling. Distal first technique was utilized and identification of perforator performed first in all the cases. Two suitable perforator (5, 7 or 10 cm) were chosen to raise the flap. After identification of perforators, proximal flap was raised. Extended flap was raised by crossing the proximal limit in most of the cases. Flap was raised by keeping the pedicle minimum 3 cm wide and perfusion was assessed at the end of procedure after deflating the tourniquet. Flap was delayed for one week and vaccum assisted closure (VAC) dressing was applied over wound. Patient was discharged after 24 to 48 hours after evaluating flap condition (by color, temperature and capillary refill) and readmitted for second surgery after one week.

In second surgery flap was raised, proximal perforator was clamped for 10 minutes, and vascularity was assessed. After adequate perfusion, proximal perforator was ligated and flap was islanded and insetted into the defect, based on single perforator.

The cases in which perfusion seemed to be inadequate, proximal perforator was ligated and flap was strategically delayed for one more week. After two weeks, flap was transferred to the defect safely.

The collected data was entered and analyzed in computer program SPSS 20. Quantitative variables like age was calculated in terms of mean ± standard deviation and qualitative variables like gender and complication were calculated for frequencies and percentages.

RESULTS

Over the period of 35 months, a total of 32 cases were enrolled who underwent delayed reverse sural artery flap. Mean age was 26.5 ±12.2 years. Twenty-four (75%) patients were males and 8 (25%) were females. Twenty-two (68.7%) cases were degloving wounds after RTA, 6 (18.7%) were diabetic foot wounds, and 4 (12.5%) sustained injury after falling from height. Seven (21.8%) patients had fracture of metatarsals that was fixed by passing k-wires. Twenty-eight flaps were transferred after one week delay, and only in 4 cases, flap were transferred after two weeks. All flaps survived completely. Complications of infection noted in 3 (9.37%) flaps, 3 (9.37%) flaps showed tip necrosis, 2 (6.25%) flaps each underwent epidermolysis and venous congestion.

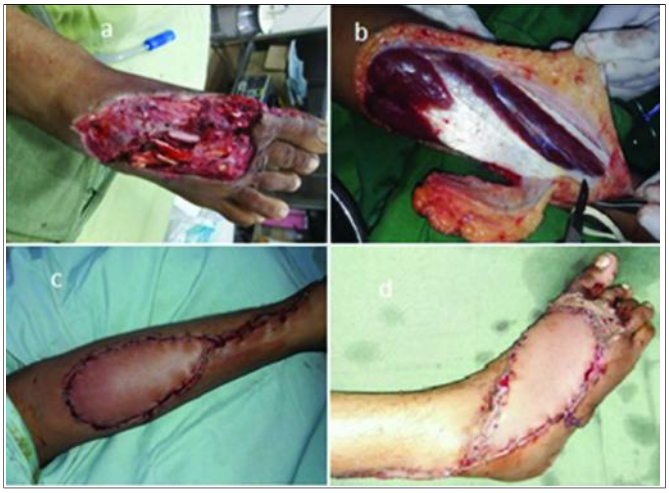

Figure 1: Foot defect covered with islanded reverse sural flap. (a) Foot defect. (b) Perop picture. (c) Flap insetted back for delay. (d) 2nd stage insetting of flap over defect.

Complications were dealt accordingly; while infection needed daily wash with normal saline, and culture-specific intravenous antibiotics given, venous congestion was minimum that settle down with leg elevation only. The flaps with necrosed tip, debridement of necrosed tip done, flap was advanced and resutured to the defect margin while in one, we did STSG (split thickness skin graft) over flap defect.

All cases showed uneventful recovery with good graft take over donor site. None complained of any painful neuroma. Two patients (6.2%) were concerned about sensory loss over lateral aspect of leg. Esthetically, 3 patients (9.3%) found the donor site appearance of concern, while rest 29 patients (90.6%) were not concerned.

DISCUSSION

In this study, road traffic accidents (68.7%) are the main causes of soft tissue defects in distal third leg, ankle, heel and dorsum of foot, which were similar to that seen in another study done by Chen *et al*.[9] For larger defects around ankle and mid foot, free tissue transfer is considered as standard option. However, it needs expertise, time limitation, and infrastructure requirements.[12] Due to unavailability of microsurgery facilities in various centers of Pakistan, Sural flap has became a

workhorse flap for resurfacing of these defects.[12] But defects located around midfoot and forefoot are difficult to cover by this flap because of limited reach of flap.[13,14]

In order to extend the reach of flap, proximal limit has to be crossed which is associated with increased flap complications in terms of distal flap necrosis 36% and 25%,[11,15] venous congestion and epidermolysis (11.2%),[16] or total flap loss19% and 9.5%.[3,15]

To overcome this issue, flap delay procedure offers a simple solution by which flap length can be increased and distant defects can be covered. A delay phenomenon, also referred to vascular delay, describes the observation that a flap undergoing partially ischemic, will undergo neovascularization and enhance its vascularity through a mechanism of increased vessel size, and reorientation.[17] It also opens up choke vessels, increasing perfusion to the most distal part of the flap preventing necrosis.[18] Yang *et al*. introduced a short incision technique to harvest the adipo-fascial variety of this flap. This has improved the cosmetic issue of donor site morbidity but did not address the issue of venous congestion and other complications.[19]

Different methods of delayed sural artery has been described, especially by Erdmann *et al*.[6] and Kneser *et al*.[7] The presently described method is simpler and superior to other methods because of increased flap survival and extended coverage area.

Complications rate, associated with this flap, has been reduced. The most common complication was the tip necrosis which has been reduced from 25-36% to 9.3%, venous congestion decreased from 11.2% to 6.2%, epidermolysis 11.2% to 6.2% and total flap loss of 9.5% to 0% in this study.

CONCLUSION

The distally based delayed islanded sural artery perforator flap is a versatile option for the reconstruction of soft tissue defects of the distal one-third of leg, ankle, heel, and upto the toes. By opting for delay phenomenon, while crossing the proximal limit of flap and based on single-perforator, flap can be made more reliable with fewer complications and extended coverage.

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ORIGINAL ARTICLE

Distally Based Medial Hemisoleus Flap: Reliable Option for Distal Tibial Wounds

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ABSTRACT

Objective: To determine the success (flap survival as a whole without necrosis or dehiscence up to two months as judged clinically) of distally based medial hemisoleus muscle flap for the coverage of distal tibial defects.

Study Design: Case series.

Place and Duration of Study: Jinnah Burn and Reconstructive Surgery Centre, AIMC, Lahore, from July 2014 to July 2017.

Methodology: Patients with middle and distal third tibial defects were enrolled and stratified according to the site of the wound in middle or distal third of tibia. Soft tissue coverage was provided with distally based medial hemisoleus muscle flap on which split thickness skin graft was applied. Postoperatively, patients were followed-up after one week of discharge and then fortnightly for at least 2 months. Outcome variable was taken as flap success.

Results: Out of 37 cases, flap was successful in 33 patients as complete flap survived with primary wound healing. Partial flap necrosis without dehiscence was seen in 3 cases and partial necrosis of flap with dehiscence in only one case that required another surgery for the defect. Complete flap loss was not seen in any case.

Conclusion: Distally based medial hemisoleus muscle flap is reliable coverage option for middle and distal third of tibial defects.

Key Words: *Distally based medial hemisoleus muscle flap. Distal medial hemisoleus flap. Distal tibial wound. Tibial defect. Dehiscence. Flap survival.*

INTRODUCTION

Distal tibial wounds usually have associated bone fracture, that requires well vascularized soft tissue coverage for early healing. The gold standard technique for coverage of large defects on distal tibia is application of free flap.[1,2] At present, the proper management of soft tissue defects of $\leq$50 cm^2 in distal tibia with minimum surgical intervention is under discussion.[3] Coverage of relatively small distal leg wounds is still a debatable issue, too. Both local and free flaps can be used. Advocates of free tissue transfer argue that one should not injure already compromised leg, which is the case in local tissue transfer. Furthermore, use of local tissue for reconstruction will make stump formation difficult, if the patient ends up with amputation. However, where free flap is not an option due to patient factors or logistics, there must be a local flap option such as reverse sural, posterior tibial artery perforator flap, and distally based hemisoleus muscle flap.

Among the local flaps, reverse soleus muscle flap (distally based soleus muscle flap) is in common use to cover middle and lower third of the leg wounds. However, sacrifice of this main ankle flexor and its limited arc of rotation, this flap has always been in debate among reconstructive surgeons.[4] So, they have been working on using part of the muscle in order to avoid aforementioned disadvantages of using entire soleus muscle. Greater arc of rotation of medial hemisoleus muscle makes insetting of this flap much easier as compared to complete soleus muscle. Furthermore, using only medial head preserves the prime function of ankle flexion. These advantages make this flap a good coverage option for suitable defects of middle and lower third of leg.[5]

The proximally based medial hemisoleus muscle flap has limited reach to a more distal defect on leg because it is not large enough at its distal end to cover a wound of $\geq$50 cm^2,[5] This obstacle might be overcome by using distally based pedicled hemisoleus muscle flap for treating middle and distal third tibial wounds.[6]

The objective of this study was to determine the success of distally based medical hemisoleus flap for coverage of distal tibial defects.

METHODOLOGY

The study was conducted at the Jinnah Burn and Reconstructive Surgery Centre, AIMC, Lahore from July 2014 to July 2017. Patients of both genders having wounds ($\leq$50 cm^2) exclusively of middle and lower third of tibia with underlying bony fracture were included in the study. Patients with history of polytrauma, high velocity injuries, diabetes mellitus, and peripheral

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vascular disease were excluded. Patients fulfilling the inclusion criteria were recruited from the outpatient and emergency departments of plastic surgery along with referral from other departments of Jinnah Hospital, Lahore. Bony fixation was done by the Orthopedic Department of the institute, mostly by external fixator.

The wound was assessed in detail and stratified according to the site of the wound in middle or distal third of tibia. Perforators at 5 cm, 7.5 cm and 10 cm proximal to the ankle joint were identified with hand-held Doppler preoperatively. After adequate debridement, incision was given 2 cm parallel and posterior to medial border of tibia. The incision was extended into already existing wound, both distally and proximally. The soleus muscle was identified and separated from gastrocnemius muscle. Medial border of the muscle was identified and elevated from the deep flexor tendons. Distal perforators entering into the flap were identified. Perforators limiting the arc of rotation were divided. It was tried to preserve as much perforators as possible. Muscle was divided proximally including the territory of perforator next to the preserved perforator. The medial half of the muscle was divided from the lateral half. Muscle flap was rotated to cover the defect and stitched. The donor site was closed primarily over suction drain. Split thickness skin graft was applied over the muscle flap. Below-knee back slab was applied and the limb was elevated postoperatively. The first dressing was changed on the fourth post-operative day. If uneventful, patients were discharged on the sixth day. All patients were followed-up after 1 week of discharge and then fortnightly for two months. On follow-up visits, patients were assessed for flap success, which was taken as flap survives as a whole without necrosis or dehiscence up to two months.

The collected data was entered and analyzed in computer program SPSS 10. Quantitative variables like age for mean ± standard deviation and qualitative variables like gender and success of primary flap healing were calculated for frequencies and percentages.

RESULTS

The selected 37 patients were in the age range of 12 to 65 years (Table I). Most of the patients were in the 3rd and 4th decade of life. Mean age was 30 ±9.78 years. Out of 37 patients, 29 (78.38%) were male and 8 (21.62%) were female patients with male to female ratio of 3.6:1.

Thirty-one patients (83.7%) had Gustilo type IIIb fractures after road traffic accidents, four (10.8%) cases were due to fall from height and one (2.7%) case each had machine crush injury and fall of heavy object on the leg. Out of the 37 patients, 8 (21.62%) had wound on the middle third of tibia, 16 (43.24%) at junction of middle and distal third and 13 (35.14%) on distal third of tibia.

Primary healing was achieved in 33 (86.8%) cases. There was partial necrosis of flap without dehiscence in three (8.1%) cases and partial necrosis with dehiscence in one (2.7%) case. All three cases of partial necrosis without dehiscence healed by secondary intension satisfactorily. Only one patient developed partial necrosis with dehiscence, requiring a second procedure for wound coverage. There was no total loss of the flap in the study. All patients, ultimately, achieved wound coverage with good cosmesis due to lesser bulk of the muscle (Figures 1 and 2).

Table I: Statistical data of patients.

Mean age (years)	30 ±9.78
Gender	
Male	29 (78.38%)
Female	8 (21.62%)
Mode of injury	
RTA*	31 (83.7%)
FH**	04 (10.8%)
FHO***	01 (2.7%)
MCH****	01 (2.7%)
Site of wound on tibia	
Middle third	08 (21.62%)
Junction	16 (43.24%)
Distal third	13 (35.14%)
Flap success	
Complete survival	33 (89%)
Partial necrosis without dehiscence	03 (2.7%)
Partial necrosis with dehiscence	01 (2.7%)
Total necrosis	0

**Road traffic accident; **Fall from height; ***Fall of heavy objects; ****Machine crush injury.*

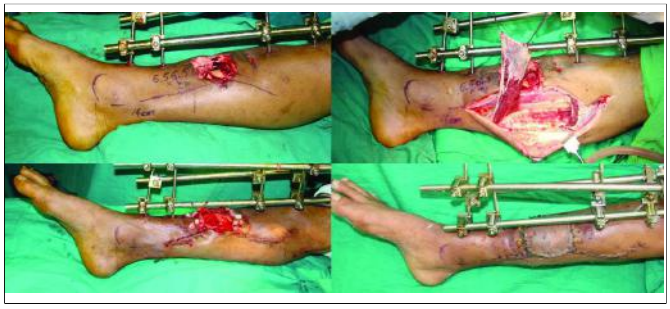

Figure 1: Picture showing defect, flap elevation, flap insetting and final picture (3 weeks follow-up).

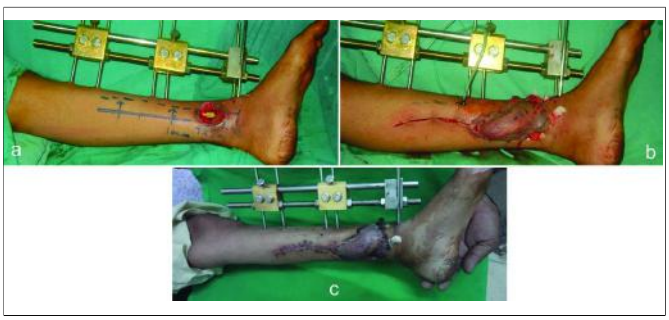

Figure 2: Distally based medical hemisoleus muscle flap: (a) preoperative, (b) immediate postoperative, (c) 6 weeks follow-up.

DISCUSSION

Due to unregulated traffic and insufficient safety measures, bike riders and pedestrians tend to be more exposed to injuries resulting in high occurrence of open fractures of lower extremity. Limb salvage cannot be guaranteed without a well vascularized soft tissue coverage of open wound in lower leg. Although, free flap transfer is considered standard option, however, some of the patients may not be candidate for such a long and extensive surgery accredited to their comorbid conditions. Furthermore, some institutes may not be well equipped for microsurgical procedures. Also wounds may be too small for free flaps. Based on previously listed facts, many authors believe use of local tissue for coverage is an acceptable option for smaller distal tibial wounds.[7,8]

Tobin reported use of distal medial hemisoleus muscle flap for first time,[9] and then by others. However, clinical utilization of distally based hemisoleus muscle flap for reconstructing defects of lower third of leg has not been advanced.[10,11]

Soleus muscle is the prime ankle plantar flexor and stabilizes ankle joint in ambulation by opposing dorsiflexion. Compensatory systems for soleus functional loss in ambulation include lessened frontward lean, earlier opposite heel strike, shorter steps and activation of synergistic muscles.[12,13] Earlier studies on the anatomy of this muscle illustrate its bipenniform structure and separate vascular supply to both lateral or medial muscle bellies.[10,11] These significant features allow to raise only half of the muscle as a flap by dividing it longitudinally across the midline muscle raphe. As the lateral half of muscle is left *in situ*, the prime function of plantar flexion of ankle is preserved. Furthermore, the arc of rotation is much more as compared to full muscle when taken as flap. Perforators arising from posterior tibial artery, supply the medial muscle throughout its length. Proximally or distally based medial hemisoleus muscle acts as a reliable flap due to presence of constant perforators.[14,15]

The distally based medial hemisoleus flap is based routinely on distal two or three major perforators arising from posterior tibial vessels. The literature shows that perforators from posterior tibial artery usually arise at 4 to 26 cm proximal to intermalleolar line.[16] Ignatiadis *et al*. found a perforator frequently present at level of medial malleolus that can easily be traced pre-operatively by hand-held Doppler.[17] It has also been demonstrated that proximal lateral half of muscle also contribute to its circulation in an antegrade fashion. If this flap is turned about 180^0, it can cover a large area on tibia.[18]

This flap should not be used in large defects of lower tibia, especially in wounds greater than 50 cm^2. If the middle or lower third of muscle is traumatized, it should not be considered as a flap. In lower limb reconstruction, angiogram can be applied in preoperative workup when there is suspicion of injury to vessels. The authors regularly use hand-held Doppler while planning for a distally based medial hemisoleus muscle flap. Using a Doppler, major perforators are identified. The pivot point of flap is designed according to position of perforators. Perforators at or close to pivot point and the ones distal to it are preserved during dissection. The pivot point of the flap will be on the most proximal perforator that was preserved during dissection. This pivot point will define the reach of the muscle. Generally, this flap can reach up to the level just proximal to the medial malleolus.

In this study, complete survival of flap was seen in 33 (86.8%) cases. This high rate of success was attributed to proper patient selection and adequate preoperative planning. These results are comparable to a study conducted by Clark *et al*., who used distally based hemisoleus flap to cover distal tibial wounds in seventeen patients and found primary healing in 94.12%. Only one patient suffered necrosis of distal tip that was treated with debridement and vacuum assisted closure.[19]

The distally based hemisoleus flap was successfully able to cover wounds up to <50 cm^2 in the middle and distal tibia. In case of wounds on distal third of leg, usually the flap was based on last one or two perforators, consistently found at 5 and 10 cm proximal to the medial malleolus in most of the cases. Flap was based on more proximal perforators when used for coverage of middle tibial wounds. Partial necrosis seen in three cases was insignificant and was treated conservatively that healed satisfactorily by secondary intension without need for any other intervention. Only one case had partial necrosis with wound dehiscence leading to exposure of fracture segment that was managed by another local flap. The flap was unable to cover the defect >50 cm^2 and also should be cautiously used in heavy smokers as distal necrosis was seen in such cases. This is also consistent with a previous study at the University of Kentucky, USA where out of the eight patients, partial flap necrosis was encountered in two patients (one paraplegic and other heavy smoker) showing success rate of 75%.[18]

In the present experience, partial necrosis was seen in high velocity injuries even though the muscle was looking viable on exploration. In high velocity injuries, there is possibly micro trauma to the muscle itself and is not an ideal option in such cases.

At the point when the measure of a tibial injury in the distal third of the leg is under 50 cm^2 in low velocity damage, reverse hemisoleus muscle flap can give enough tissue to deal with the injury reliably with good contour of leg, unless a part of the flap has already been damaged.[7,13,18,20,21]

Another point of concern is using soleus muscle as flap which is jeopardizing the ankle function, as this muscle is prime ankle flexor. This problem was solved by using part of muscle only, i.e. medial hemisoleus muscle. By this technique, the remaining muscle can perform ankle flexion efficiently. The fact that using part of muscle does not interfere with ankle movement is well known and was demonstrated in a study by Tobin.[9] No problem was found regarding movement of ankle in any of the cases, that is consistent with the previous study. The gait was assessed for any limp or weakness while walking. Free flap should in any case be considered for a larger wound in lower third of leg or for extensive wounds when other local flaps are in zone of trauma.

CONCLUSION

Although, free flap is still considered the gold standard for extensive wounds, distally based medial hemisoleus muscle flap is reliable option for coverage of middle and distal third tibial wounds. Limitations for the use of this flap is high velocity injuries, wound larger than 50 cm^2, and local trauma to the muscle itself.

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REVIEW ARTICLE

Proteomic Profile of Lymphoid Leukemia

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ABSTRACT

Lymphoid (or lymphocytic/lymphoblastic) leukemia, one of two major types of leukemias (lymphoid and myeloid), is divided into two subtypes, acute lymphoid leukemia (ALL) and chronic lymphocytic leukemia (CLL), depending on the maturation stage and speed of multiplication of the bone marrow lymphocytes. Early diagnosis and treatment can make the difference between life and death. Advancements in the field of proteomics may allow the development of early biomarkers and more effective agents to combat both these types of cancer, and to better understand the underlying mechanisms of the disease. The aim of this review was to elucidate the pathophysiology of lymphocytic leukemia using cancer proteomics techniques from 2007 until 2017. Only relevant original research articles archived in the Science Direct, PubMed and/or the Google Scholar databases within this period were included, which were a total of 30 studies. The role of proteomes in the detection, diagnosis and treatment of ALL and CLL was examined separately. Overall, the findings of this study confirm the viability of proteome analysis in profiling lymphocytic leukemia; and highlight novel leukemia biomarkers and potential therapeutic targets.

Key Words: *Lymphoid leukemia. Proteomics. Profiling. Acute lymphoid leukemia. Chronic lymphoid leukemia. Biomarker. Therapeutic target. Leukemia incidence.*

INTRODUCTION

Leukemia is a cancerous disorder of the hematopoietic cells that originate in the bone marrow. The classification of leukemias is based upon multiple criteria, including morphology, karyotype, cytochemistry, and a limited immunophenotype of 10-15 cluster of differentiation antigens.[1] Unlike myelogenous leukemia, in which longer-lasting abnormal granulocytes and monocytes accumulate in the blood, lymphoid or lymphocytic/lymphoblastic leukemia is characterized by the fast-multiplication of immature acute lymphocytic leukemia (ALL), and the maturation and differentiation of long-lived abnormal chronic lymphocytic leukemia (CLL) lymphocytes in the circulation. Due to its rapid progression, treatment for ALL must be started immediately. CLL, on the other hand, might take years before it overruns the natural defense mechanisms of the body. However, aggressive forms have also been characterized.[2] The prognosis is very variable for CLL as some patients end up having almost normal life expectancy even without treatment, while others may die from therapy-resistant form within a year or so.[3]

Proteomics techniques allow for disease-related discrepancies to be investigated on a genome-wide scale.[4] A large number of cancer-related changes present themselves at the functional level only, like sub-cellular localization, post-translational modification, protein cleavage and protein-protein interaction disorders.[5] Hence, cancer patients with no typical/known cytogenetic mutation cannot have a valid prognosis with the use of morphological, immunophenotyping or genetics techniques alone.[6] Clinical applications of proteomic techniques and markers have been impeded by a lack of knowledge regarding the proteomic profiles of the different types and subtypes of leukemia.[7]

Acute lymphoid leukemia: ALL is characterized by the accumulation of immature lymphoid neoplasm that immunophenotypically and morphologically resemble B- and T-progenitor cell lineages in the bone marrow and peripheral blood.[8] The internationally recognized four subtypes are Precursor B-cell (PCB) ALL (most common), Precursor T-cell ALL, Burkitt-type ALL and BCR-ABL fusion ALL. ALL affects both children and adults, with an incidence peak between the ages of 2 and 5 years, making up nearly 26% of all pediatric cancers.[9] A total incidence of 3 to 4 per 100,000 cases is reported annually among children in the United States, with the highest incidence rate for those aged 1 to 4 years.[10] The prevalence of ALL among adults is less than one per 100,000 cases per year; males are at a slightly greater risk than females, regardless of age.[8]

Several studies have reported strong association with predisposing genetic disorders, such as Fanconi anemia, Bloom syndrome, and Down syndrome.[11,12] Clinical symptoms correlate well with the degree of bone marrow failure and leukemic cell burden.[9] Besides, more than half of ALL patients present with fever caused by leukemia or infection, bleeding diathesis due to thrombocytopenia and fatigue, pallor and lethargy as a result of anemia.[8,13] Substantial progress has been

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made in terms of treatment approaches for pediatric ALL, yet therapy remains inadequate in some cases.[14]

Choronic lymphoid leukemia: CLL encompasses a group of mature lymphoid neoplasms in the bone marrow, peripheral blood and secondary lymphoid organs, such as the lymph nodes and the spleen.[11] The disease previously described as T-CLL is now referred to as T-cell prolymphocytic leukemia.[1] According to the Surveillance, Epidemiology and End Results (SEER) database on the US population between the years 2009 and 2013, CLL is more common among adults than children, with as many as 28% of the cases being diagnosed in those aged 65 to 74 years. CLL accounts for 1.1% of all new cancer cases annually, with an incidence of 4.6 per 100,000 for both genders. Men are at a significantly higher risk than women. B-CLL is characterized by high levels of CD5+ B lymphocytes in the peripheral blood that progressively infiltrate the bone marrow and the secondary lymphoid tissues.[15] Western countries record a significantly higher incidence rates annually than most Asian and African countries.[16] The traditional staging system and prognosis tools in every stage of CLL rely on clinical features and have been known to be occasionally unreliable.[17] Early in the disease course, most CLL patients may present with asymptomatic lymphocytosis detected merely by incidental blood tests. As the disease progresses, patients have been shown to develop lymphadenopathy, hepatosplenomegaly and bone marrow infiltration, leading to bone marrow failure with anemia and thrombocytopenia.[18]

This review aimed to summarize the advances made in the field of lymphoid leukemia profiling from 2007-2017. The findings cited here will hopefully highlight novel proteomic treatment, prognosis and monitoring targets that may be exploited in the future for a better overall healthcare system for leukemia patients.

METHODOLOGY

The review methodology adopted in this paper focused on relevant works indexed by Google Scholar, PubMed and/or Science Direct databases. Keywords, including lymphoid leukemia and proteomics, were used to search publications between the year 2007 and 2017. Books, conference proceedings and reviews were excluded. Proteomic research aimed at understanding the mechanisms of action or demonstrating the efficacy of therapeutic agents was taken as outside the scope of this review. On the other hand, studies aiming to determine the expression levels of pre-selected proteomes in ALL or CLL cells were included. Overall, it included 30 original research articles on CLL and ALL, divided into the above two categories.

Findings: A total of 15 articles were found to be published in the past ten years on the profiling of acute lymphoblastic leukemia. The same was also available on the profiling of the chronic form of the disease. A list of the primary objectives, major findings, and study limitations of all publications cited in this work is presented in Tables I and II. Most studies were conducted using primary samples derived from patients, such as bone marrow aspirates, peripheral blood, serum, and CSF. Only two studies concerned with the profiling of ALL reported *in vitro* results obtained in leukemic cell lines without the use of primary cells for validation; and only one study on CLL profiling reported findings obtained in mice alone. Works related to ALL originated from various institutions worldwide, located in 14 different countries: The Czech Republic, the UK, the USA, the Netherlands, India, China, South Korea, Greece, Singapore, Italy, Spain, Belgium, Russia and Australia. However, almost half of the proteomics studies on ALL were found to have been carried out in a total of three countries: China (three publications), USA and UK (two publications each). Being a disease with significantly higher prevalence in the Western world, it is not surprising that 10 out of the 15 studies cited here on

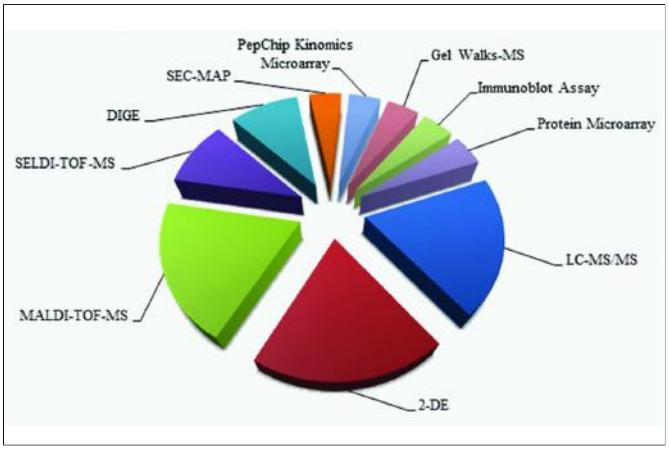

Figure 1: Proteomic techniques utilized for acute lymphoid leukemia profiling in the past ten years (2007-17) according to the frequency of use as primary screening tools.

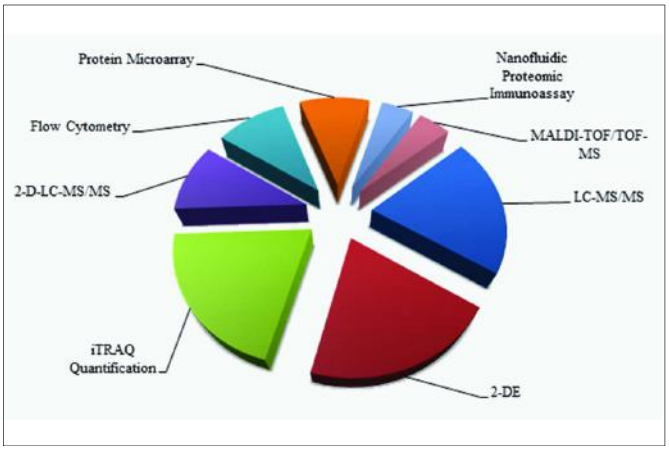

Figure 2: Proteomic techniques utilized for chronic lymphoid leukemia profiling in the past ten years (2007-17), according to the frequency of use as primary screening tools.

Table I: Summary of studies investigating the proteomic profile of acute lymphoid leukemia.

Target	Methodology	Major findings	Limitations	Reference
To validate SEC-MAP as a high-throughput proteomic technique.	SEC-MAP for bone marrow aspirates of 57 pediatric patients.	Significant ($P < 0.05$) correlation between SEC-MAP and flow cytometry. DBN1, PAX5 or PTK2 ove-rexpressed in β–cell precursor ALL. LAT, SH2D1A or STAT5A over-expressed in T-cell ALL.	Absence of optimal antibodies for all tested markers. False negative due to suboptimal spatial molecular arrangement in protein complexes.	[19]
To detect key differences between GC sensitive/ resistant ALL cell lines.	2DL-MS/MS. PreB 697 cell line and its GC-resistant clones (R3C3, R3G7, R3F9, R4C10 and R3D11).	Low expression of PAX5 (resulting in less CD19) in GC-resistant ALL cell lines JNK activation high in GC-resistant ALL cells. GC-Resistance reversible with JNK inhibition.	The data was not obtained in primary cells.	[20]
To identify potential markers for localized CNS thrombosis in L-asp treatment.	LC-MS/MS using CSF of 5 pediatric patients.	Antithrombin III and plasminogen levels decreased markedly prior to a thrombosis event.	The results need to be confirmed using a larger cohort.	[21]
To showcase kinome profiling proteomics as a useful tool to a better understanding of ALL mechanisms.	PepChipTM Kinomics microarray for PB & BM of 20 patients.	RON expression critical to ALL cell survival.	*In vitro* kinomics may not reflect in vivo phosphorylation mechanisms due to the loss of intrinsic spatial arrangements. Utilization of short peptide sequences in phosphorylation.	[22]
To conduct a comparative study of B-lymphocytes between ALL patients & healthy.	2-DE and DIGE for PB & BM of 27 patients & 10 control subjects.	79 differentially regulated proteins participating in proteostasis; cytoskeletal organization; cellular energy and redox homeostasis; signal transduction; RNA splicing; and regulation of transcription.	The findings may not account for expression discrepancies between ALL subtypes.	[23]
To establish a proteomic classification model capable of accurately differentiating all AL types.	SELDI-TOF-MS for BMA of 151 patients & 38 control subjects.	A proteomic signature identified for each subtype of AL (M1593, M2121, M2536 & M1016 for APL, AML-Gra, AML-Mon & ALL, respectively). A decision tree presented capable of defining the origin and proteomic-based subtype of leukemia blasts.	The study did not explore expression discrepancies between ALL subtypes.	[7]
To determine the expression levels of three splicing factor proteins (NSRP70, SRSF1, and HNRNPA1) between AML and ALL.	Semiquantitative I A & IFS for BMA of 48 AML and 23 ALL patients & 116 control subjects.	NSRP70 showing excellent diagnostic accuracy (92%) as a marker in ALL. The highest molecular weight form of NSRP70 signaling adverse genetic abnormalities in AML. NSRP70, SRSF1 & HNRNPA1 more prevalent in ALL than AML; and weakly represented by their mRNA expression levels.	A larger sample size is required to confirm some of the findings, especially in terms of the disappearance of the splicing factors upon remission.	[24]
To examine protein expression levels using comparative proteomics in primary ALL vs. healthy lymphocytes.	2-DE followed by MALDI-TOF-MS for BMA of 15 ALL and 15 control pediatric subjects.	Fifteen differentially expressed proteins. Glutathione S-transferase P and Prohibitin dramatically upregualted in ALL patients. Low expression of Peroxiredoxin 4, 60s acidic ribosomal protein P0; Cytoplasmic actin; Pyridoxine-5 ' -phosphate oxidase; and Triosephosphate isomerase 1 in ALL patients.	The protocol did not allow for the identification of nearly half of the differentially expressed proteins detected.	[25]
To identify differentially expressed proteomes in pediatric ALL patients that could be markers for disease aggressiveness.	2-DE followed by MALDI-TOF-MS for PB & BM of 45 children with ALL and 7 controls.	Vitamin D-binding protein and S10A9 up- and down-regulated respectively in ALL patients. Gelsolin and afamin down-regulated in high and low risk patients, respectively. Ceruloplasmin, clusterin, PT, ?-1-m/b p, and F-3 upregulated in high-risk patients. Kininogen-1 upregulated in low-risk patients.	The findings may not account for expression discrepancies between ALL subtypes.	[26]
To demonstrate the applicability of label-free alignment-based quantitative LC-MS/MS data to the elucidation of protein markers using AML and ALL models.	LC-MS/MS for BMA. Primary cells isolated from 4 AML and 5 ALL patients, as well as 8 control subjects.	10, 14, and 3 proteins differentiated ALL from CD34+, ALL from AML, and AML from CD34+. Nicastrin up-regulated in AML only.	The results need to be confirmed using a larger cohort.	[27]
To identify prognostic markers to predict prednisolone treatment outcome in pediatric ALL patients.	2-DE followed by MALDI-TOF/TOF-MS for BMA. REH, 697, Sup-B15, RS4;11 cell lines and 43 pediatric ALL.	77 vs. 17 differentially expressed proteins in prednisolone-sensitive vs. -resistant cells. In sensitive cells, western blot confirmed PCNA, cofilin1, PA28? (downregulated) and VDAC1 (upregulated). PCNA clinically proved to be capable of predicting treatment outcome.	PCNA usefulness was confirmed in patients with B-lineage ALL. Further confirmation is necessary in more patients spanning the less common sub/types of ALL.	[28]
To reveal the underlying mechanisms of ALL invasion of the CNS prior to and post relapse.	Gel Walks & MS for BMA. SD1, SupB15 and REH cell lines.	94 upregulated and 81 downregulated proteins in SD1 cells. AEP partially involved in ALL invasion of the CNS. AEP, RAC2 and ICAM1 necessary for CNS invasion.	Trypsin digestion was not optimal for AEP quantification as it created peptides that were undetectable using MS.	[29]
To profile the phosphorylation status of 92 signaling proteins in childhood acute lymphoblastic leukemia	RPPM for BMA of 118 pediatric patients and SEM, RS4;11, REH and NALM6 cell lines.	Cyclin E, Annexin 2, AMPKb (S108) and AMPKα over-activated in case of MLL-rearrangement. AMPK pathway plays a major role in resistance to chemotherapy via BCL-2 activation. LCK kinase hyperactive in PPR patients and suppressed in PGR patients. Cyclin E is a marker for disease aggressiveness.	No healthy control subjects were used.	[14]
To identify potential protein biomarkers for pediatric ALL	SELDI-TOF-MS followed by LC-MS/MS for 94 ALL and 30 AML, and 54 controls.	PF4 and PBP are down-regulated in ALL. Two fragments of C3a are up-regulated in ALL.	Confirmation in the test set was not made for all differentially expressed proteins.	[30]
To compare the cytosolic protein profiles of four leukemia and lymphoma types (APL, CLL, T-cell ALL, and Burkitt's lymphoma)	DIGE coupled with MALDI-MS. HL-60, MEC1, CCRF-CEM, and Raji cell lines.	Myeloperoxidase, phosphoprotein 32 family member A, ras related protein Rab-11B, protein disulfide-isomerase, ran-specific GTPase-activating protein, nucleophosmin and S10A4 differentially expressed in HL-60 only. Ezrin overexpressed in Raji cells only. C-1-tetrahydrofolate synthase, elongation factor 2, ?- and ?-tubulin, transgelin-2 and stathmin differentially expressed in both Raji and MEC1. Gamma-Enolase upregulated in both MEC1 and CCRF-CEM Ubiquitin-conjugating enzyme E2 N overexpressed in both HL-60 and CCRF-CEM.	No primary samples or control were used.	[31]

SEC-MAP = Size exclusion chromatography-microsphere-based affinity proteomics; ALL = Acute lymphoid leukemia; AML = Acute myeloid leukemia; IFS = Immuno-fluorescence staining; IA = Immunoblot Assay; PB = Peripheral blood; BM = Bone marrow; DIGE = Difference in-gel electrophoresis; BMA = Bone marrow aspirate; CSF = Cerebrospinal fluid; RPPM = Reverse phase protein microarray; AL = Acute leukemia; α-1-m/b p = alpha-1-microglobulin/bikunin precursor; F-3 = Ficolin-3; PT = Prothrombin; L-asp = L-asparaginase.

CLL were found to have originated from USA and UK (five publications each). The search further indicated that over half of the publications on ALL profiling were published in the period between 2013 and 2015. As depicted in Figures I and 2, quite a number of techniques have been used over the past decade to elucidate the proteomic profiles of ALL and CLL. The most commonly used methods for ALL profiling were

Table II: Summary of studies investigating the proteomic profile of chronic lymphoid leukemia.

Target	Methodology	Major findings	Limitations	Reference
To detect proteomic differences in Burkitt's lymphoma and CLL.	LC-MS for E?-myc and E?-TCL1 mouse models, in addition to wild-type mice.	695 peak intensities upregulated in both types of leukemia. High levels of methylating enzymes in E?-myc tumors. Cytoskeletal components downregulated in E?-myc tumors. ER stress response proteins and two IL-5 subunits upregulated in E?-TCL1 tumors. IL-5 implicated as a factor of tumorigenesis via TCL1-mediated activation of the AKT pathway.	The findings were not obtained in primary samples.	[32]
To examine the impact of del(13q14), del(17p13.1), trisomy 12, and NOTCH1 mutations on the expression of 224 signaling proteins.	Panorama Cell Signaling Kit for PB of 14 CLL patients & 63 controls.	Del(13q14) linked to the overexpression of JUN and cyclin A, B1 and D1. Del(13q14) linked to the downregulation of PRKC and RAF1. RAF/MEK downregulated in cells with del(17p13.1). del(17p13.1) linked to the upregulation of CDKN2A, and JNK. NOTCH1 associated with the upregulation of CDK4 and the downregulation of cyclin B1 and synuclein.	The results need to be confirmed using a larger cohort.	[33]
To identify biomarkers for progressive CLL.	iTRAQ labeling coupled with 2D-LC-MS/MS for PB of 77 CLL patients.	84 differentially expressed proteins between stable and progressive CLL. 12 differentially expressed molecules corresponded to CLL subtypes and prognosis.	No control was used.	[34]
To determine whether the expression of ZO-1 reflected the progress of CLL	Flow cytometry & western blot for PB of 113 CLL patients & 50 controls.	ZO-1 and Cx43 downregulated in CLL patients. Low ZO-1 levels associated with poor prognosis. ZO-1 gene silenced in malignant CLL cells.	The exact role of ZO-1 in CLL was not extensively investigated.	[35]
To investigate the impact of IGHV mutation on the pathogenesis of CLL	iTRAQ-MS quantification for PB of 138 CLL patients.	35 proteins downregulated in the unmutated IGVH group. Unmutated cells more likely to cause lymphadenopathy in patients.	Confirmation of the results in the larger cohort was not made for all differentially expressed proteins.	[36]
To detect proteomic differences of prognostic value in B-cell CLL	2-D nano LC coupled with MALDI-TOF/TOF-MS for PB of 39 CLL patients	728 proteins identified. Thyroid hormone receptor-associated protein 3, T-cell leukemia/lymphoma protein 1A, and S100A8 linked to high-risk CLL. Myosin-9 downregulated in high risk CLL.	No control was used.	[37]
To conduct proteomic profiling of CLL (indolent vs. aggressive)	iTRAQ quantification for PB of 12 CLL patients & healthy donors	22 deregulated proteins in aggressive vs. mild CLL. 70 differentially expressed proteins between healthy and CLL samples. cH2A (overexpressed in CLL) is of myeloid origin.	The results need to be confirmed using a larger cohort.	[38]
To elucidate the role of B-cell receptor in cellular survival.	2-DE-MS for PB of 55 CLL patients & 4 controls.	The LMWK form of Kininogen overexpressed in CLL. No presence of LMWK in control samples. LMWK linked to shorter median survival.	The specificity of LMWK to CLL must be validated as it was not detected in absolutely all CLL patient samples.	[39]
To determine whether MMP-9 has an impact on B-CLL cells' survival	Flow cytometry for PM, BMA, LN from 4 and 2 patients, respectively from 35 CLL patients	Binding of (pro) MMP-9 and MMP-9's hemopexin domain to the ?4?1 integrin and CD44v receptors promotes survival.	No control was used.	[40]
To investigate the effects of CXCL12 and its receptor, CXCR4, on the pathogenesis of CLL	LC-MS/MS for PB of 5 CLL patients.	PDCD4 and HSP27 overexpressed in CXCL12-stimulated cells.	The method used could not support an extensive evaluation of the phosphoproteins expressed by leukemic B-cells.	[41]
To detect differentially expressed proteins in patients with mutated and unmutated *IGVH*	2-DE-MS for PB of 11 CLL patients.	14 spots differntialy expressed between mutated and ummutated samples. IGVH in the unmutated form linked to high levels of ribosomal associated protein and nucleophosmin 1. Nucleophosmin 1 a major transcription regulator.	The results need to be validated in a larger cohort.	[42]
To provide a proof of concept for a novel nanofluidic proteomic immunoassay	Nanofluidic proteomic immunoassay for PB of Primary CML and CLL cells.	MYC oncoprotein and BCL2 successfully quantified. The detected the phosphorylation of ERK1, ERK2, MEK, STAT3, STAT5, JNK and caspase-3 in imatinib-treated CML cells. ERK2 isomer phosphorylation status successfully detected.	The efficiency of the technique needs to be ascertained.	[43]
To elucidate the differences in histone isoform distribution in CLL patients	LC-MS for PB of 40 CLL patients & 4 controls.	Histone H2A variants (H2AFL and H2AFA/M) underexpressed CLL cells. H3 variants and two other unknown proteins upregulated in most CLL samples. Major peaks detected corresponded to H2AFC/D/I/N/P, H2AFL and H2AFA/M.	The low mass accuracy of the identification method used, LC-ESI-TOF-MS.	[44]
To detect the antigens bound by cancerous B-lymphocytes	2-DE and flow cytometry for PB of 28 CLL patients.	High reactivity towards Sm, Ku, snRNP A, BB', and C, CENP-B. CLL cells' survival depended on smIg and endosomal TLR co-signaling.	No control was used.	[45]
To detect differentially expressed cytokines and cytokine receptors in CLL cells	Antibody array method for PB of 5 untreated CLL patients & 3 controls.	174 cytokines and cytokine receptors detected in CLL cells. Cytokines and cytokine receptors have no effect on CLL B-cells' survival.	The results need to be confirmed in a larger cohort.	[46]

LN = Lymph node; CLL = Chronic lymphoid leukemia; CML = Chronic myeloid leukemia; LC-MS/MS = Liquid chromatography-tandem mass spectrometry; TOF-MS = Time-of-flight mass spectroscopy; PB = Peripheral blood; BM = Bone marrow; BMA = Bone marrow aspirate.

LC-MS/MS; 2-DE; and MALDI-TOF-MS in 9 out of 15 studies. Conversely, the most common proteomic techniques used for the profiling of CLL were found to be LC-MS/MS; 2-DE; and iTRAQ labeling, followed by 2-D-LC-MS/MS, flow cytometry and protein array. Such methods seem to have become mainstream in terms of detecting and identifying leukemia biomarkers. Nevertheless, 20% of the studies reviewed on ALL also utilized DIGE and SELDI-TOF-MS in conjugation with these techniques.

DISCUSSION

In 2014, SELDI-TOF-MS was shown to be sufficient on its own to establish a proteomic classification model capable of accurately differentiating all types of acute leukemia. Research done with other techniques has mainly focused on providing a proof of concept using ALL or CLL as a model, or on detecting the activity/ expression status of pre-selected sets of proteins. The Western blot and flow cytometry were routinely used in the studies cited in this work to confirm selected results obtained in the primary analysis and to add useful input to the interpretation of the main findings.

Researchers set out to investigate whether size exclusion chromatography-microsphere-based affinity proteomics (SEC-MAP) could match or exceed flow cytometry in robust detection of large numbers of proteins in patient samples. Flow cytometry-based immunophenotyping (FACS), western blot (WB) and real-time PCR (qPCR) were used to validate the results. Out of 31 markers used, a significant ($p<0.05$) correlation was found for 18 of those between SEC-MAP and FACS. The technique was shown to be arguably superior to WB and qPCR in certain aspects. It enabled the detection of 51 characteristic protein entities capable of differentiating different types of childhood acute leukemia. Overall, SEC-MAP appears to be a high-content, reproducible, accurate tool for the detection of large numbers of proteins in childhood ALL.[19]

In another study, PreB 697 (or EU-3), a pediatric ALL cell line, was cultured in RPMI 1640 medium, supplemented with 10% (v/v) foetal bovine serum. GC-resistant clones (R3C3, R3G7, R3F9, R4C10 and R3D11) were obtained thereafter by continuous culture in a dexamethasone-containing medium. The proteomic profiles of GC-sensitive and -resistant ALL cells were compared before and after 24-hour treatment with dexamethasone (0.1 µmol/L). iTRAQ proteomics coupled with LC-LCMS/MS was used to analyze the nuclear lysates. Differences were revealed in the expression of PAX5 and IRF4 between GC-sensitive and GC-resistant clones, confirmed using WB. Using qPCR, PAX5 was shown to be upregulated in GC-sensitive cells at both the mRNA and the protein levels. Resistant EU-3 clones mainly exhibited reduced basal levels of PAX5 due to post-transcriptional regulation, but had higher levels of active JNK, whose inhibition seemed to restore GC-sensitivity by 30 folds. The study suggested that JNK activation influences PAX5 levels, cell maturation and, ultimately, GC resistance.[20]

A pilot study was carried out to demonstrate that proteomics could identify markers of prognostic value in childhood leukemia treated with L-Asparaginase. CSF was collected from 4 ALL and 1 LL patients on days 0, 8, and 29 following treatment; and immuno-depleted peptide digests were prepared. One-dimensional LC, coupled with MS/MS, was used to determine the relative peptide abundance and protein expression levels in those digests. All patients were observed for a period of six months. The levels of proteins involved in coagulation differed in all patients on L-Asparaginase treatment. A child who had multiple CNS thrombi 59 days after induction exhibited markedly low antithrombin III and plasminogen levels, in addition to reduced basal levels of plasma serine protease inhibitor and coagulation factor X. The results indicated that coagulation factors of the serpins superfamily could serve as reliable markers capable of predicting a CNS thrombosis event in pediatric ALL patients undergoing L-Asparaginase treatment.[21]

Kinome profiling, a subset of proteomics, has the potential to facilitate the development of novel therapeutic agents that target key signaling pathways involved in the progression of ALL. Researchers attempted to profile kinase proteins in children with ALL to better understand the mechanisms of the disease. A total of 20 B-cell progenitor ALL (BCP-ALL) and T-cell ALL (T-ALL) blood and bone marrow samples were studied. The mononuclear leukemic cells were isolated; and the profile of signaling-pathway proteins was determined using the PepChipTM Kinomics microarray system. Cell lines such as Jurkat (T-ALL), Molt 4 (T-ALL), Nalm 6 (BCP-ALL), and RCH-ACV (BCP-ALL) were used to validate the results obtained in primary cells on the HGFR growth factor receptor. Analysis showed 250 peptides to be activated in all samples, with 66 being related to the MAPK-signaling pathway, 49 to the PI3K/Akt-signaling pathway, and 33 to cell cycle regulation and p53 functionality. ALL cell growth and survival were found to be dependent on high RON expression and activation.[22]

To detect proteomic differences of prognostic value in cancerous vs. healthy B-lymphocyte, a group of researchers utilized 2-DE to study samples from 27 ALL patients and 10 healthy individuals. Difference in-gel electrophoresis (DIGE) and WB were also used to study 3 BCP-ALL and 3 control, and 4 BCP-ALL and 2 control samples, respectively. Pure CD19+lymphoblasts were obtained from each sample using density gradient centrifugation to allow for the preparation of protein

lysates and total proteins for analysis. Separated proteins were identified using matrix-assisted laser desorption/ionization (MALDI). A total of 35 up-regulated and 25 down-regulated proteins were reported in leukemic B-cells, most of which were involved in proteostasis, cytoskeletal organization, and cellular energy and redox homeostasis. In general, stress-response proteins were found to be over-activated, while transport/translocation proteins were suppressed. The proteins identified in this study seem to be key to the transformation of healthy B-lymphocytes into ALL; and may potentially be exploited as treatment targets.[23]

A study was conducted to develop a reliable proteomics-based classification methodology. The characteristic proteomic expression of leukemia markers in different sub/types of acute leukemia was elucidated using 151 primary bone marrow samples. The samples were categorized into four acute leukemia sub/types (AML-Gra, APL, AML-Mon, ALL) based on flow cytometry immunological analysis and cytogenetic data. Mononuclear cells were then isolated from each sample using centrifugation; and protein signatures from 500 to 20 kDa were determined using SELDI-TOF-MS. Characteristic proteomes were detected for each of the sub/types of acute leukemia (M1593, M2121, M2536 and M1016 for APL, AML-Gra, AML-Mon and ALL, respectively); and the applicability of the resulting classification models was confirmed using 23 more patient samples. The study provided evidence of differentially expressed molecules that can potentially be utilized as markers or treatment targets.[7]

Researchers set out to examine the potential diagnostic value of the proteomic vs. genetic expression levels of three splicing factor proteins (NSRP70, SRSF1 and HNRNPA1) in AML and ALL patients. Samples from 187 adults (48 AML, 23 ALL, and 116 controls) were collected in Wonkwang University Hospital and Chonnam National University Hospital, South Korea, to be analyzed using immunoblotting and immuno-fluorescence staining. Moreover, serial bone marrow aspirates were collected from five patients in complete remission. Band intensity was higher in the patients group compared with the control, and in ALL patients as compared with AML ones, with no observable mRNA level differences between leukemic and healthy subjects. Abe-sum-NSRP70, Abe-SRSF1 and Abe-HNRNPA1 were found to be reliable markers for ALL that disappeared upon remission, whereas Abe-hi-NSRP70 was associated with poor prognosis in AML. In conclusion, the levels of the splicing proteins examined in this work, and not their mRNA levels, appear to be useful markers for the detection and treatment of ALL.[24]

A study was conducted to detect protein expression differences in ALL pediatric patients vs. healthy children. Bone marrow aspirates and peripheral blood samples were respectively collected from a total of 15 ALL patients and 15 controls. The lymphocytes were isolated from all samples using a standard kit, after which the cells' protein contents were extracted using a lysis buffer. Two-dimensional gel electrophoresis was used to perform protein separation and detected spot intensity levels. Overall, 15 significantly and consistently different protein spots were found, 11 of which were down-regulated, while 4 were over-expressed. An attempt to identify these proteins was made using MALDI-TO-MS; and identification was possible for 8 proteins as follows: (1) Glutathione S-transferase, (2) Prohibitin (both up-regulated in ALL), (3) Peroxiredoxin 4, (4) 60s acidic ribosomal protein P0, (5) Cytoplasmic actin, (6) Pyridoxine-5'-phosphate oxidase, (7) Triosephosphate isomerase 1, and (8) hypothetical protein FLJ26567 (all down-regulated in ALL). It is plausible that those proteins may serve as markers in the future to enable early diagnosis of ALL or as treatment targets for novel anticancer agents.[25]

A comparative proteomics study was executed to detect differences in protein expression levels in high-risk (HR) vs. low-risk (LR) ALL pediatric patients. Bone marrow and peripheral blood plasma samples were withdrawn from a total of 45 children with ALL and 7 healthy control subjects. Patients aged 1 to 9 years with a WBC count below $50x10^9$/L and L2 under 20% (based on FAB classification), with no L3 blasts or CNS involvement were considered to be the low-risk group (19 patients in total), while the rest (26 children) made up the high-risk group. Following high-abundance protein depletion, proteomes from all samples were separated using 2-DE and identified via MALDI-TOF-MS. Western blot analysis was performed to confirm some of the findings. The results revealed the presence of 18 and 16 differentially expressed proteins in high- and low-risk groups, respectively. Vitamin D-binding protein and S10A9 were respectively shown to be up- and down-regulated in both groups. In contrast, a suppressor protein, Gelsolin was found to be under-expressed in the HR population, whereas ceruloplasmin, clusterin, prothrombin, alpha-1-microglobulin/bikunin precursor, and ficolin-3 were all over-expressed in the same group. The LR group rather showed high levels of kininogen-1 and low levels of afamin. Further evidence suggested that ficolin-3 and kininogen-1 were of particular interest as distinctive biomarkers for ALL aggressiveness. Overall, this work provided useful data on differentially expressed proteins between LR and HR ALL groups, between different sample origins, and between patient and healthy subjects. The proteins highlighted in this work, including β-actin, catalase and apolipoprotein E, A4 and A1 were rightfully reported as potential diagnostic/prognostic markers for ALL.[26]

Research was performed to prove that label-free alignment-based quantitative LC-MS/MS data could

differentiate AML from ALL, and leukemic from healthy cell lines in many genetically-different individuals at once. Leukemic and healthy CD34+ cells isolated from bone marrow aspirates of 4 AML and 5 ALL patients; and the peripheral blood of 8 healthy individuals were respectively retrieved from the repository at Fred Hutchinson Cancer Research Center, WA, USA, in a random manner. All cells were lysed and analyzed using quadruplicate micro-capillary HPLC-MS/MS. The leave-one-out approach was used to determine and confirm markers distinctive to each cell group (AML, ALL and non-leukemic). A total of 91, 71 and 17 proteins were found to distinguish ALL from CD34+; ALL from AML; and AML from CD34+. Of these, 10, 14, and 3 proteins were shown to be strictly found in one group and not the other for each pair, respectively. Furthermore, nicastrin expression was shown to be specific as a marker to AML. The study indicated that the method and algorithm used in this work could robustly serve as a reliable tool for acute leukemia diagnosis and classification in larger sample sizes.[27]

Researchers set out to determine the proteomic cellular changes that can predict a good/poor response to prednisolone treatment in pediatric patients by the 8th day of treatment – a standard prognostic point. For this purpose, cell lines representing ALL subtypes (REH, 697, Sup-B15 and RS4;11) were treated with prednisolone for 4 days. REH cell line was shown to be resistant to treatment. Next, protein lysates were prepared from those and control cells to be separated using 2-DE and analyzed. Protein spots with intensity alteration higher than 30% were excised and digested to be identified using MALDI-TOF/TOF-MS. A total of 77 and 17 proteins were found to be differentially expressed in prednisolone-sensitive vs. -resistant cells. Western blot confirmed the findings pertinent to four proteins, of which proliferating cell nuclear antigen (PCNA) was deemed to be of particular interest due to its known role in cell cycle regulation and survival. It was shown to be downregulated in sensitive cells. Hence, PCNA was quantified in a cohort of 43 pediatric patients before/after treatment, 35 of which had responded well to prednisolone (i.e., PGR patients). The findings confirmed that PCNA was downregulated in all 35 patients as compared to the other 8 (PPR patients); and could, hence, be a reliable tool to predict the extent of prednisolone-caused apoptosis in ALL patients regardless of the subtype of the disease.[28]

A study was carried out to elucidate the proteomic changes that underlie the ability of pre-B lymphoblasts to spread ALL to the CNS based on data obtained in previous works. The role of asparaginyl endopeptidase (AEP), a prominent mediator of cancer aggressiveness, was investigated using a relative quantitative MS protocol. SD1, SupB15 and REH cell lines were used to study invasion mechanisms *in vitro*. SD1 cells, the most invasive, shown upregulation of AEP. However, higher AEP levels in REH increased the cells' invasiveness moderately, drawing the conclusion that the protein was partially involved in CNS invasion mechanism. Thereafter, 94 upregulated and 81 downregulated plasma membrane proteins were identified in SD1 using two labeling techniques (SILAC and iTRAQ), coupled by flow cytometry and western blot. The effect of a selected candidate, RAC2, was tested in mice by cell engraftment. The findings indicated that the presence of all of AEP, RAC2 and other factors, such as ICAM1, would be necessary to confer the pathogenesis of ALL on CNS. This was confirmed in primary bone marrow-derived CD10/CD19 samples. Overall, the study provided a valuable insight into possible mechanisms responsible for CNS involvement in ALL cases. Further research is warranted, however, to confirm the findings clinically.[29]

A search effort attempted to unveil potential treatment targets for individualized therapy in pediatric ALL patients. A set of 92 signaling proteins was chosen to detect changes related to protein phosphorylation/ activation using a reverse-phase protein microarray technique. No correlations were found between protein activation and patient characteristics, though some proteins were differentially phosphorylated in patients with MLL-rearrangement vs. those with no translocation, and in PGR patients vs. those with PPR. Four proteins, namely Cyclin E, Annexin 2, AMPKb (S108) and AMPKα, were over-activated in MLL-rearranged subjects. Other proteins related to the AMPK pathway were also found to be deregulated, indicating that the pathway plays a major role in resistance to chemotherapy via BCL-2 activation – a major anti-apoptotic regulator. As to changes pertaining to response to prednisolone, further findings in patient samples and REH cell line revealed that LCK kinase was hyperactive in PPR patients and suppressed in PGR patients – constituting a potentially reliable prognostic tool. Lastly, a relapse-free survival analysis showed that increased cyclin E activity was strongly linked to an increased risk of early relapse, suggesting its potential use as a marker for disease aggressiveness.[14]

Researchers attempted to identify differentially expressed proteomes as useful to diagnose ALL non-invasively. Serum samples were collected from a cohort of 178 children. Next, the samples were randomly divided into two groups: a training set (45 ALL patients + 34 controls) and a test set (49 ALL patients + 30 AML patients + 20 controls). The samples were centrifuged at 1500 x g for 10 min before being analyzed using a SELDI-TOF-MS technique. Peak intensities of seven peaks of interest in the training set were evaluated using the biomarker pattern software to elucidate the proteomic profile characteristic of ALL patients vs. healthy subjects. Four markers were chosen to be

purified from samples in the test set and separated using HPLC. Later, those markers were identified using LC-MS/MS and confirmed using ProteinChip Immunoassays. The assay revealed that CTAP III, a fragment of pro-platelet basic protein precursor, and platelet factor 4 were down-regulated in ALL patients; whereas, the other two markers, fragments of C3a, were upregulated. The study highlighted a number of proteins that were shown to be specific to ALL and capable of differentiating between ALL and AML.[30]

A study was conducted to compare the cytosolic protein profiles of different leukemia and lymphoma types. The proteomes of four cell lines, HL-60, MEC1, CCRF-CEM, and Raji – respectively representing APL, CLL, T-cell ALL, and Burkitt's lymphoma – were sub-fractionated to prepare cytosolic extracts. The extracts were further purified by protein precipitation and suspended in a DIGE, buffer. Ultimately, they were labeled with fluorescent CyDyes and combined with an internal standard for proteins to be later separated and identified using DIGE, coupled with MALDI-MS. Differentially expressed cytoplasmic proteins in each of these cell lines were profiled. One hundred and sixty-six different protein species were detected, of which principle component analysis identified 17 as differentially expressed proteomic species between all four cell lines i.e., a total of 22 proteins. Many of these appeared to be differentially expressed in HL-60 only, such as myeloperoxidase, disulfide-isomerase, and S10A4. These proteins may be key contributors to myeloid differentiation. Ezrin was upregulated in Burkitt's lymphoma cells only. Other proteins, such as C-1-tetrahydrofolate synthase and stathmin were deregulated in two B-cell lines, but not in the T-cell line, indicating a potential role in B-cell development. Overall, the findings of this work provided a valuable insight into the molecular basis of important leukemia types.[31]

Review of studies on the proteomic profile of chronic lymphoid leukemia: An experiment was devised to detect proteomic differences in two types of B-cell leukemia, Burkitt's lymphoma and CLL. Plasma samples were withdrawn from mouse models representing both diseases, Eμ-*myc* and Eμ-*TCL1* respectively, in addition to wild-type mice. The samples were analyzed using LC-MS. High abundance proteins were removed using size-dependent protein fractionation. Peak intensities of 10,000 proteins were detected, of which 695 were found to be commonly upregulated in both types of leukemia. A number of methylating enzymes were found in high levels in Eμ-*myc* tumors, while many cytoskeletal components appeared to be downregulated. On the other hand, ER stress response proteins and a few signaling components were shown to be upregulated in Eμ-*TCL1* tumors. Both subunits of interleukin-5 (IL-5) receptor were also upregulated; and further findings implicated IL-5 as a factor of tumorigenesis via TCL1-mediated activation of the AKT pathway. Overall, the study provided a valuable insight into the nature of the biomarkers present in the plasma of leukemic cells, and the cellular mechanisms shared by, and those unique to Burkitt's lymphoma and CLL. A better understanding of the role of the differentially expressed proteins, cited in this work, is expected to further our general understanding of the origins of B-cell leukemias.[32]

Researchers set out to examine the impact of common CLL cytogenetic alterations, namely del(13q14), del(17p13.1), trisomy 12, and NOTCH1 mutations, on the expression of 224 proteins involved in intracellular signaling. Leukemic B-cells isolated from the peripheral blood of 14 CLL patients (representative of all the mutations above) and 63 healthy individuals were analyzed using the Panorama cell signaling kit. Del(13q14) was linked to the overexpression of JUN and cyclin A, B1 and D1; and the downregulation of PRKC and RAF1. Moreover, RAF/MEK signaling was found to be suppressed in cases presenting with del(17p13.1), with the upregulation of CDKN2A, a p53 degradation-inhibitor, and JNK. Contrary to the other tested mutant B-cells, the trisomy 12 cytogenetic defect had no impact on relative protein abundance. NOTCH1 was associated with the upregulation of CDK4 and the downregulation of cyclin B1 and synuclein. Furthermore, p21 CDK inhibitor was observed as being downregulated in both del(17p13.1) and NOTCH1. The results were validated using an LC-MS/MS quantitative proteomic technique based on spectral counting (emPAI). Lastly, PKC family members were shown to be significantly and consistently downregulated in 75% of the samples. This, the authors hypothesized, could be a part of the underlying mechanisms that make CLL progress slowly.[33]

A study aimed to determine differentially expressed protein biomarkers in progressive CLL. Peripheral blood samples from 27 patients were collected at the Royal North Shore Hospital, NSW, Australia, and the University of Leicester, UK. Initial screening was performed using an iTRAQ labeling technique and 2D-LC-MS/MS. A total of 84 differentially expressed proteins were identified between stable and progressive CLL. The results of the initial screening were scrutinized using primary CD19+ cells obtained from 50 CLL patients. The cells were categorized into three groups: stable, slow-progressive and progressive. Selected reaction monitoring (SRM) was used to transform the profiles of CD19+ cells into clusters and identify biomarkers for the different subtypes of CLL. Two main clusters emerged distinctly containing most of the progressive and stable CLL samples. No distinct cluster was shown for slow-aggressive CLL. Further findings provided evidence of 12 differentially expressed molecules that corresponded to CLL subtypes and prognosis, and that may serve as markers for disease aggressiveness in patients with/without prior treatment.[34]

The expression level of Zonula Occludens protein-1 (ZO-1), an associate of connexin Cx43 involved in cell function regulation, was assessed in primary CLL samples. Ammonium chloride erythrocyte lysis was performed for peripheral blood samples taken from 113 CLL patients and 50 controls. Next, to assess the expression of ZO-1 and Cx43, the whole-cell extracts were analyzed using flow cytometry and western blot. Analysis showed that both ZO-1 and Cx43 were correlated in terms of expression, and downregulated in CLL patients compared with the control subjects. The levels of ZO-1 were shown to be lowest in patients with the worst prognosis in the cohort. Further findings showed that the expression levels of CD38 and Zap-70, proteins associated with cell cycle regulation, were inversely proportional to those of ZO-1, suggesting that the ZO-1 gene in malignant CLL cells is silenced. Later, the effect of Cx43 on apoptosis was evaluated in leukemic cells treated with gap junction inhibitors and anti-Cx43 antibodies. The results indicated that cells with depreciated levels of ZO-1 were more likely to undergo apoptosis when treated with a gap-junction inhibitor. The proteins highlighted in this work, namely ZO-1, CD38 and Zap-70, were rightfully reported as potential prognostic tools for CLL.[35]

The effect of *IGHV* mutation on the pathogenesis of CLL was investigated to elucidate the underlying mechanisms that make unmutated forms of CLL cells more aggressively retained in the lymph nodes. Peripheral blood mononuclear cells from a total of 18 CLL patients (9 with mainly mutated cells and 9 with unmutated ones) were isolated via centrifugation and labeled with iTRAQ. Differentially expressed proteins were identified using MS. CLL samples with and without IGVH mutations revealed the presence of 39 deregulated proteins, of which 35 were shown to be significantly downregulated in the unmutated IGVH group. These proteins had direct effects on cell migration, which suggested that unmutated cells were more adhesive than mutated ones. The results were verified by means of western blot using 3 differentially expressed proteins. Furthermore, analysis in a cohort of 120 CLL patients confirmed that unmutated cells were more likely to cause lymphadenopathy in patients due to increased adhesion to the lymph nodes. Overall, the study demonstrated the potential of iTRAQ-MS to showcase the pathogenetic mechanisms of leukemia and reveal potential treatment targets.[36]

To detect proteomic differences of prognostic value in B-cell CLL, a group of researchers utilized two-dimensional nano liquid chromatography coupled with MALDI-TOF/TOF-MS to study samples from 12 CLL patients. Cellular extracts from the samples were analyzed and a total of 728 proteins were identified. Quantified proteins were subjected to hierarchical cluster analysis to detect protein expression patterns. A cohort of 39 patients (including the original 12) was used to confirm the findings pertaining to 4 differentially expressed proteins using antibodies. The results revealed that thyroid hormone receptor-associated protein 3, T-cell leukemia/lymphoma protein 1A, and S100A8 were linked to high-risk CLL. Moreover, myosin-9 was shown to be downregulated in the high-risk group. The proteins identified in this study seem to be key to further understand the pathogenesis of CLL and may potentially be exploited as treatment targets.[37]

Researchers investigated the protein expression patterns between indolent and aggressive CLL forms; and between CLL patients and healthy subjects. Whole blood samples from 12 adults (equally distributed as aggressive and mild CLL) and healthy donors above the age of 50 were collected in Ghent University Hospital, Belgium, to be analyzed using iTRAQ quantification. Twenty-two proteins were found to be deregulated between the aggressive and the mild form of the disease, as opposed to 70 between the healthy and the CLL samples. Differences in the expression of a proteolytic (clipped) product of histone H2A (cH2A) between CLL patients and healthy subjects were deemed of importance. The peptide cH2A had been reported earlier as being upregulated in CLL patients, though with lacking information regarding its significance. However, in this study, analysis of CLL cells, and *in vitro* tests during which myeloid THP-1 cells were forced to differentiate into macrophages; indicated that though cH2A was overexpressed in CLL patients, it was actually of myeloid origin. Overall, the study provided a valuable insight into H2A V114 clipping but further research is needed to extensively elucidate the significance of this epigenetic phenomenon.[38]

Addressing an ambiguity in terms of the proteins associated with CLL cells' survival, a study was conducted to elucidate the role of B-cell receptor in cellular survival. Peripheral blood mononuclear cells were purified from three CLL patients who have not been treated for at least 3 months. Next, 2-DE coupled with MS was used to determine the protein expression levels in those cells following artificial activation of the receptor. The proteomic analysis showed that kininogen was overexpressed in all patients. Immunoblotting confirmed that the LMWK form of the protein was indeed elevated in all the tested CLL samples; and tests in 4 healthy patient samples showed no presence of LMWK. Evaluation of 52 CLL patient samples revealed basal expression of LMWK in 71% of the patients. Those who were positive for the protein generally had shorter median survival. The findings confirmed that kininogen was critical to the persistence of a CLL cell, and a novel therapeutic target for the disease.[39]

A study set out to determine whether cell-bound Matrix metalloproteinase-9 (MMP-9; gelatinase B), the major

MMP protease in B-cells with multiple cleaving functions, has an impact on B-CLL cell survival. Peripheral blood samples were collected from a total of 35 CLL patients. In addition, bone marrow aspirates and lymph node samples were collected from 4 and 2 patients (of the same cohort), respectively. CD5+ B-lymphocytes were isolated from all samples. The function of MMP-9 in B-cells and its role in apoptosis were then evaluated using flow cytometry. The study provided evidence to indicate that the catalytic function of the enzyme had virtually no effect on apoptosis. The findings indicated that MMP-9 contributed to cell survival and CLL persistence through a pathway triggered by the binding of (pro)MMP-9, a catalytically inactive proMMP-9 mutant, or the enzyme's hemopexin domain to its docking receptors $\alpha 4\beta 1$ integrin and CD44v. The effect was shown to be a halt of apoptosis. The pathway is believed to involve Lyn activation, STAT3 phosphorylation, and Mcl-1 upregulation. Exploiting (pro)MMP-9 as a treatment target is expected to yield much greater therapeutic effects than seen with the use of common MMP-9 inhibitors.[40]

The role played by the chemokine CXCL12 and its G-protein coupled receptor, CXCR4, in the pathogenesis of CLL cells was investigated. For this purpose, quantification of phosphopeptides was performed using LC-MS/MS in unstimulated and CXCL12-stimulated primary CLL cells derived from the peripheral blood samples of 5 CLL patients. Over 700 phosphoproteins were detected, some of which had previously been linked to CLL, including BCNP1, FMNL1, Hcls1, HSP-90 alpha, Lyn, Mcm2, PLZF, SHIP-1, and Stathmin 1. Both programmed cell death factor 4 (PDCD4), a tumor suppressor, and heat shock protein 27 (HSP27), an anti-apoptotic factor, were found to be overexpressed in CXCL12-stimulated cells. To validate the results, the cells were purified by using a negative selection technique via magnetic associated cell sorting to be analyzed using western blot. The study revealed that in all the CLL cells tested, PDCD4 was a novel phosphorylation target downstream of CXCL12 signaling, whereas HSP27 was present in only a subset of the samples. Stabilizing molecules for PDCD4 are expected to be beneficial agents for CLL.[41]

Differentially expressed proteins were profiled in patients with mutated and others with unmutated IGVH. Healthy primary CD19+ cells and CLL B-cells belonging to 5 patients with mutated IGVH and 6 patients with unmutated IGVH; were retrieved from the Royal Liverpool University Hospital, UK. Proteomic analysis of the samples was performed using a 2-DE-MS technique. One hundred and forty-three spots were found in all gels and chosen as the test set. Fourteen spots were found to be significantly different between the mutated cases and the unmutated samples. Western blot and other immunological methods were used to further clarify the results. Ribosomal associated protein and nucleophosmin 1 were shown to be upregulated in CLL patients with unmutated IGVH. Co-immunoprecipitation experiments revealed a correlation between nucleophosmin 1 and ribosomal proteins. Immunocytofluorescence showed that nucleophosmin 1 underwent nucleo-cytoplasmic shuttling in CLL from the nucleoplasm to the cytoplasm. Taken together, the findings suggest that nucleophosmin 1 is involved in the pathogenesis of CLL as a major transcription regulator, which makes it a prime candidate for novel therapeutic agent development.[42]

A study was carried out to provide a proof of concept for a novel nanofluidic proteomic immunoassay capable of quantifying total and low abundance oncoproteins. Primary CML and CLL cells were purified from peripheral blood samples of patients. The protocol comprised of lysed samples analyzed in a firefly system equipped with a micro-scale capillary. Isoelectric focusing was used to resolve the peptide peaks generated through the system. The proteins eluted were detected using multiple monoclonal antibodies, and quantified by peak analysis software. MYC oncoprotein and B cell lymphoma protein-2 (BCL2) were successfully quantified. The protocol was able to detect discrepancies in terms of the activation of extracellular signal-related kinases-1 (ERK1) and ERK2, mitogen-activated kinase-1 (MEK), signal transducer and activator of transcription protein-3 (STAT3) and STAT5, c-Jun N-terminal kinase (JNK) and caspase-3 in imatinib-treated CML cells. Differences in the activation pattern of an ERK2 isomer, associated with good response to imatinib, were successfully detected. Moreover, tests in additional samples showed that the proposed method was sensitive to decreases in STAT3 and STAT5 phosphorylation in lymphoma patients receiving atorvastatin treatment. The study indicated that nanofluidic proteomic immunoassay was a reliable tool to study differentially expressed proteins in different leukemia types.[43]

Histone isoform distribution in CLL patients was elucidated. Leukemic and healthy mononuclear cells were isolated using density gradient centrifugation from peripheral blood samples obtained from 40 CLL patients and 4 healthy subjects. Using bovine calf thymus histones as standards, analysis using LC-MS showed that histone H2A variants (H2AFL and H2AFA/M) were significantly underexpressed in primary CLL cells as compared with normal B cells. Moreover, there was a significant upregulation of H3 variants and two other unknown proteins in most CLL samples. High mass accuracy identification of the deregulated histones using LC-ESI-TOF-MS revealed that the main peaks detected corresponded in mass to H2AFC/D/I/N/P, H2AFL and H2AFA/M. The method used in this work was validated and proven to be highly robust by eliminating the effects of possible interference due to instrumental parameters,

sample preparation and biological variations over time. It is plausible that the chromatin modifications observed in this work may serve as highly valuable prognostic tools for CLL.[44]

To detect the antigens bound by cancerous B-lymphocytes and elucidate the role of some apoptosis products, autoantigens, on the etiology of CLL, a group of researchers utilized 2-DE to study samples from 28 CLL patients (19 with unmutated *IGVH* and 9 with mutated *IGVH*). Peripheral blood samples were withdrawn from to enable the purification of mononuclear cells and analysis using flow cytometry. Most unmutated cells were shown to exhibit antigenic polyreactivity, binding multiple unrelated autoantigens. Great reactivity was detected with quite a number of autoantigens involved in systemic autoimmunity, including Sm, Ku, snRNP A, BB', and C, CENP-B. The results revealed that smIgs, especially in patients with aggressive CLL, recognized ubiquitous conserved autoantigens. This seem to be key to the etiology of CLL as leukemic cells appeared to originate from a B-cell subset that usually facilitates the clearance of cellular debris and metabolic byproducts. Evidence presented in this work suggest that survival of CLL cells depended on smIg and endosomal TLR co-signaling, making those pathways potential treatment targets for CLL.[45]

The differential expression of endogenous cytokines and cytokine receptors was assessed in CLL cells by using an antibody array technique. CLL and healthy mononuclear cells were purified from the peripheral blood samples of 5 untreated patients and 3 healthy donors, respectively, by capturing non-B cells via magnetic beads coated with antibodies against CD2, CD14, CD16, CD36, CD43 and CD235a. Out of 174 cytokines and cytokine receptors detected in CLL cells, only 2 were found to be deregulated as compared with normal B-cells: IL-6 was found to be downregulated, whereas eotaxin was upregulated in CLL samples. Overall, the levels of cytokines and cytokine receptors secreted from the leukemic cells *in vitro* were only slightly different from healthy ones. This begs the conclusion that cytokines secreted by CLL B-cells do not have any tangible impact on the survival of leukemic cells.[46]

CONCLUSION

Molecular genetics has been the primary tool used to study aspects related to leukemia treatment, prognosis, classification and progression.[47] However, proteomics techniques are becoming increasingly more popular due to the fact that many forms of cancer manifest primarily on the cellular functional level.[19] However, mRNA expression does not always truly represent protein expression in a mammalian cell.[5,6,48] Such techniques are able to expand our knowledge about the mechanisms of action of chemotherapeutic agents in ways that are not possible with genomic experiments alone.[49] Yet, unlike molecular genetics protocols, sensitive and efficient proteomic analysis techniques are yet to be established as standard methods for clinical studies involving primary leukemic samples.[19] Proteomic approaches to diagnose, monitor, classify and treat lymphoid leukemia are still in their early stages.[33] Almost none of the studies cited in this review was properly replicated and validated by other research groups in countries in/outside the location of the study. The impact of racial, age and gender differences, as well as the differences related to the disease's subtypes on the proteomic profiling of lymphoid leukemia were often unexplored. Cell lines are highly homogenous as compared with primary cells purified from patient samples; however, their use has been known to generate less useful data, which can, on occasions, be misleading.[41] This may explain why 90% of the studies in this review presented results obtained in primary cells or used patient samples to validate results obtained in cell lines. This analysis indicates that LC-MS/MS and 2-DE are among the most utilized proteomic techniques in the field of leukemia profiling. Though 2-DE has been found to be a frequently used tool in proteomics related to lymphoid leukemia, Issaq made the observation that 2-DE coupled with MS provided a rather limited coverage of the proteome and generated data that could not be reproducible.[50] MALDI-TOF-MS, and iTRAQ labeling based methodologies, in addition to 2-D-LC-MS/MS, flow cytometry and protein array have also demonstrated high potential for the detection of low abundance proteins in the past ten years. We note that proteomic profiling techniques intrinsically generate a staggering amount of data,[51] often more than can be adequately processed within the scope of one study, which leaves the possibility of subtle, yet important, discrepancies that may have otherwise proved useful. To establish routine proteomics-based approaches for the diagnosis, prognosis, and treatment of leukemia on a worldwide scale, future research ought to focus on establishing the efficiency, sensitivity and clinical applicability of some of the most prominent protein signature candidates cited in previous works, in addition to optimizing the proteomic approach(s) best suited for the detection of such markers in primary samples.

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Sofosbuvir Adverse Events Profile in a Subset of Pakistani Population

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ABSTRACT

Objective: To determine frequency and pattern of adverse events reporting in a subset of Pakistani population being treated for chronic hepatitis C with sofosbuvir combination therapy.

Study Design: Descriptive study.

Place and Duration of Study: Department of Medicine, Gastroenterology Division, Shalamar Hospital, Lahore, from September 2015 to May 2016.

Methodology: Patients who were offered sofosbuvir therapy for treatment of chronic hepatitis C were randomly enrolled. The study subset included both treatment naïve as well as retreatment groups. Patients were screened for subjective as well as objective evidence of adverse events at regular intervals. Frequency was determined.

Results: Among 196 patients with chronic hepatitis C, 192 patients received dual therapy consisting of ribavirin and sofosbuvir. The most frequent complaints in these subjects were fatigue, fever, myalgias and nausea accounting for 55%, 42%, 44.2% and 50%, respectively. Twenty-seven percent of patients reported with drop in hemoglobin of >2g/dl, while absolute neutropenia and moderate to severe thrombocytopenia was observed in 3% and 5% of patients, respectively. One patient died as a result of severe pancytopenia. Later derangements were all observed in patients with decompensated disease.

Conclusion: Sofosbuvir showed less severe adverse effects in terms of symptomatology and less frequent neutropenia and thrombocytopenia as compared to previous regimens. Careful monitoring is required, especially in those with decompensated liver disease.

Key Words: *Sofosbuvir. Chronic hepatitis C. Direct acting antiviral drugs. Sofosbuvir adverse events.*

INTRODUCTION

Treatment of chronic hepatitis C had always been challenging, continuously undergoing new trials and tests with an objective to achieve better SVR rates. Previously conventional interferon was the standard treatment for chronic hepatitis C with several limitations and less than adequate SVR rates. With the advent of new direct acting antiviral drugs, the above targets have been achieved in a much improved manner.

DAA are divided into four categories, i.e. NS3/4A protease inhibitors, NS5A protease inhibitors, NS5B nucleoside type polymerase inhibitors and NS5B non-nucleoside type polymerase inhibitors. This group of drugs is not indicated as monotherapy and is always recommended to be given in combination with pegylated interferon and ribavirin to minimize resistance rates. Combination regimens containing protease and polymerase inhibitors have achieved SVR rates of upto 95% and 99%, respectively.[1-8]

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Sofosbuvir belongs to class 3 direct acting antivirals. It is actually a prodrug, metabolized into direct acting antiviral metabolites that inhibit HCV NS5B RNA-dependent polymerase, which is required for viral replication. Sofosbuvir has activity against genotypes 1, 2, 3 and 4.[9] On the other hand metabolites of sofosbuvir do not have inhibitory effects on human DNA or RNA polymerases, which probably is the reason for better safety profile.[10] Majority of the drug is excreted through kidneys, hence limitation of its use in patients with advanced renal disease and a scarce data availability in this aspect. Above all, studies conducted in western population have proven to be far safer when using sofosbuvir combination therapy as compared to interferon-based regimen, particularly neuropsychiatric symptoms and bone marrow suppressive effects.[11,12]

The purpose of this study was to analyze the adverse events related to sofosbuvir in Southeast Asian population and to compare it with western data whether the two sets of populations differ in their representation which may be further related to different metabolic rates and renal excretion in widely separate ethnicities.

METHODOLOGY

The study was carried out in the Department of Medicine, Gastroenterology Division, Shalamar Hospital, Lahore from September 2015 to May 2016. It was a prospective

observational study. Patients of chronic hepatitis C who were visiting the gastroenterology outpatient department and started on sofosbuvir combination therapy were enrolled by simple convenient sampling. Equal number of male and female patients were selected. Study group included both treatment-*naïve* and patients undergoing retreatment, majority being treatment-*naïve*.

Patients were followed at baseline, i.e. before start of treatment and thereafter at 4-week, 12-week and 24-week, respectively. A questionnaire comprising of a set of symptomatology was introduced and asked each time they reported on proposed visit. The subjects were enquired about these complaints either not present previously or reported as aggravation of previous symptoms. Similarly, patients were also directed to mark severity of symptoms as mild, moderate or severe, mild being not limiting daily life activities and severe as a consideration to discontinue treatment. Patients were serially screened for cytopenias, derangements in liver and renal functions on each visit. Documentation of a new or worsening of already existing co-morbid major organ dysfunction was done by thorough clinical examination. Patients were also screened for HCV genotype as well as status of underlying liver disease. Those with comorbid conditions were directed to give complete information regarding other medications they were currently taking. Patients with end-stage renal disease (ESRD) were excluded from the study.

Data was entered and analyzed in SPSS version 20. Mean and standard deviation was calculated for age. Percentages and frequencies were described for subjective complaints. Frequency of absolute neutropenia ANC <1500/mm^3, marked thrombo-cytopenia <50,000/mm^3, drop in hemoglobin >2 g/dl, rise in ALT >2 times UNL and bilirubin elevation >2 mg/dl that developed during therapy was also described. Number of deaths resulting from above complications were noted. P-value was calculated using test for two populations propotions, any value of $\leq$0.05 was taken as significant.

RESULTS

A total of 200 patients with chronic hepatitis C receiving sofosbuvir-based regimen were enrolled in the study. Initially, equal number of males and females were selected. Out of these, three female and one male patients were lost to follow-up. The mean age was 46 ± 10 years. One hundred and two (52%) patients were treatment-naïve, while rest of individuals (94%) were on retreatment either because of non-responder status or relapse of HCV. Fifty-seven (29%) patients had decompensated liver disease. Four (2%) patients received triple therapy consisting of sofosbuvir, ribavirin, and peg INF; while rest of patients received dual therapy. All the study patients were of genotype 3 except 2% of patients receiving triple therapy who had genotype 1.

Table I: Comparative analysis of subjective symptoms.

Symptom	Ribavirin+ Sofosbuvir[a] (n=250)	Ribavirin+ Sofosbuvir[b] (n=192)	p-value
Fatigue	75 (30%)	106 (55%)	<0.0001
Myalgia	22 (9%)	85 (44.2%)	<0.0001
Fever	10 (4%)	81 (42%)	<0.0001
Chills	5 (2%)	27 (14%)	<0.0001
Flu	15 (6%)	4 (2%)	0.044
Headache	75 (30%)	72 (37.5%)	0.096
Insomnia	40 (16%)	98 (51%)	<0.0001
Asthenia	52 (21%)	40 (20.8%)	0.992
Nausea	32 (13%)	96 (50%)	<0.0001
Rash	22 (9%)	32 (16.6%)	0.012
Pruiritis	67 (27%)	65 (33.8%)	0.107
Diarrhoea	30 (12%)	15 (8%)	0.149

Comparison done between study groups receiving similar regimens; a=data from Gilead Sciences, December 2013[10]; b=data from study at Shalamar Institute of Health Sciences.

Table II: Comparative analysis of hematological parameters.

Parameters	Ribavirin$^+$ Sofosbuvir[a] (n=250)	Ribavirin$^+$ Sofosbuvir[b] (n=192)	p-value
Fall in hemoglobin			
>2 g - 5g/dl	15 (6%)	53 (27.6%)	<0.0001
>5 g/dl	2 (0.8%)	5 (3%)	0.131
Neutropenia			
<1500/mm^3	0%	6 (3%)	0.004
<500/mm^3	0%	1 (0.52%)	0.254
Thrombocutopenia			
<50,000/mm^3	2 (0.8%)	10 (5%)	0.004
<25,000/mm^3	0%	1 (0.52%)	0.254

Comparison done between study groups receiving similar regimens; a=data from Gilead Sciences, December 2013[10]; b=data from study at Shalamar Institute of Health Sciences.

Subjective symptoms like fatigue, myalgia, fever, insomnia and nausea were more frequent. On comparative analysis with a similar study group, a p-value of <0.0001 was recorded; while less common symptoms were flu, diarrhea and rash (Table I). However, none of the symptoms were severe enough to warrant discontinuation as reported by the patients. Among hematological parameters, anemia was the most frequent abnormality developed during treatment. Drop in hemoglobin was seen in 147 (75%) patients (p=0.001), whereas severe anemia having Hb fall >5 g/dl was seen in 8 (4%) of total patients. Absolute neutropenia and thrombocytopenia was noted in 8 (4%) and 12 (6%) individuals, respectively. In later case, all patients were with decompensated cirrhosis. Among these, one patient taking sofosbuvir and ribavirin died due to severe pancytopenia after two months of treatment. Since majority of the study group received dual therapy, so further break up and comparative analysis of this subgroup was done (Table II). No worsening of any of comorbid condition was noted.

DISCUSSION

Treatment of chronic hepatitis C, one of the major burdens of infectious diseases and associated with life

threatening complications, is undergoing continuous research process with an aim to achieve an optimum combination of therapy and minimal or no associated adverse events. Until now, direct acting antiviral drugs have come out with near-desirable results. One of the class of direct acting antivirals has activity against NS5B polymerase, which is essential for viral replication. sofosbuvir is one of them. As already described, it is a prodrug and needs hepatic conversion to active metabolites. Its metabolite uridine analog triphosphate acts as chain terminator. Sofosbuvir has activity against genotype 1, 2, 3 and 4 of hepatitis C. Majority of the agent is excreted via renal pathway and a small percentage may be excreted in feces.[9] Although many studies propose that there is no significant difference in pharmacokinetics of this agent in variant ethnicities, gender, and age groups; but ongoing studies and research process is required to validate it.[13] The purpose of this study was to evaluate adverse events secondary to sofosbuvir-based therapy in a subgroup of Pakistani population.

Chronic hepatitis treatment has always faced challenging side effects with past therapies, especially with interferon and ribavirin. Ribavirin induced anemia could not be off set yet because of its indispensible requirement in chronic HCV therapy until recently, although newer guidelines by American Association of Study of Liver Diseases (AASLD) have recommended treatment regimens containing other newer antivirals in place of ribavirin; but such regimens have limited availability in developing countries at present. However, interferon has been definitely replaced with much more better options.[14,15] Previously, 10-17% of patients turned out with serious adverse events due to interferon. Similarly, 18-27% and 3-4% reported with neutropenia and thrombocytopenia, respectively.[16,17] So apart from neuropsychiatric symptoms, bone marrow suppression was one of the major contributor to pre-mature termination of treatment.

Although among various classes of direct acting antiviral agents, treatment related adverse events are also variable. Still sofosbuvir has been reported to have a better safety profile with very smart SVR rates. Fatigue, headache and nausea have been reported in 30%, 30% and 13% of individuals receiving sofosbuvir and ribavirin for 24 weeks, respectively.[10] The present study reported these symptoms at a frequency of 55%, 37% and 50%, respectively. The percentage of symptomatology rises proportionately on addition of pegylated-INF. Less reported complaints were rash, flu and chills comprising 16%, 2% and 14%, respectively as compared to 6%, 9% and 2% in international studies.[10] Although the study population showed higher frequencies of subjective complaints, however, none of the above symptoms were severe enough which resulted in treatment discontinuation. This reflects that metabolism of sofosbuvir may not be significantly different between these two racial origins, but psychosocial behavior of populations living in underdeveloped countries may vary widely.

The other important area that needs attention is effect on bone marrow and hemoglobin. About 2/3rd of patients receiving ribavirin experience drop in hemoglobin but severe anemia is reported in <1% of individuals.[18] In another study, the percentage of anemia was found to be 6-8% in sofosbuvir-based regimens and 14-23% when peg INF was added.[9] In our study, 27% of patients receiving sofosbuvir and ribavirin combination reported with drop in hb >2g/dl from baseline; out of these, 3% had severe anemia with hb fall >5g/dl. Absolute neutropenia and severe thrombocytopenia were seen in 3% and 5%, respectively. Mild to moderate anemia alongwith neutropenia and thrombocytopenia is more frequent in our population. The last two abnormalities are reported in <1% of patients in studies conducted in the West.[10] However, statistical difference was not significant between two populations when severe cytopenias were analysed. But it has been observed that fall in hemoglobin was more frequent and severe in patients with decompensated cirrhosis as compared to compensated liver disease (hb fall >2g/dl 50% vs. 19%). All cases reporting with severe thrombocytopenia and neutropenia were those with decompensated disease. One of those patients died due to complications as a result of severe pancytopenia during treatment.

There had been no evidence of any untoward effect on various comorbid conditions, 42% of our patients had comorbities, majority being diabetic, hypertensive and a small percentage had ischemic heart disease and hypothyroidism. No worsening of underlying condition was noted.

Sofosbuvir-based regimens have not shown a marked decline in frequency or pattern of symptomatology in this set of population but do reflect improved tolerance and compliance owing to lesser severity of these symptoms. Psychosocial behavior of people living in under-developed countries may be a contributing factor in undue reporting of symptoms. Similarly, ribavirin appears as the major determinant of anemia in this population, again supported by evidence of high percentage of anemia in dual therapy group. In order to further validate these results, larger populations and multicenter studies in this region need to be conducted.

CONCLUSION

In short, mild to moderate anemia and cytopenias were more frequent in the study population as compared to the West and severe cytopenias should be anticipated, especially in those with decompensated liver disease.

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Improving Outcomes of Emergency Bowel Surgery Using NELA Model

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ABSTRACT

Objective: To find outcomes of emergency bowel surgery and review the processes involved in the care of these patients on the same template used in National Emergency Laparotomy Audit (NELA).

Study Design: An audit.

Place and Duration of Study: Surgery Department, The Aga Khan University Hospital, Karachi, from December 2013 to November 2014.

Methodology: Patients undergone emergency bowel surgery during the review period were included. Demographic data, type of admission, ASA grade, urgency of surgery, P-POSSUM score, indication of surgery, length of stay and outcome was recorded. Data was then compared with the data published by NELA team in their first report. P-value for categorical variables was calculated using Chi-square tests.

Results: Although the patients were younger with nearly same spectrum of disease, the mortality rate was significantly more than reported in NELA (24% versus 11%, p=0.004). Comparison showed that care at AKUH was significantly lacking in terms of proper preoperative risk assessment and documentation, case booking to operating room timing, intraoperative goal directed fluid therapy using cardiac output monitoring, postoperative intensive care for highest risk patients and review of elderly patients by MCOP specialist.

Conclusion: This study helped in understanding the deficiencies in the care of patients undergoing emergency bowel surgery and alarmingly poor outcomes in a very systematic manner. In view of results of this study, it is planned to do interventions in the deficient areas to improve care given to these patients and their outcomes with the limited resources of a developing country.

Key Words: *Emergency laparotomy. Outcome. Bowel surgery. NELA model.*

INTRODUCTION

Emergency laparotomy have a high rate of complication than same procedures done in elective setting.[1,2] Outcome depends on structural factors in the area and process factors.[3] The process factors can vary in care of different patient in a single institute resulting in different outcomes. More than 30,000 emergency laparotomies are performed every year in England alone.[4,5] 30-day death rate after emergency bowel surgery is reported to be 15% consistently in reports all around the world, which is 5 times higher than any other type of surgery including elective bowel surgery.[6,7] Modifiable factors in these patients, which can alter the outcome of emergency bowel surgery, are: timely diagnosis, preoperative resuscitation, prompt intervention and perioperative care. These factors vary in different centers all around the world, and can significantly impact the outcome.

National Emergency Laparotomy Audit (NELA), funded by NHS England and Welsh Government, was commissioned in 2011, aimed to collect and publish high-quality comparative information from hospitals in England and Wales. It is a prospective ongoing audit and publishes its report annually. In 2015, it published its first annual report online, which includes patients undergoing emergency bowel surgery in 193 hospitals all over England and Wales from November 2013 to October 2014.[8] In the first report, NELA team has documented some policies which are being followed in high performance centres of NHS, and has labeled these key to their better outcomes.

This study was planned to audit the outcomes of the patients who underwent emergency bowel surgery at AKUH, Karachi during the same period as in first report by NELA and compare these with the same report to see the differences in the two audits. The aim was to find the areas where improvements can be made.

METHODOLOGY

Permission from NELA team was obtained to use the proforma and Inclusion/exclusion criteria as being used by NELA. Exemption for this audit (3849-Sur-ERC-15) was obtained from Ethical Review Committee of Aga Khan University Karachi. Proformas were filled by the investigators after retrospectively reviewing the medical records of patients who underwent emergency bowel

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surgery at AKUH from 1st December 2013 to 30th November 2014. Patients undergoing emergency laparoscopic cholecystectomy, appendectomy, abdominal sepsis due to perforated appendix or emergency hernia repair without bowel resection anastomosis were excluded from the study.

Data was retrospectively collected with the help of Health Information Management System. Files of patients and computerized booking in operating room was used to record exact time of booking the case. Anesthesia preoperative evaluation form was used to record preoperative risk stratification. Preoperative and postoperative P-POSSUM score for predicted morbidity and mortality was calculated using online calculator (www.riskprediction.org.uk).[9]

Data was analysed in SPSS. Mean +/- SD and medians with interquartile ranges were calculated for continuous data, where appropriate, Frequencies with percentages were calculated for categorical variables. Significance calculated by p-value using Chi-square test. P-value <0.05 was considered statistically significant. These variables and outcomes were then compared with the charts in the first report of the national emergency laparotomy audit to find out the compliance of AKUH for each variable, example as in Figure 1.

RESULTS

The total number of files extracted were 89. Fifteen were excluded as those patients had non-obstructing, irreducible hernias, where bowel was not resected. The files included in the first analysis were 74; and after excluding the penetrating trauma patients, the number decreased to 50 in the final analysis. The 193 NHS hospitals had a total of 20,183 patients in that period ranging from <10 patients to 351 patients per institute. So AKUH was 153rd out of 194 institutes with respect to number of patients per year.

Mean age of patients was 53.72 ±18.9 years (Table I). Two-thirds patients were male. Fifty-eight percent population was in younger than 60 years of age. Seventy percent patients belonged to ASA II and ASA III groups. Urgency of surgery was <2 hours in 30%. Eighty-nine percent patients underwent emergency laparotomy as a primary procedure, while 11% needed emergency laparotomy for treatment of a complication of a recent procedure. P-POSSUM predicted mortality risk was >10% in 48% of patients and <10% in 52% of patients.

Most frequent indication of emergency surgery was intestinal obstruction, followed by intestinal perforation. Most frequent primary procedures were stoma formations, followed by small bowel resections.

Mortality rate was 24% in the population as compared to 11% in NELA (p=0.004) (Table II). This rate increased to

Table I: Baseline characteristics.

Characterisation n	NELA 20,183	AKUH 50	p-value
Gender			0.03
Female	10,375 (51%)	18 (36%)	
Male	9,808 (49%)	32 (64%)	
Age in years			0.007
18-39	2,188 (11%)	12 (24%)	
40-49	1,939 (10%)	8 (16%)	
50-59	2,707 (13%)	9 (18%)	
60-69	4,197 (20%)	6 (12%)	
70-79	5,084 (25%)	12 (24%)	
80-89	3,537 (18%)	3 (6%)	
≥90	531 (3%)	0	
Hospital admission type			0.02
Emergency	18,693 (93%)	42 (84%)	
Elective	1,490 (7%)	8 (16%)	
ASA grade			0.29
1	2,097 (10%)	1 (2%)	
2	6,793 (34%)	17 (34%)	
3	7,108 (35%)	18 (36%)	
4	3,747 (19%)	12 (24%)	
5	438 (2%)	2 (4%)	
Urgency of surgery			0.004
<2 hours	1,976 (14%)	15 (30%)	
2-6 hours	5,498 (39%)	13 (26%)	
>6 hours	6460 (46%)	22 (44%)	
Procedure			1.00
Primary procedure	18,034 (89%)	45 (90%)	
Surgery for a complication of a recent procedure	2,149 (11%)	5 (10%)	
Preoperative predicted risk of death within 30 days of surgery (P-POSSUM)			0.54
<5%	7,709 (38%)	15 (30%)	
5.0 - 9.9%	3,315 (16%)	11 (22%)	
10.0 - 24.9%	3,828 (19%)	8 (16%)	
25.0 - 49.9%	2,589 (13%)	9 (18%)	
≥50%	2,742 (14%)	7 (14%)	
Indication for surgery			0.007
Intestinal obstruction	9,811 (49%)	23 (46%)	
Perforation	4,744 (24%)	9 (18%)	
Peritonitis	4,116 (20%)	1 (2%)	
Ischaemia	1,720 (9%)	4 (8%)	
Abdominal abscess	1,332 (7%)		
Sepsis: other	1,474 (7%)	1 (2%)	
Haemorrhage	819 (4%)	1 (2%)	
Colitis	748 (4%)		
Anastomotic leak Intestinal	618 (3%)	1 (2%)	
Fistula	326 (2%)	3 (6%)	
Abdominal wound dehiscence	116 (0.6%)	1 (2%)	
Abdominal compartment syndrome	55 (0.3%)		
Other	1,809 (9%)	6 (12%)	
Surgical Approach			0.06
Open	17,573 (87%)	50 (100%)	
Laparoscopic	1,208 (6%)		
Laparoscopic converted to open	1,215 (6%)		
Laparoscopic-assisted	187 (1%)		
Primary operative procedure			0.014
Small bowel resection	3,420 (17%)	12 (24%)	
Adhesiolysis	3,379 (17%)	4 (8%)	
Colectomy: right	2,573 (13%)	4 (8%)	
Hartmann's procedure	2,562 (13%)	1 (2%)	
Stoma formation	1,148 (6%)	13 (26%)	
Peptic ulcer - suture or repair of perforation	1,138 (6%)	3 (6%)	
Colectomy: subtotal	1,113 (6%)		
Drainage of abscess/collection	588 (3%)		
Colectomy: left (including anterior resection)	578 (3%)	2 (4%)	
Washout only			
Repair of intestinal perforation	532 (3%)	5 (10%)	
Colorectal resection - other	454 (2%)		
Exploratory/relook laparotomy only	440 (2%)	1 (2%)	
Gastric surgery - other	408 (2%)	1 (2%)	
Intestinal bypass Haemostasis	327 (2%)	1 (2%)	
Peptic ulcer oversew of bleed	302 (2%)		
Not amenable to surgery	245 (1%)		
Enterotomy	210 (1%)		
Stoma revision	185 (1%)		
Abdominal wall closure	159 (1%)		
Laparostomy formation	161 (1%)	1 (2%)	
Resection of other intra-abdominal tumour(s)	121 (<1%) 77 (<1%)		
Pancreatic necrosectomy	63 (<1%)	1 (2%)	

53% if age of patient was >70 years. Median length of stay was 8.5 days (IQR 5.75-15).

Table II: Outcome.

Variable	NELA	AKU	p-value
Length of stay			
Days (Median)	11.3 (6.5-20.4)	8.5 (5.75-15)	
Mortality			
Overall	(2254/20183) 11%	(12/50) 24%	0.004
<70 years	(662/11031) 6%	(4/35) 11.4%	0.18
>70 Years	(1592/9152) 17.4%	(8/15) 53%	<0.001

Table III: Postoperative predicted mortality versus actual mortality.

Post op predicted P-Possum mortality risk	No. of patients	Deaths	Actual mortality
<5%	16	0	0%
5.0-9.9%	5	2	40%
10-24.9%	14	4	28.5%
25-49.9%	8	3	37.5%
>50%	6	2	33%

**One patient had on table death.*

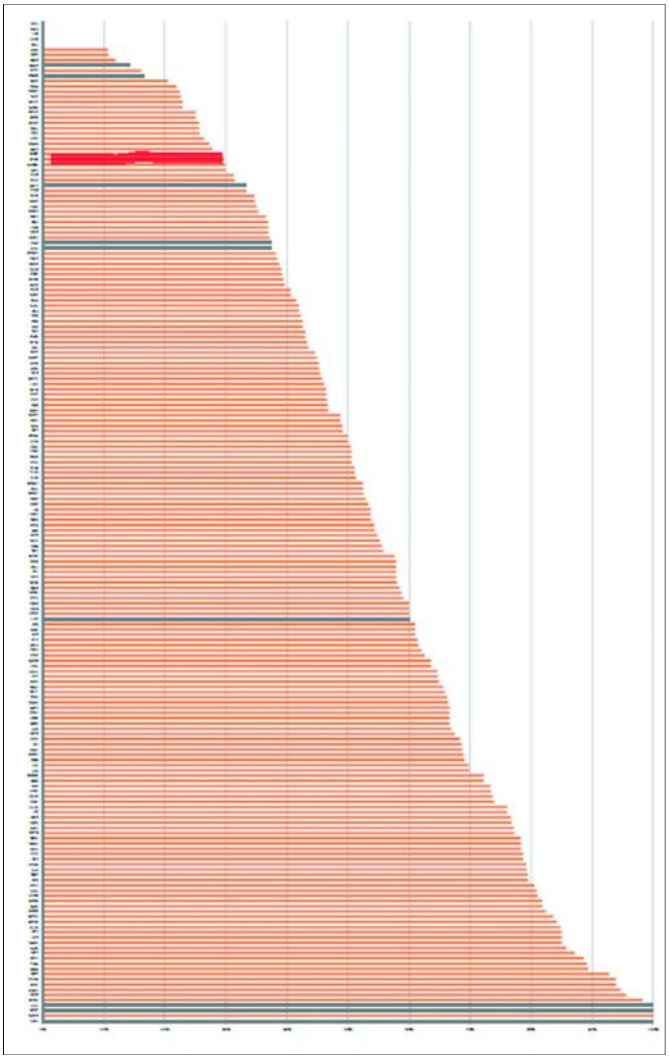

Figure 1: Proportion of patients with risk documented preoperatively. Red line shows position of AKUH among 193 NHS hospitals (30% documentation of risk,156th position). Figure modified with permission from NELA team.

Table IV: Postoperative shifting and outcome.

Post op predicted P-Possum mortality risk	No. of patients	Shifted post op to	Mortality
<5%	16	Ward → 2	None
		SCU → 14	
		ICU → 0	
5.0-9.9%	5	SCU → 5	2
10-24.9%	14	SCU → 9	1
		ICU → 5	1
25-49.9%	8	SCU → 4	2
		ICU → 4	2
>50%	6	SCU → 1	1
		ICU → 5	3

***Out of 6 patients who eventually died, shifted in SCU postoperatively, 2 were made DNR in SCU (due to disseminated malignancy and mesenteric ischemia) while 4 were intubated and shifted to ICU and expired in ICU.*

DISCUSSION

Pakistan is a developing country with poor emergency health care system. Only 44% public secondary and 25% private secondary hospitals have designated emergency rooms.[10] The Aga Khan Univeristy Hospital (AKUH) is a JCIA accredited, 600 bedded tertiary care hospital, providing 24-hour emergency surgery facility in Karachi. General surgery department has a team of residents and an attending consultant-on-call every day. The hospital has two designated emergency operating theaters, functional 24-hour a day with a dedicated team of anesthetists. There is a dedicated Surgical ICU with seven fully equipped beds to provide intensive care under supervision of an intensivist.

This audit showed that although the population was younger than NELA population and risk for mortality is similar of NELA population, the mortality rate was more than twice. Further analysis of mortality patients was done which showed that mortality rate was greater in high risk group than anticipated (Table III), while it was same or even lower than predicted in higher risk group. This poor outcome in relatively lower risk patients lead to the evaluation of AKUH for standard practices which are being followed in NHS high permorfance hospitals.

First practice is review of patients by consultant surgeon within 12 hours of their admission. This variable could not be measured at AKUH because of retrospective nature of audit and the time seen by consultant was missing in 84% of the files. Second practice is reporting of preoperative CT scan by consultant radiologist before surgery. At AKU preoperative CT scan was done in 82% of patients, out of which 66% were reported preoperatively by a consultant radiologist (103rd position, range 95%-3%). Third practice is preoperative risk stratification. At AKU, 80% patients were seen preoperatively by consultant anesthetist. Preoperative risk labeling was one only in 30% (156th position, range 100%-11%). There was no objectivity in this risk stratification.

Fourth practice is booking to operating room, time should be the same as documented at the time of booking of case. At AKUH, it was same in 80% of cases (127th position, Range 100%-48%). Fifth practice is direct supervision of surgery by a consultant surgeon. At AKU 100% of surgeries were directly supervised by the consultant surgeon (first position, range 100-36%). Sixth practice is provision of goal directed fluid therapy intraoperatively using cardiac output monitoring in high risk patients. At AKU, this was not done in any of the patients using cardiac output (176th position). In 60% patient, central venous line and arterial line were used, and in 40% of patients intraoperative monitoring was non-invasive.

Seventh practice is provision of intensive care postoperatively to highest risk patients (predicted mortality ≥10% and age ≥70 years). Twenty-eight percent of all patients undergoing emergency bowel surgery were shifted to ICU postoperatively (176th position, range 96-23%). Fifty percent of patients with predicted mortality ≥10% were shifted directly to ICU postoperatively (178th position, range 100-52%). While only 53.3% of patients aged more than 70 years were shifted to ICU postoperatively. The eighth variable was postoperative review of patients aged ≥70 years by MCOP physician. None of the patients was seen by a geriatric specialist at AKU postoperatively (151st position, range 100-0%).

This study identified a number of variables where there were deficiencies as compared to NHS hospitals. Although the patients are younger and spectrum and severity of disease similar to those reported in NELA, there is very high mortality in this population. The practices which were being practiced by the high performance centres of NHS were deficient at AKUH.

Preoperative evaluation by a consultant surgeon within 12 hours of admission is important for early decision making.[12,13] Delay in review can result in delayed diagnosis or intervention in some patients.[14,15] Being JCIA accredited hospital, the policy at AKU is consultant review within 24 hours of admission.

To improve outcomes, it is now a consensus among General Surgery, Anesthesia and ICU departments to change the practices. All CT scans will be reported by consultant radiologist preoperatively, after making some changes in radiology oncall system and hospital has provided home access to PACS to the radiology consultants. Now, it is mandatory to calculate mortality risk using P-POSSUM score and documenting it preoperatively. This information will help in shared decision-making for families and physicians. Compliance of booking-to-OR-time will be improved by operating room coordinators by making separate queue for emergency bowel surgery patients and giving them preference on routine add on patients.

There is an agreement with intensivists to daily review all these patients in HDU until they are stabilized. This will help in improving outcome of these high risk patients and to predict the need of ventilator support rather than providing it when the patient has crashed. Internal medicine department had been requested to nominate a specific internist with interest in geriatrics to help us.

There is a plan to reaudit emergency laparotomy result prospectively and see the effect of these changes. Although this study was evaluating the data which was prospectively recorded, yet it had some artifacts of retrospective study which will be covered in the next audit. The model provided by NELA is a simple and applicable model to every hospital providing emergency surgical care and that every hospital should audit its outcomes upon this model and find its way forward towards improving outcomes.

CONCLUSION

This study helped in understanding the deficiencies in the care of patients undergoing emergency bowel surgery and alarmingly poor outcomes in a very systematic manner. In view of results of this study, it is planned to carry out interventions in the deficient areas to improve care given to these patients and their outcomes with the limited resources of a developing country.

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CASE REPORT

Bladder Distension as a Cause of Abdominal Compartment Syndrome

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ABSTRACT

Abdominal compartment syndrome (ACS) is increasingly identified in critically ill patient and its harmful effects are well documented. The disparity among the pressure, volume in abdominal cavity and its contents, results in ACS. The actual incidence of ACS is not known. However, it has been observed predominantly in patients with severe blunt and penetrating abdominal trauma, ruptured abdominal aortic aneurysms, retro- and intra-peritoneal hemorrhage, pneumoperitoneum, neoplasm, pancreatitis, ascites and multiple bone fracture. We present a case of 40-year female who underwent emergency cesarean section and developed abdominal compartment syndrome due to urinary bladder distension secondary to blockade of urinary catheter with blood clots. This is a very unusual cause of ACS.

Key Words: *Abdominal compartment syndrome. Intra-abdominal pressure. Urinary catheter.*

INTRODUCTION

Abdominal compartment syndrome (ACS) has been increasingly recognized in critically ill patient. If unrecognized and untreated, it is associated with high mortality.[1] ACS may be caused by conditions that increase the pressure in the abdomen due to increase in abdominal or retro-peritoneal volume/contents.[2] This increased intra-abdominal pressure (IAP) adversely affects cardiovascular and respiratory systems with resultant decrease in venous return, cardiac output, and increased airway pressures with hypoxia and hypercapnia along with decreased chest compliance. As a result of increased intra-abdominal pressure, perfusion to major abdominal organs is compromised and renal circulation also reduce with resultant decrease in urine output.[3,4]

Classical examples of intra-abdominal hypertension (IAH) and ACS were described after damage control surgery in trauma and in those patients undergoing massive fluid resuscitation for any reasons. Incidence of ACS after abdominal surgery varies from low after elective surgery to considerably high after emergency procedures.[5] The clinical suspicion of raised intra-abdominal pressure in patients with associated risk factors is the key for the early diagnosis and subsequent treatment of ACS by abdominal decompression, in order to avoid multiple organ failure.[6]

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We report a case of abdominal compartment syndrome caused by postoperative urinary bladder distension secondary to blockade of urinary catheter with blood clots in a 40-year female patient who underwent emergency cesarean section. As per our knowledge, this is the only case report of abdominal compartment syndrome due to bladder obstruction.

CASE REPORT

A 40-year female with no known comorbid condition, was planned for elective cesarean section due to type IV placenta previa, poly hydramnios and poor obstetric history.

Two days earlier to her planned procedure, she presented in labour room with a history of four hours of labour pain for which emergency lower cesarean section was undertaken. Her preoperative assessment was normal and she underwent surgery under general anaesthesia.

Intraoperative course was complicated by massive blood loss (6-7 liters) for which she was given 13 units of packed red blood cell (PRBC), 11 units of fresh frozen plasma (FFP), 8 units of platelets, 8 units of cryoprecipitate, 12 litres colloid, and 3 litres of crystalloid. Hemostasis was difficult to achieved so a cesarean hysterectomy was performed as a rescue. A diagnosis of disseminated intravascular coagulopathy was made clinically; abdominal closure was difficult, therefore, the wound was left open and covered with soaked abdominal packs. She required ionotropic support with dopamine at 20 mcg/kg/min and epinephrine at 0.05 mcg/kg/min. She was electively ventilated postoperatively. Patient was kept sedated. On inotropic support with dopamine and epinephrine, she was maintaining her systolic arterial pressure between 90-110 mmHg, with a CVP of 5-7 cm H_2O. Her abdominal girth was monitored hourly. She received 6 units of red cells concentrate, 10 units of cryoprecipatate, 4 units of fresh frozen plasma and six units of platelets within first

18 hours postoperatively. She became oliguric followed by an increase in the abdominal girth from a baseline of 93 cm to 101 cm. Subsequent to that, her CVP also dropped to 2 cm H_2O and she became severely hypotensive with a blood pressure of 56/32 mmHg and heart rate went up to 135/minute. A surgical review was sought immediately and provisional diagnosis of intra-abdominal bleeding was made. For the management of this life-threatening peri-arrest condition, she was immediately transferred to operating room. Re-exploratory laparotomy was negative for any active bleeding with minimal amount of intraperitoneal blood stained fluid. The most striking finding was an enormously distended urinary bladder, extending from pubic symphysis up to umbilicus, which was confirmed by needle aspiration. On examination, it was found that urinary catheter was blocked with blood clots which was replaced and drained about 1400 ml urine.

Following the decompression of urinary bladder, immediate improvement was noticed in her blood pressure with decreasing requirement of dopamine. Her blood pressure increased to 140/70 mmHg and heart rate came down to 110 per minute, and the urine output increased to 200 ml/hour. The peak airway pressure also dropped from 30 cm H_2O to 20 cm H_2O. Her CVP increased to 12 cm H_2O. It was decided to put abdominal pack and not to close the abdominal wound at this stage. Patient was transferred to post anaesthesia care unit for elective ventilation. On the very next day, her inotropes were successfully tapered off. Two days later, patient was again taken to operating room for removal of abdominal packs and wound closure. She remained stable and was extubated on the next day and shifted to high dependency unit and later discharged home after 10 days in good condition.

DISCUSSION

ACS is a disorder associated with significant morbidity and mortality, refers to organ dysfunction resulting from increased intra-abdominal pressure (IAP).[7] ACS, defined by world society of abdominal compartment syndrome as intra-abdominal pressure of at least 20 mm Hg with dysfunction of at least one thoraco-abdominal organ.[8] This increase in intra-abdominal pressure leads to organ dysfunction involving primarily heart and lungs.[9] The reason for cardiac symptoms are multifactorial with decreased preload due to compression of both the portal vein and inferior vena cava, and an increased afterload due to increased systemic vascular resistance, leading to a decreased stroke volume and thus decreased cardiac output and cardiac arrest with pulseless electrical activity (PEA). The main reasons for respiratory compromise are the combination of cephaled displacement of the diaphragm, with resultant decrease in total and residual lung volume and lung compliance.[7]

The standard recommended method for diagnosis and management of IAH and ACS is based on accurate and repeated measurements of its surrogate pressure; intra-vesical urinary bladder pressure.[3] But in peri-arrest situations, when there is no time to measure IAP, clinical signs of organ dysfunctions, and hypoperfusion with low cardiac output, hypoxia, tense distended abdomen, progressive oliguria or anuria are sufficient to justify emergency decompression.[10]

In this case, the diagnosis was also made by clinical parameters only because of deteriorating and near crash situation which developed suddenly. The intra-vesical pressure was not measured as she had to be rushed to operating room while cardiopulmonary resuscitation was actively going on.

This patient had signs and symptoms that could suggest hypovolemic/septic shock (tachycardia, severe hypo-tension, tachypnea, fever and decreased urine output). However, following emergency surgical decompression, the patient had a prompt normalization of her vital signs and urine output. In view of the above, a distended urinary bladder, due to catheter block, was the most likely diagnosis of her deterioration. There was no case report of ACS secondary to urinary catheter obstruction.

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Congenital Synechia and Syngnathia: Two Case Reports

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ABSTRACT

This case report presents two cases of rare congenital malformation, i.e syngnathia. First case is of 2-day infant with bilateral fusion of maxilla and mandible, leaving a small anterior portion. After consultation with other concerned specialties, early intervention was planned and fusion was released to facilitate feeding. The infant suffered from frequent respiratory tract infections and subsequently died at the age of ten months. The second case is of 8-month baby girl with unilateral congenital maxillomandibular bony fusion without any other anomaly. She underwent general anesthesia for thorough examination and release of soft tissue union. Second surgery was performed after few months for removal of bony fusion. Good mouth opening was seen on 1 month follow-up.

Key Words: *Congenital unilateral maxillo-mandibulo-zygomatic fusion. Syngnathia.*

INTRODUCTION

There are many craniofacial anomalies that can affect not only quality of life but also jeopardize individual's survival. Syngnathia is a rare condition of fusion of jaws. When it involves fusion of soft tissues only, it is called synechia and when it involves bony fusion of maxilla and mandible, it is termed as synostosis.[1] The exact incidence and etiology is unknown. Fusion can be partial or complete, unilateral or bilateral, depending upon the extent of the fusion. Syngnathia can occur independently or it can occur with other organ anomalies. It may be associated with other facial malformations such as facial hemiatrophy, temporomandibular joint ankylosis, cleft lip and palate, microglossia, micrognathia or limb anomalies.[2] It can lead to limited or no mouth opening, feeding and airway difficulties. The limited number of reported cases restricts establishment of its treatment protocol. Early intervention is required to avoid and treat nutritional, feeding and growth related problems.[3] If left untreated, it can lead to severe malnutrition, ectopic eruption of teeth, severe jaw deformities, which will require extensive corrective surgeries later on.

CASE REPORT

Case 1: First patient of syngnathia was seen in January 2006. A call was recieved from neonatal Intensive Care Unit (ICU), Military Hospital, Rawalpindi, that there is a 2-day infant whose mouth did not open. Infant's examination revealed that he had syngnathia, i.e fusion of maxilla and mandible bilaterally, leaving only small anterior opening. The case was discussed with pediatrician and neonatologist who suggested early surgery to facilitate feeding and to prevent aspiration of secretions. Pre-anesthesia evaluation was carried out and surgery was planned (Figure 1). Initially, inhalational anesthesia was induced with the help of nasopharyngeal airway. Once the mouth opening was created, orotracheal intubation was carried out and general anesthesia was continued. Soft tissue incision was given at the fusion region of maxilla and mandible. Bone was separated by gentle strokes of chisels and mallets. Recovery was uneventful. The infant started to take feed and had good mouth opening and remained on regular follow-up (Figure 2). He had recurrent respiratory tract infections, for which he was managed by the pediatrician. However, he eventually died of respiratory tract infections at the age of 10 months.

Case 2: A 3-day female was referred from a Military Hospital in Tarbela to Armed Forces Institute of Dentistry due to inability to open her mouth. Her weight at birth was 2.7kg and there was late first cry. She was born with normal delivery. She was second child of her parents. Her elder brother suffered from G-6PD deficiency. Her parents were distant relatives. Her mother had a history of fall from motorbike during 6th month of pregnancy. There was no history of alcohol consumption or ingestion of any drug during pregnancy except iron supplements and multivitamins.

The physical examination revealed synechia of left upper and lower gums starting from incisor region. Examination of palate and tongue could not be achieved preoperatively. There was no mouth opening and baby was being fed by a nasogastric tube. Examination of rest of baby's body revealed no other deformity; thus was referred to a neonatologist to evaluate any other congenital cardiac, neural or respiratory deformity. There was no sign of any other congenital deformity.

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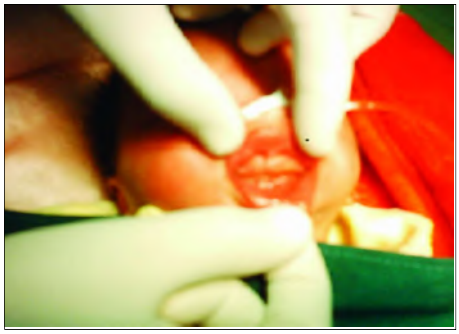

Figure 1: Examination under general anesthesia revealed fusion of upper and lower arches leaving small anterior portion.

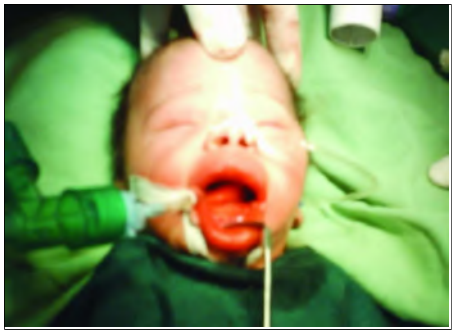

Figure 2: Adequate mouth opening achieved after removal of bony fusion.

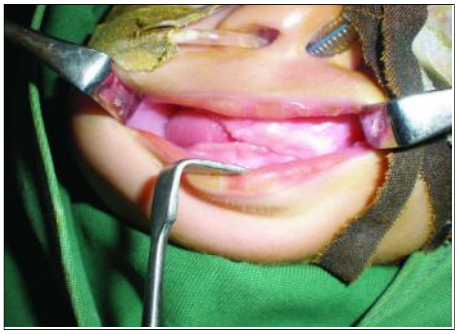

Figure 3: Examination under general anesthesia revealed fusion of upper and lower arches.

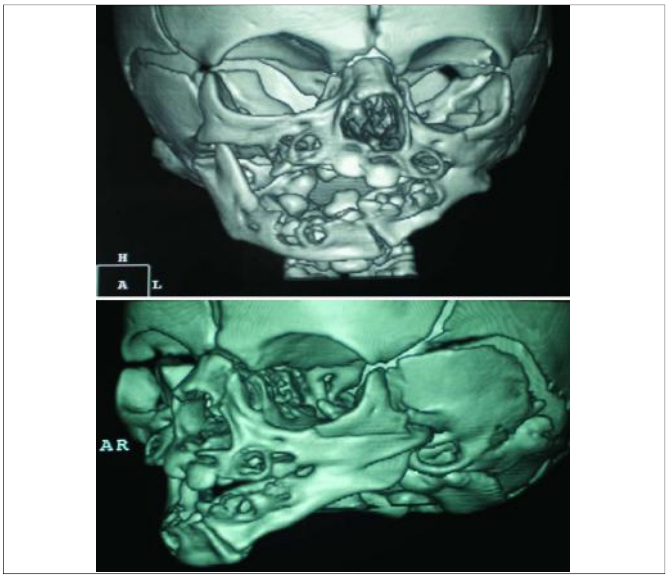

Figure 4: CT scan.

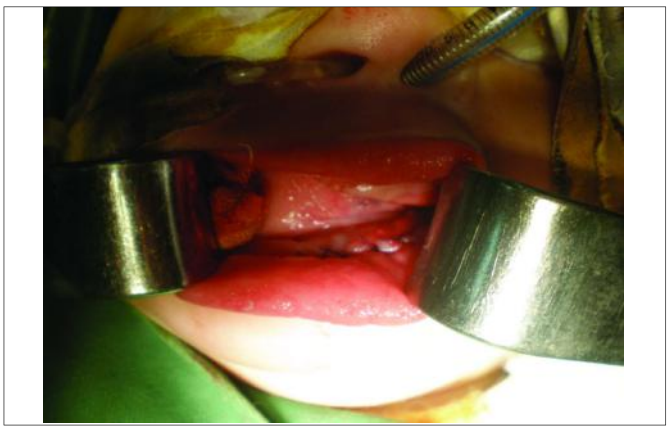

Figure 5: Mouth opening of 18mm was achieved after removal of bony fusion.

The baby girl underwent general anesthesia for release of synechia and detailed examination (Figure 3). Synechia in anterior region was released but mouth opening could not be achieved. As there was no breathing and feeding difficulty so the operation was delayed upto the age of 8 months. The surgery was performed to prevent secondary growth effects due to bony fusion.

Computer tomography (CT) scans with axial and coronal sections were ordered with 3D reconstruction, which revealed bony fusion between maxilla, mandible and zygomatic complex on left side. Temporomandibular joint space was normal and no ankylosis was present. Mandible was hypoplastic on left side.Tooth buds were present (Figure 4).

The baby was operated again at 8 months of age with blind awake intubation. Intraoral U-shaped incision starting from lower alveolar ridge along external oblique ridge to upper alveolar ridge was given, mucoperiosteal flap was raised and bony fusion was identified. Coronoid process and ramus on affected side were fused with zygoma. The alveolar processes of maxilla and mandible were not fused. Condyle was easily identifiable. Bony fusion was removed using piezosurgery and small chisels and mallets. Mouth opening of 18 mm was achieved (Figure 5). Incision was closed with 3.0 vicryl. Periodontal dressing was placed between maxillary and mandibular arches to prevent fusion. After one week of operation, mouth opening exercises were performed under sedation once, and later by the parents. Mouth opening remained same at 10th day and 1 month follow-up.

DISCUSSION

Congenital maxillomandibular fusion is rare and its extent variable. It can be present along with syndromes or it can manifest alone.[4] The exact etiology and pathogenesis is unknown, and several hypothesis have been proposed. Because of its rarity and limited knowledge, standardized management protocol of such patients is not available. Some cases have been operated within few hours or few days of birth, others at few months to even 74 years of age.[5] Its management is both challenging and risky. The greater the extent of bony union, the greater the risk involved in surgery.

Dawson, *et al*.[6] classified syngnathia as follows:[6]

Type 1: simple syngnathia with other anomaly in head and neck.

Type 2: complex syngnathia.

Type 2a: syngnathia co-existent with aglossia.

Type2b: syngnathia co-existent with agenesis or hypoplasia of proximal mandible.

However, Laster, *et al*.[7] proposed following classification of syngnathia:[7]

Type 1a: simple anterior syngnathia-bony fusion of ridge only and without other congenital deformity in head and neck.

Type 1b: complex anterior syngnathia-bony fusion of alveolar ridges only and associated other congenital deformity in head and neck.

Type 2a: simple zygomatico-mandibular syngnathia-bony fusion of mandible to zygomatic complex causing only mandibular micrognathia.

Type 2b: complex zygomatico-mandibular syngnathia-bony fusion of mandible to zygomatic complex and associated with clefts and tmj ankylosis.

The patients presented here had zygomaticomandibular syngnathia with no other abnormality and belonged to type 2a category of classification by Laster, *et al*.[7]

Difficulty in feeding, difficulty in intubation, risk of aspiration, rarity of this disease, presentation of patients at late age, all have resulted in surgical treatment at different ages.[8] Some cases treated at late stage had resulted in severe malnutrition and stunted growth, asymmetry as well as disordered eruption of dentition away from alveoli.[9] A case of old lady treated at 74 years of age with no considerable asymmetry has also been reported. She, however, was edentulous and had no history of tooth eruption or extraction.

Rarely, syngnathia is associated with duplication of the craniofacial midline including hypophyseal duplication.

General anaesthesia is a challenge in such cases because of impossibility of orotracheal intubation. Nasotracheal intubation or indirect laryngoscopy, using fibre-optic visualization, is method of choice. If this results in failure, availability of equipment and experienced personnel for an emergency tracheostomy is mandatory.

In conclusion, early surgical intervention should be carried out to avoid feeding difficulty and risk of aspiration and to reduce cosmetic deformity, which may require further surgical management.

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Lupus Enteritis: An Atypical Initial Presentation of Systemic Lupus Erythematosus

Hina Mushtaq, Shahla Razzaque and Kamal Ahmed

ABSTRACT

Systemic lupus erythematosus may rarely present initially with gastroenteric features labelled as lupus enteritis, that may lead to serious complications, if it remains undiagnosed for a long period of time. It is difficult to diagnose because the clinical picture of lupus enteritis mimics gastroenteritis. The diagnosis is made on radiological findings, rather than histopathology, and supported by autoimmune profile. Here is a case of a 40-year lady who presented with diarrhea and vomiting that was unresponsive to treatment with intravenous antibiotics. The diagnosis of lupus enteritis was made on the basis of CT scan abdomen, that showed classic target sign due to bowel edema. There was non-specific inflammation found in the biopsy specimen taken on colonoscopy and her autoimmune workup showed ANA and anti-ds-DNA positive. She was treated with high dose of intravenous steroids and recovered.

Key Words: *Systemic lupus erythematosus. Gastroenteritis. Colonoscopy. Steroids. Histopathology.*

INTRODUCTION

Lupus enteritis is a very rare and atypical initial presentation of systemic lupus erythematosus (SLE) with a reported incidence of 0.2% to 53%,[1] and a mortality rate as high as 53%, if complicated, or if treatment is delayed.[2] There is vasculitis involving the small bowel, with non-specific clinical features like those of simple gastroenteritis. CT scan abdomen is the gold standard for diagnosis, as histopathology does not add much to the diagnosis. It is steroid-responsive, with preference for intravenous route.

CASE REPORT

The present report describes this uncommon presentation of SLE in an adult lady. This is a case report of a 40-year lady who presented with two weeks history of colicky abdominal pain, loose stools and vomiting. She had 15-16 episodes of loose stools per day, watery in consistency with no blood or mucus in it. Vomiting was bilious in nature and was not associated with hematemesis or melena. At the time of presentation in emergency, the patient was tachycardiac, afebrile and had distended, non-tender abdomen with diminished bowel sounds.

Her laboratory workup showed WBC=20X109/l, Hb=11.2 g/dl, hematocrit=46%, neutrophils=92% and platelet count=221X109/l, PT=13 sec and INR=1.3. Serum biochemistry showed serum sodium of 146 meq/ml and potassium of 3.5 meq/ml. Blood urea nitrogen and creatinine were 80 mg/dl and 1.4 mg/dl, respectively. ESR was 6 mm after 1st hour. C-reactive protein was elevated. Liver function tests were within the normal limits. Subsequent blood cultures, stool cultures, urinalysis, chest radiographs came out to be normal. Abdominal radiographs showed non-specific gas pattern, with no signs of bowel dilatation or perforation. Abdominal sonography showed mild ascites. Colonoscopy showed patchy pan-colitis. Colonic biopsy showed mild chronic non-specific inflammation. Computed tomography (CT) showed mild ascites, small bowel moderately dilated, thickening edematous small bowel loops and target sign (Figure 1).

The patient was admitted and was given intravenous hydration and broad spectrum antibiotics for presumed infectious enteritis; however, the symptoms did not improve and she continued to have abdominal pain and loose motions. After excluding other causes of enteritis, the patient's autoimmune profile was sent, thinking of lupus enteritis as a differential, based on CT image findings. Laboratory data showed a low serum complement level (C3=24.6mg/dl, C4=4.8mg/dl), positive antinuclear antibody ++, antinucleosome ++, antihistones ++, AMA-M2 ++ and positive anti-ds-DNA antibodies with a titer of 102.65 +ve.

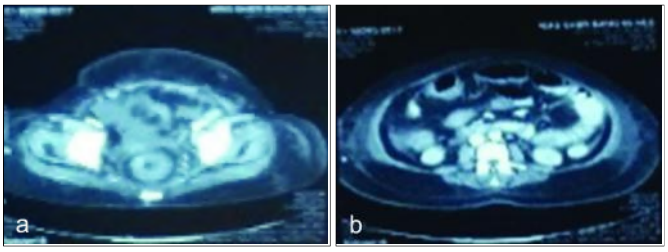

Figure 1: CT scan image showing (a) bowel wall thickening and (b) increased mesenteric fat attenaution.

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The diagnosis was made as lupus enteritis on the basis of autoimmune profile and CT scan findings. She was given intravenous methylprednisolone 1 gram per day for three days, followed by 80 mg per day. She had a dramatic response on steroids, and her symptoms resolved.

DISCUSSION

SLE is an autoimmune disease with diverse manifestations, that can be indolent to fulminant, affecting any organ system. In a woman of childbearing age presenting with fever, rash and joint pain, makes one peep into the the diagnosis of SLE.[3] Other presentations can be renal, neuropsychiatric, pulmonary, cardiac, hematologic or gastrointestinal symptoms. Gastrointestinal involvement by SLE is likely to be misdiagnosed, as it mimics abdominal tuberculosis or chronic gastroenteritis.[4] Lupus enteritis is a dreadful complication of SLE, that can be life-threatening if not treated promptly. It can lead to ischemia, perforation, and infarction.[5] The overall incidence of lupus enteritis is reported to be 0.2-53% in SLE patients.[6] A retrospective study conducted in St. Louis Hospital, Paris, France concluded that the clinical symptoms of lupus enteritis mostly include abdominal pain, followed by vomiting and less commonly diarrhea with fever as the least common presentation.[5] The study also highlighted the laboratory features of the lupus activity including low complement levels, anemia, leukocytopenia or lymphocytopenia, thrombocytopenia, normal CRP and proteinuria.

There is extensive bowel wall involvement of any vascular territory. It mostly presents with jejunal (80%) or ileal (85%) involvement.[7] The pathogenesis still remains unclear, but immune complex deposits leading to complement activation are thought to be the culprit for bowel wall edema.[8] Histopathologic diagnosis is made very infrequently on endoscopic biopsies, possibly because only superficial tissue is obtained, making the features of CT scan abdomen, a key to the diagnoses.[5] Biopsies can be done to rule out alternative diagnosis like tuberculosis.[5] The three classic patterns suggestive of lupus enteritis on CT scan include target sign which is the bowel wall thickening >3 mm, Coombs sign is the engorgement of vascular bed, and there is increased attenuation of mesenteric fat.[9]

The first line treatment for lupus enteritis is high-dose intravenous (IV) methylprednisolone along with bowel rest.[1] The supportive therapy include IV fluids and proton pump inhibitors along with heparin, in the presence of antiphospholipid antibodies.[5] Surgical exploration should be considered in case of complications like necrosis or perforation. Oral corticosteroids are started after remission of the acute phase clinically. The use of cyclophosphamide or mycophenolate mofetil is recommended in cases resistance to corticosteroids or when there is another organ, like nervous system involvement.[5] For long term maintenance therapy hydroxychloroquine, mycophenolate mofetil and azathioprine are given along with low dose corticosteroids. Cyclophosphamide is also recommended in cases of relapse. In cases of cyclophosphamide failure, no further treatment option is yet recommended, although there is a case of recurrent and resistant lupus enteritis reported by Shirai, *et al*. that responded well to tacroliumus.[10]

This case was successfully treated with intravenous methylprednisolone. Early diagnosis is of great importance for the optimal management of lupus enteritis, as occurred in this case.

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Endobronchial Mucosal Neuroma with Sarcoidosis

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ABSTRACT

A first case of endobronchial mucosal neuroma with sarcoidosis is hereby reported. A 67-year female patient, who was diagnosed as sarcoidosis previously, was admitted to our hospital with symptoms of dyspnea, chest pain and fatigue. Middle lobe atelectasis and endobronchial lesion were observed in thorax computed tomography (CT). Fiberoptic bronchoscopy revealed polypoid lesions and histopathological examination of biopsy material showed clustered nerve bundles consistent with mucosal neuroma and non-necrotising granulomas consistent with sarcoidosis. Mucosal neuromas are pathognomonic features of multiple endocrine neoplasia (MEN) type 2B. But other components of MEN type 2B such as medullary tyroid carcinoma or pheochromocytoma were not detected in our patient. Hence, a diagnosis of solitary mucosal neuroma and sarcoidosis in the bronchi was made.

Key Words: *Neuroma. Sarcoidosis. Bronchus. Mucosa.*

INTRODUCTION

Mucosal neuromas are benign lesions originated from peripheral nerves.[1] Histologically, clustering bundles of nerve cells are observed in submucosa. Mucosal neuromas are pathognomonic features of multiple endocrine neoplasia (MEN) type 2B or MEN3. MEN 2B is a rare autosomal dominant disease with components of medullary thyroid cancer, pheochromocytoma and mucosal neuromas. Mucosal neuromas, as an isolated finding without other components of MEN 2, are very rare and have been reported as case reports in the literature. Solitary mucosal neuromas are reported in rectosigmoid colon, bronchi, conjunctiva, larynx, tongue and hard palate.[1-6] No report of a mucosal neuroma, together with sarcoidosis located in the bronchi, is reported in literature till date.

CASE REPORT

A 67-year female, previously diagnosed as sarcoidosis, was admitted with dyspnea, chest pain and fatigue of 3 months duration. She was diagnosed as sarcoidosis on transbronchial biopsy 11 years ago and received corticosteroid therapy 2 times because of activation of disease. Patient's daughter was also diagnosed as pulmonary sarcoidosis. Physical examination was normal. Laboratory tests were in normal limits. Pulmonary function tests were obstructive. Chest X-ray was compatible with stage 2 sarcoidosis according to Scadding stage. Middle lobe atelectasis and endobronchial polypoid lesions were observed in computed tomography (CT) of thorax (Figure 1). Fiberoptic bronchoscopy (FOB) was performed. Widespread mucosal edema in both bronchial systems, narrowing of right upper lobe bronchus by external compression and mucosal white polypoid lesions were observed on right middle-lower lobe division carena. Forceps biopsy was performed in this area and histopathological examination and immunohistochemistry study reveled clustered nerve fiber bundles in lamina propria

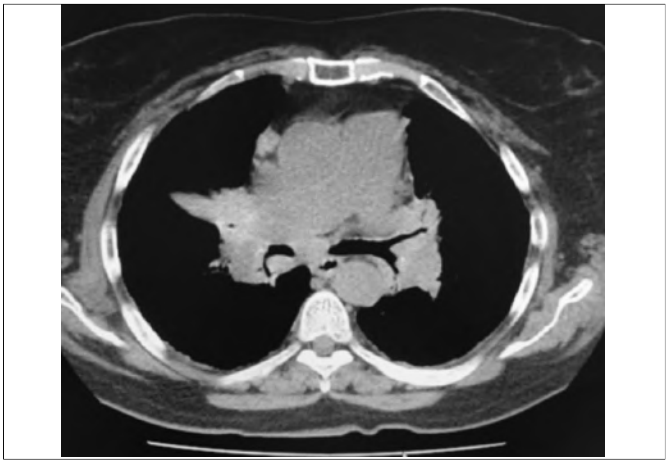

Figure 1: Thorax CT revealed endobronchial lesion in the middle lobe bronchus.

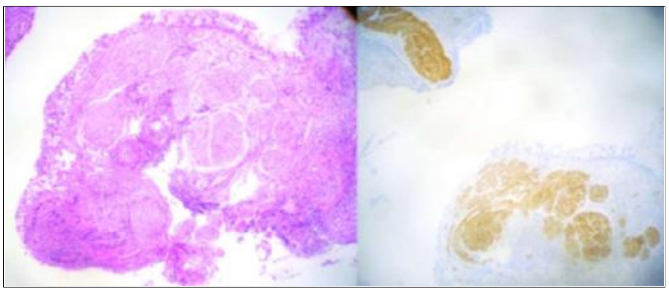

Figure 2: Hematoxylin eosin staining and synaptophysin study revealed clustered peripheric nerve bundles in the lamina propria.

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consistent with bronchial neuromas and granulomas with no necrosis consistent with sarcoidosis (Figure 2). Screening for MEN 2B showed that there were no other features of MEN 2B such as pheochromocytoma or thyroid carcinoma. Hence, the diagnosis of mucosal neuroma with sarcoidosis in the bronchi was made.

DISCUSSION

As we reviewed the literature, this is the first case of isolated mucosal neuromas with sarcoidosis in the bronchi. It is thought that origin of neuromas is from the abnormal growth of the end portion of vagus nerve. Whether heredity plays any role in etiology is unclear.[3] Sarcoidosis is a benign disorder effecting multiple systems characterized by non-caseating granulomas. Etiology of sarcoidosis is also unknown. Linkage between sarcoidosis and neuroma is a question.[7]

MEN, type 2B, is rare disease with components of medullary thyroid carcinomas, pheochromocytomas and mucosal neuromas. Mucosal neuromas are benign and asymptomatic lesions, often seen at birth or first few years of life. As mucosal neuromas are regarded as a component of MEN, type 2B, screening was made and no other components were present. At the end, a rare entity of solitary mucosal neuromas without MEN 2B was diagnosed.

Endobronchial abnormalities are also commonly observed in patients with sarcoidosis, and sarcoid granulomas can involve any part of the respiratory tract. In previously reported cases of bronchial mucosal neuromas, polypoid lesions were observed at the bifurcation of right upper lobe bronchi and truncus intermedius with FOB.

In our case, FOB revealed white polypoid lesions at the division of right middle lobe and lower lobe bronchi. Histopathological examination, and immunohistochemistry study showed nerve bundles arranged in streams in lamina propria and non-caseating granulomas. So, the diagnosis of neuroma and sarcoidosis was proven. It is important for both bronchoscopists and pathologists to consider the possibility of neuromas, particularly when faced with ploypoid lesions of the bronchus in patients with sarcoidosis.

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Isolated Involvement of Penis in Fournier's Gangrene: A Rare Possibility

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ABSTRACT

Fournier's gangrene is an idiopathic progressive inflammation of scrotum and perineum, causing widespread necrosis of skin and subcutaneous tissue. Penis may be secondarily affected in some cases; however, primary isolated involvement of penis is rare. A 48-year male smoker presented with pain and blackish discoloration of the distal part of penis for the last 4 days which developed following rupture of a papulo-vesicular lesion over the prepuce of penis. It rapidly progressed to involve half of the skin of the penis. The patient was hospitalized and broad spectrum antibiotics were administered parenterally. Emergency wound debridement and urinary diversion by suprapubic cystostomy was done. After repeated wound debridement and dressings, the wound healed. Our case was unusual as the penis was the sole site of affection, which is very unusual and only few such cases are reported in the literature.

Key Words: *Fournier's gangrene. Penis. Prepuce.*

INTRODUCTION

Fournier's gangrene is a fulminant, spreading necrotising infection of the skin and subcutaneous tissue of the scrotum, genitalia and/or perineum.[1] Initially described as idiopathic disease, etiology may be identified in more than half of the cases. Incidence is around 1.6 cases per 100,000 males.[2] Mortality upto 20-40% has been reported in past studies; however, recent reports show better outcome with mortality below 7.5%.[2] Primary site of affection is mostly scrotum, due to minor abrasion or trauma, which then progresses to perineum, external genitalia and anterior abdominal wall.[3] Primary isolated involvement of penis is very rare.

CASE REPORT

A 48-year male patient presented with a papulo-vesicular lesion over the prepuce of the penis for seven days, associated with watery discharge. The lesion spread circumferentially and became blackish for the last 4 days. The blackish area was initially wet to start with and later on became dry. There was no history of dysuria, trauma, redness or discharge in the scrotal or perineal region. Patient was smoker for 20 years, but not diabetic. On general examination, the patient was febrile and tachycardic. Local examination revealed dry gangrene of the distal half of the penile shaft skin and prepuce, with a clear line of demarcation proximally (Figure 1). Glans was spared and there was no associated erythema of the scrotum.

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Routine hematological examination revealed leucocytosis and neutrophilia. Urine microscopy and culture revealed no abnormality. Random sugar, blood urea, and serum creatinine were within normal limits. The patient was put on broad spectrum antibiotics including cefoperazone sulbactum and metronidazole parenterally. Suprapubic cystostomy was performed to divert urine from the surgical site, anticipating repeated debridement. Emergency wound debridement and excision of gangrenous tissue was done, preserving the glans, which was not involved in the process (Figure 2). Two days later, desloughing was done again. Growth of *Escherichia coli* and *Klebsiella pneumoniae* was obtained on wound culture. Following repeated debridement and dressings, the wound bed became healthy and was covered with split skin graft after 3 weeks.

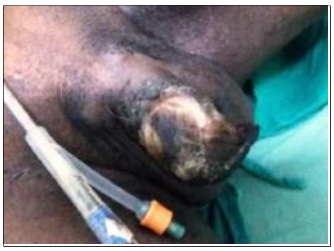

Figure 1: Fournier's gangrene of distal half penis with sparing of scrotum, along with suprapubic catheter *in situ*.

Figure 2: Post-debridement appearance of primary Fournier's gangrene of penis, glans remained unaffected.

DISCUSSION

Penile gangrene may be dry or wet type. Former type is mainly seen in systemic vasculitis, chronic renal failure (CRF), hypertension, diabetes and coronary artery disease (CAD).[4] Latter type or Fournier's gangrene is a polymicrobial infection with multiple aerobic and anaerobic bacteria. The primary pathophysiology

Table I: List of all reported cases of primary penile Fournier's gangrene.

Author, year	Age (years)	Penile involvement	Comorbidity	Inciting cause	Management	Outcome
Temiz MZ, 2015	62	Partial	Diabetes, CRF, hypertension	Scotch over glans	Subtotal penectomy f/b dartos flap closure	Good
Yecies T, 2013	Adult	-	ESRD	Calciphylaxis	-	-
Talwar A, 2010	45	Whole	-	None	Wound debridement f/b skin grafting	Good
Anchi T, 2009	23	Whole	-	Oral sex	Wound debridement f/b skin grafting	Good
Tauro LF, 2005	50	Whole		None	Wound debridement f/b skin grafting	Good
Schneider PR, 1986 (2 cases)	46	-	-	Urethral stricture/ periurethral abscess	-	-
	52	-	-	Phimosis	-	-
Our case, 2016	48	Partial	Smoker	None	Wound debridement f/b skin graft	Good

CRF = Chronic renal failure; ESRD = End-stage renal disease.

includes superficial necrosis due to arteriolar ischemia, promoting further bacterial growth and rapid spread of infection.[5] The comorbid conditions associated with it are diabetes mellitus (32-77%), alcoholism, immunosuppression (including AIDS), malignancy, obesity, malnutrition, and intravenous drug use.[6] Our case had none of the above risk factors. Association with periurethral infection resulting from stricture disease, instrumentation with urinary extravasation, and abnormal sexual practices have been reported in few cases.[7]

It remains a life-threatening disease that requires early recognition with aggressive surgical debridement, resuscitation and broad spectrum antibiotics as the cornerstones of therapy.[8] We managed the case similarly with optimal outcome. Previous reports of isolated penile involvement have been summarized in Table I. Most of the cases were in middle age group, except one. Entire penile shaft was affected in 3 cases, while only partial involvement was seen in 2 cases. Co-morbidities were not uniformly present in all the cases, unlike a classic description. Inciting cause could be identified in half of the cases only. Almost all cases had good recovery after surgery and satisfactory reconstruction, using either skin graft or local scrotal flap.[9]

Primary penile involvement in Fournier's gangrene is a rare, but possible occurrence. It spreads rapidly along the shaft and may affect abdominal wall also. Urgent repeated debridement and antibiotic therapy is the key to successful management. Reconstruction, using scrotal flap or skin graft, gives excellent coverage.

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Descriptive Epidemiology of Congenital Clubfoot Deformity in Sri Lanka

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Abstract

A retrospective descriptive study was conducted, based on database of Sri Lanka Clubfoot Program, under the International Clubfoot Registry. Patients with Clubfoot deformity treated at Orthopaedic unit of Lady Ridgeway Children's Hospital (LRCH), Sri Lanka were evaluated from June 2012 to March 2015. There were a total of 354 patients with male: female ratio of 2.7:1. Bilateral deformity was detected in 48% (171) with positive family history in 14% (49). Majority was hospital births (95%) and 14% were preterm deliveries-pregnancy, and birth-related complications were found in 28.5% (101) and 11% (39), respectively. Cause of clubfoot was idiopathic in 87% (309) and syndromic in 13%. None of the mothers were smoker; three mothers have consumed alcohol during pregnancy. No significant associations among sex of the patient, laterality of clubfoot and the cause of the clubfoot, and there was no seasonal variation among births of clubfoot patients.

Key Words: *Sri Lanka. Clubfoot. Epidemiology.*

Clubfoot or congenital *talipes equinovarus* (CTEV) is a well-recognized congenital deformity of the foot since the time of ancient Egyptians.[1] Forefoot adduction, midfoot *cavus*, hind foot *equinus* and *varus* are the main components of the CTEV deformity (Figure 1). Commonest cause of clubfoot is idiopathic; however, it may also be associated with syndromes such as spina bifida, spinal muscular atrophy, sacral agenesis, myelomeningocele or arthrogryposis.[2] Estimated birth prevalence of this deformity is 1 per 1,000 live births with bilateral involvement in 50% of cases. Nearly 80% of these cases occur in developing countries. Birth prevalence of clubfoot varies by race/ethnicity with low rates (about 0.6 per 1,000 live births) among Asians and high rates (more than 6 per 1,000 live births) among the Pacific Islanders.[3]

The aim of treatment in clubfoot is to obtain anatomically and functionally normal feet in all patients, i.e. a pliable plantigrade foot. Treatment should be started early to ensure better outcomes allowing optimal growth of bone (particularly the talus) and maintenance of joint mobility.[4] The Ponseti method developed by Dr. Ignacio Ponseti, is the "gold standard" treatment for clubfoot at present.[4]

Despite being a common congenital deformity, the situation of clubfoot in Sri Lanka is not well documented in literature. The aim of this study was to analyze the data of clubfoot patients treated in the orthopedic unit of the largest children hospital in Sri Lanka.

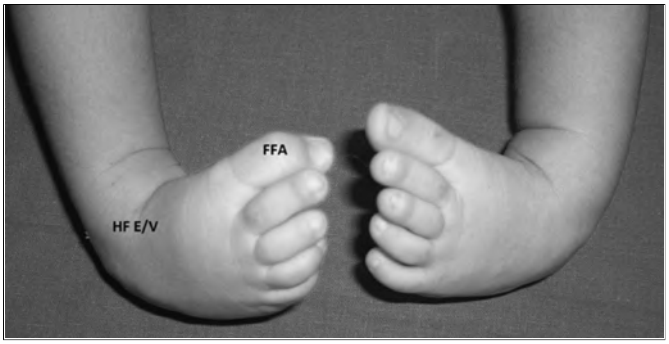

Figure 1: Bilateral club foot deformity (anterior view). FFA: Forefoot adductus, HF E/V: hindfootequinus and varus.

LRCH Orthopedic unit has joined the Sri Lanka Clubfoot Program under the International Clubfoot Registry since 2012. All the children who have been treated for clubfoot in LRH Orthopedic unit from June of 2012 till March 2015 were included in the study. IBM SPSS Statistics for Windows, Version 22.0 was used for the statistical analysis and p-value <0.05 considered as statistically significant.

In all, 354 clubfoot patients were treated from June 2012 to March 2015. There were 73% (257) males and 27% (97) females: male to female ratio was 2.7:1. Forty-eight percent (171) of the cases presented with bilateral clubfoot deformity, while 52% (183) of the cases had unilateral deformity. Among unilateral deformities, 21% (74) had the left foot involvement and 31% (109) had the right foot deformity. Among 354 families, 14% (49) reported to have a positive family history of clubfoot (Table I).

Ninety-five percent (337) births had taken place in hospitals and 83% (295) were referred from obstetric

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Table I: Clubfoot patients treated at LRH Orthopaedic Unit from June 2012-March 2015.

Total No.of patients	n=354	%
Race		
White (Caucasians)	2	0.56
African (Black)	1	0.28
Asian (Indian)	78	22.03
Asian (Sri lankan)	273	77.12
Sex		
Male	257	72.60
Female	97	27.40
Laterality		
Unilateral left foot	74	20.90
Unilateral right foot	109	30.79
Bilateral	171	48.31
Place of birth		
Hospital	337	95.20
Home	1	0.28
Unspecified	16	4.52
Family history		
Affected relatives	49	13.84
No affected relatives	303	85.59
Unspecified	2	0.56
Cause of clubfoot		
Idiopathic	309	87.29
Syndromic	45	12.71
Period of gestation		
Term	303	85.59
Preterm	51	14.41
Extreme preterm	<28W	2 (3.9%)
Very preterm	28-31	6 (11.8%)
Moderate preterm	32-37	43 (84.3%)
Maternal alcohol consumption		
Yes	3	0.85
No	349	98.59
Unspecified	2	0.56
Maternal smoking		
Yes	0	0.00
No	353	99.72
Unspecified	1	0.28
Pregnancy related complications		
Yes	101	28.53
No	252	71.19
Unspecified	1	0.28
Birth related complications		
Yes	39	11.02
No	312	88.14
Unspecified	3	0.85
Previous treatment of clubfoot		
Yes	40	11.30
Casting above knee	33 (82.5%)	
Casting below knee	2 (5%)	
Physiotherapy	1 (2.5%)	
Other	4 (10%)	
No	311	87.85
Unspecified	3	0.85
Other congenital abnormalities/birth defects		
No other abnormality	263	74.29
Associated abnormalities	91	25.71
Head	16 (17.5%)	
Heart/lung	10 (10.9%)	
Urinary/alimentary	10 (10.9%)	
Skin	1 (1.1%)	
Spine	12 (13.2%)	
Hip	10 (10.9%)	
UL	11 (12.1%)	
LL	17 (18.6%)	
Neurologist	4 (4.3%)	
Referal source		
Hospital clinic	295	83.33
Mid wife	3	0.85
Word of mouth	34	9.60
Promotional maternal	1	0.28
Other	21	5.93
Age at presentation		
0-3 months	331	93.50
3-6 months	15	4.24
6-12 months	7	1.98
>1 year	1	0.28

units and neonatal clinics in the hospital. Only 14% (51) of clubfoot cases were born before full term.

Eighty-seven percent 87% (309) cases were idiopathic and 13% (45) were secondary to a syndrome. Pregnancy related complications were reported in 28.5% (101) of cases but only 11% (39) patients had birth related complications. Nearly 75% (263) of patients had isolated clubfoot deformity and every one out of four patients had another deformity associated with the clubfoot. These anomalies included head, heart/lung, urinary/alimentary, skin, hip, upper limb, lower limb and neurological conditions. Out of the patients with other anomalies, nearly 20% was associated with another lower limb anomaly.

In 89% of cases, the deformity was detected at birth and nearly 93% (331) of patients presented for orthopedic treatment before the age of 3 months. Out of 354 cases, 88% were for their primary treatment and only 40 patients presented for secondary treatment with an unsuccessful previous treatment.

There was no seasonal variation related to births of clubfoot babies when comparing distributions of clubfoot births in 2013 and 2014. There was no significant association between gender and the laterality of clubfoot, or the cause of the clubfoot and the laterality of clubfoot.

The results of this study support the previously reported data in the literature indicating that males are twice as likely as females to be affected by clubfoot, indicating the possibility of a genetic influence for male gender as a risk factor for clubfoot.[5] In concordance with the literature, approximately half of children with clubfoot were affected with bilateral deformity and majority was idiopathic clubfoot.[4]

There was a significant difference between the sides affected in unilateral clubfoot since there was a 10% increase in right side clubfoot than left side (n=183). Right-sided clubfoot is more prevalent than left side when it comes to unilateral deformity.[1]

Premature delivery has been shown to have significant association with clubfoot in previous studies.[5] Among these patients, 14% births were preterm deliveries. Although some previous studies have identified maternal smokers as a risk factor for clubfoot deformity,[6] none of the mothers were smoker in this series. Even though many studies have considered maternal alcohol consumption as significant risk factors for congenital clubfoot, there were only three mothers with a history of alcohol consumption prior to pregnancy.[6] There may be an association in other cultures where maternal smoking and alcohol consumption is high.

Higher rate of hospital births must be the reason for early detection of the deformity and referral to orthopedic clinics. This is an indirect indicator of the better level of health service and the trust of the patients towards the health sector.

Previous studies have reported an increased prevalence of infants with clubfoot born in the winter season. In the southern hemisphere, the winter season lasts from March until August,[7] but this data have not found any seasonal variation.

Family history of clubfoot has also been considered as a significant risk factor.[8] In this series 14% (n=49) patients had a positive family history; but out of them, there were more males than females.

Most of the patients presented for their primary treatment. Among those patients who had previous treatment elsewhere, 82% (n=40) had above knee casting, probably the same Ponseti technique which may have failed. Only seven patients have received unaccepted mode of treatments such as casting below knee, physiotherapy and other modes of native treatments, indicating that majority of patients received the ideal treatment for the clubfoot deformity as their primary treatment.

Most of these results are, therefore, showing the same epidemiological pattern in Sri Lanka as in the rest of the world. There were very few cases of maternal smoking and alcohol consumption in this study to draw any conclusion of their association with the deformity. These results can be further substantiated by comparison with future epidemiologic studies in Sri Lanka and other countries.

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Important Decision for an Oral Physician to Make in High-Risk Chronic Kidney Disease Patients: It is Not Only to Record the Associated Resistant Hypertension, but Whether to Use Manual Sphygmomanometer or Automated Blood Pressure Device?

Sir,

Chronic kidney disease (CKD) patients are categorized as high-risk individuals when associated with the risk factors like diabetes and hypertension. Recent reports suggest that, during a dental treatment, resistant hypertension (RH) may act as one such risk factor and adverse cardiovascular (CVS) events could occur when RH is misinterpreted as primary hypertension.[1] Therefore, an oral physician (OP) should be extremely cautious in terms of anxiety and stress management during the dental-care of a high-risk CKD patient. In this perspective, the diagnosis plays a major role and the commonly used manual sphygmomanometer serves as a most feasible diagnostic tool for hypertension. However, the accuracy of diagnosing RH is questionable.[2] The next question for the reader is that should an OP record the associated RH in a CKD patient undergoing dental treatment? If at all recorded, why cannot a manual sphygmomanometer be used?

We must admit that the mercury sphygmomanometer has been a gold standard in diagnosing hypertension,[3] but the complexity in the health status of patients with CKD forces us to seek the aid of an automated office blood pressure (AOBP) device. The main principle of an AOBP device is to record multiple readings in a fully automatic manner with the patient resting alone in a quiet room. Meanwhile, usage of AOBP among OPs is quite limited because of the false perceptions like inaccuracy, cost, and technical difficulties enforcing them to switch to manual recordings.[4] The main concern about manual sphygmomanometer is the lack of adherence to recommended techniques for the blood pressure (BP) measurement, which usually culminates to erroneously high readings, resulting in the misclassification of CKD patients as being hypertensive, consequent to white coat hypertension (WCH). There is increasing evidence from recent reports that AOBP is not influenced by the WCH, and provides more accurate and reliable results.[2,3]

Furthermore, RH is not a rare event in the CKD population and also considered to be the highest risk for CVS events.[1,5] Thus it is sensible for a patient, showing true signs of RH, to delay the invasive oral procedures. Considering the adverse outcomes, it is necessary to identify RH, rather than defining it as a medical problem. A dental visit in such cases may be an opportunity for the patients to be diagnosed and refer them early to our medical colleagues. Though BP measurement has been synonymous with mercury sphygmomanometer for more than a century, it is important to implement this new technique to maintain the best quality of care.

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Letter to the Editor Regarding the Manuscript Titled 'Surgical Outcome of Pancreaticoduodenectomy in Pancreatic and Periampullary Neoplasms'

I read with great interest the recently published article titled "Surgical outcome of pancreaticoduodenectomy in pancreatic and periampullary neoplasms," by Shah and colleagues.[1] The authors should be congratulated for their fine work demonstrating early postoperative outcomes after pancreaticoduodenectomy (PD) in a cancer hospital in Pakistan. Although there is limited published data on PD outcomes from Pakistan, in recent years, both short and long-term outcomes have been reported.[2,3] The authors have shown impressively low morbidity and mortality, which emphasizes further the need for development of specialized hepatopancreatobiliary units in the country. However, a few points merit further exploration.

1. Out of 65 patients, who underwent a trial of dissection, 15 (23%) were irresectable at the time of surgery. Given the high sensitivity (100%) and positive predictive value (89%) of a good quality CT scan in identifying resectable disease, this is a large fraction of patients not receiving curative treatment.[4] It would have been worthwhile to know reasons for failure to proceed with curative surgery.

2. Postoperative complications were defined as those occurring within 30 days of the surgical procedure. Since many of the postoperative complications do not meet their eventual fate within the first 30 days; in recent times, it has been recommended that 90-day should be used as a cut-off for early postoperative morbidity and mortality for hepatic and pancreatic resections.[5]

3. It would also be interesting to know what bilirubin cut-off was used for preoperative biliary drainage since a high percentage of patients underwent this procedure.

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Author's reply

Sir,

Many thanks for your interest in our paper "Surgical outcome of pancreaticoduodenectomy in pancreatic and periampullary neoplasms."

1. Regarding pancreatic cancer surgery, the percentage of resectability is a relevant term as it depends on the stage of tumours which are accepted for resection. In our setup, we accept all T3 tumours including locally advanced tumours involving portal or superior mesenteric vein with a view to perform a trial of dissection and subsequent portal vein resection-reconstruction. In our series, four (8%) patients underwent successful portal vein reconstruction. On the trial of dissection, if a long segment of portal vein (more than 3 cm) or superior mesenteric vein was found involved, close to small bowel mesentry where distal control for reconstruction is not possible, then those patients are managed with palliative bypass. If locally advanced tumours were refused a trial of dissection based on CT findings of portal vein involvement, then around 8% lesser patients would have a chance of curative resection.

2. This study focused on short-term outcome, therefore, we only considered a 30-day mortality. However, we intend to publish our medium-term outcomes, where 3-month morbidity and mortality will be included.

3. Finally, most of our patients are referred to us after being stented in other hospitals for biliary obstruction, therefore, no uniform bilirubin criteria was applicable for stent placement to our patient population.

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Utilization of Unconventional Methodologies for Teaching Anatomy in Medical Colleges: Challenging Fixed Mindsets

Sir,

Keeping in pace with global advances in medical education, the medical institutes in Pakistan encourage active learning techniques.[1] Although this upgrade of educational strategies is a step forward in the positive direction, the real challenge is bridging the gap that students face as they leap from a traditional learning environment in their initial years of education to an active learning environment that is introduced at a later stage. In my opinion, the lack of uniform method of education is a challenge that needs to be overcome, if we want to achieve our students' full potential with relevance to changing times.

In Pakistan, the vast majority of students from both public and private sectors are required to pass a national examination administered by a regional Board of Intermediate and Secondary Education (BISE). Upon successful completion, at the end of grade 9 and 10, they are awarded the 'Secondary School Certificate' (SSC) or 'matriculation certificate'. Upon completion of grades 11 and 12, students are awarded the 'Higher Secondary School Certificate' (HSSC). This examination system drives a teaching curriculum that promotes memorization of factual knowledge rather than students' understanding and analytical thinking.[2,3] In addition to this, there is no unified policy of the government to incorporate technology related modern tools into teaching and learning methods.[4] Obviously, these factors develop a passive learning attitude in the students.

Alternative qualifications in Pakistan are available and managed by examination boards other than BISE. The most common alternative is the General Certificate of Education (GCE), where SSC and HSSC are replaced by Ordinary Level (O Level) and Advanced Level (or A Level), respectively. These are managed by British examination boards of CIE (Cambridge International Examination) and/or Edexcel International of the Pearson PLC. These curricula promote creativity and independent thinking in the learners,[5] but these programs are expensive and not suitable for entrance into professional colleges and universities, which largely favor the BISE examination system.

According to my personal teaching experience, such a fixed stagnant mindset of the students that develops before entering medical colleges, proves to be a barrier while incorporating self-learning strategies and interactive sessions at the professional level. In a system where the stakeholders, namely the parents and faculty members still have reservations towards dynamic learning environments, bringing the students to a problem-solving and critical mental approach is an even more daunting task.

On an experimental basis, I conducted a flip classroom in a lecture hall of 100 students in a private medical college.[6,7] The students were provided the learning material and lecture beforehand. The teaching session included different class activities using the multimedia powerpoint and slide projector. The class displayed enthusiasm and active participation during the session. At the end of the session, I conducted verbal and written feedback from the students. Surprisingly, even though nearly all the students enjoyed the interaction and group work, yet they still preferred the traditional method of teaching in which they had to largely rely upon the teacher for conveying the key concepts and knowledge related to a particular topic.

During teaching anatomy in large group sessions, I have employed discussion, game-learning, interactive videos, spot quizzes, and various other strategies to increase student participation; but other than temporary sparks of reciprocation, the students repeatedly tend to fall back to their passive comfort zone.

Recently, I have been using 3-dimensional (3D) digital models in large group sessions to enhance spatial understanding of gross anatomical structures. The use of dissected cadavers for teaching anatomy is also being applied in our department. I focus on inviting the students to come forward and discuss these 3D models with their fellow learners. My concern is a lack of confidence and creativity of the students, except for a small number of students from O and A levels education system, who voluntarily participated and showed logical thinking skills. The difference in learning behaviors from the two separate education systems is clearly visible.

Sadly, the mode of assessment in the professional colleges and universities here in Pakistan is still summative and teacher-centered. These passive non-cognitive learning behaviors are reinforced when bad study habits, like memorizing paragraphs from prescribed textbooks, using model and guess paper guides, are rewarded in the annual examinations. Students who regurgitate theoretical contents perform well in recall-based assessments. This strengthens the conventional teaching and learning mindsets.

In conclusion, it is my personal opinion that unless we establish active learning environments at the grassroot level, from pre-school all the way up to professional colleges and universities, we will not be able to satisfactorily produce independent and motivated learners. Most, if not all, attempts of changing the mindsets in adult learners by introducing unconventional teaching methodologies will be futile unless their learning behaviors and habits are nurtured from the beginning, especially during the stages of secondary and higher secondary education. A reformation of the assessment strategies at all levels of education is also essential in this context.

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Anti-NMDA Receptor Encephalitis: A Masquerade Ball of Neuropsychiatric Symptoms

Sir,

Anti-NMDA (N-methyl-d-aspartate) receptor encephalitis, categorized officially in 2007, is a rare disorder which is potentially fatal at one end of the spectrum but may demonstrate astounding recovery, especially if the disease is mild and detected early.[1] Being a relatively modern disease, it poses great challenges in the diagnosis and subsequent management.

A 39-year lady presented with two episodes of typical generalized tonic clonic seizures. She had a preceding two weeks history of low-grade fever and headache. At presentation, she was vitally stable. Apart from slight agitation, general and systemic examinations were unremarkable. Plain CT scan of the brain was normal. Cerebrospinal fluid (CSF) examination showed pleocytosis with 93% lymphocytes. CSF was negative for bacteriologic and fungal cultures, acid-fast bacilli, mycobacterial DNA, and viral Polymerase chain reaction (PCR). She was started on ceftriaxone, vancomycin and acyclovir at presentation for the suspicion of partially treated bacterial meningitis or viral encephalitis. She was not showing any significant improvement. Repeat CSF examination after two days showed rising proteins. She was started on antituberculous medications with steroids but she continued to deteriorate. Later, patient started to develop behavioral and cognitive changes. She then developed muscular rigidity and autonomic instability. Thus, suspicion of autoimmune encephalitis was raised and her CSF was sent for anti-NMDA receptor antibodies which came out to be positive. She was diagnosed to have anti-NMDA receptor encephalitis. Full body imaging did not show any tumor including ovarian teratomas. Patient was started on high-dose methylprednisolone and IVIGs for 5 days, which did not bring any improvement. Because of the non-responsiveness, she was then placed on rituximab and cyclophosphamide, which dramatically improved the patient condition. She continued to show improvement in her clinical and cognitive condition and was shifted under care of the rehabilitation team. On follow-up one year after the presentation, she had good performance status without any neurological or intellectual disability.

Anti-NMDA receptor encephalitis manifests in a variety of overlapping clinical patterns. Some reviews describe a simplified sequence of prodromal phase, psychotic and/or seizure phase, unresponsive phase and finally hyperkinetic phase.[2] Testing for the specific anti-NMDA receptor antibodies in CSF should be performed for confirmation.[3,4] As it is frequently associated with tumors in the body, especially ovarian teratoma, full body imaging is suggested.[3] Specific treatment involves glucocorticoids, IVIGs, plasma exchange, rituximab and cyclophosphamide, either in sequence or in combination.[3]

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Aneurysmal Bone Cyst of Mandible with Classical Histopathological Presentation

Sir,

Aneurysmal Bone Cyst (ABC), described as a distinct entity in 1942 by Jaffe and Lichenstein, is a rare cyst that seldom affects maxillofacial jaw bones.[1] ABCs tend to grow rapidly and destruct cortical plates resulting in bony expansion and facial asymmetry.[2] Histopathologially, these are classified as false cysts and characterized by numerous sinusoidal blood filled spaces, multinucleated giant cells, areas of new bone formation, and varying amounts of hemosiderin.[3]

An 18-year male presented to our Institution with the chief complaint of painful swelling of his right lower back region of the jaw for 8 months, which was small initially and gradually increased to the current size. Family history and past medical history of the patient were non-relevant to the present swelling. Past dental history revealed an extraction of tooth #46 due to secondary dental caries 1 year back. There was no history of trauma. Extra-oral examination revealed facial asymmetry and swelling on rightside of the face, measuring about 2 x 1.7 cm. Intra-oral examination revealed a diffuse swelling extending from #45 to the distal surface of #47, measuring 2.5 x 1.5 cm with expansion of cortical plate. On palpation, it was firm and tender. Panoramic radiograph revealed a multilocular radiolucency extending from the roots of #47 covering impacted #48 and going distally to the ramus of the right mandible (Figure 1a). A provisional diagnosis of Keratocystic odontogenic tumor (KCOT) was given. The curettage of the lesion was performed with the extraction of #47 and impacted #48, under general anesthesia, and the defect was restored with titanium angled reconstruction plates. The excised tissue was sent for the histopathological examination. Follow-up period of 1 year was uneventful. Histopathological examination revealed fibrocellular connective tissue stroma consisted of numerous sinusoidal spaces filled with red blood cells (Figure 1b), abundant hemosiderin deposition and newly formed bony trabeculae lined by osteoblasts (Figure 1c). Numerous multinucleated giant cells were also noted. Based on histopathological features, a final diagnosis of ABC was given. ABCs are commonly found in long bones and vertebral column. Only 1.9% incidence has been reported in jaw bones.[2] ABC exhibits extremely variable clinical presentation ranging from a small asymptomatic lesion to painful giant lesion with massive bone loss.[4] The radiographic features of ABCs are varied and often simulate different lesions, i.e. central giant cell granuloma, ameloblastoma, odontogenic myxoma, KCOT, and central hemangioma.[3] Hence, histopathological examination provides the gold standard for the diagnosis. In the present case, the provisional diagnosis was KCOT.

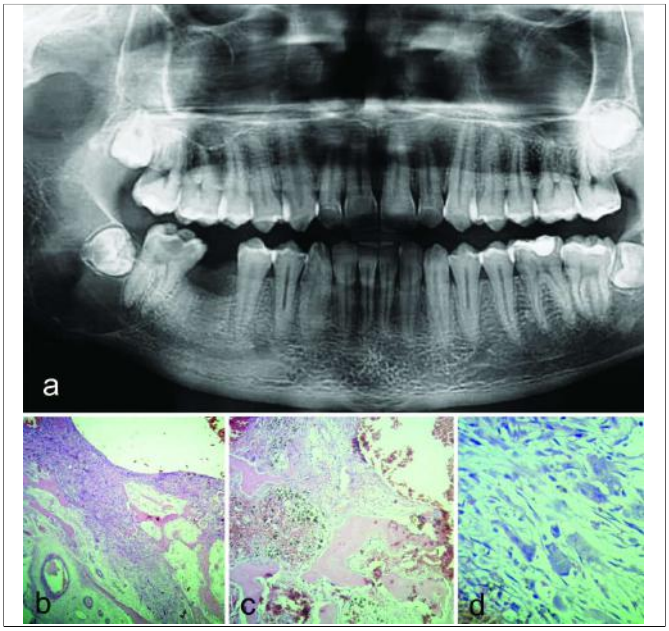

Figure 1: (a) Panoramic radiograph shows a large multilocular lesion of right posterior mandible. **(b)** Sinusoidal spaces filled with red blood cells in a fibrocellular stroma, (hematoxylin and eosin stain X20). **(c)** Newly formed bony trabeculae with abundant hemosiderin pigmentation, (hematoxylin and eosin stain X20). **(d)** High power view shows multinucleated giant cells, (hematoxylin and eosin stain X40).

The case presented here depicts the importance of histopathology in the diagnosis of ABC. Although rare, ABC should be included in the differential diagnoses of large radiolucent lesions of the jaw.

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Benign Migratory Glossitis

Sir,

Benign migratory glossitis (BMG) is a benign, immune-mediated, chronic inflammatory disorder of unknown etiology, usually characterized by asymptomatic erythematous patches separated by white irregular borders. This condition is also known as geographic tongue, erythema migrans, glossitis exfoliativa and wandering rash of the tongue. The central erythematous patch represents atrophy of the filiform papillae. The white border is composed of regenerating filiform papillae and a mixture of keratin and neutrophil aggregates within the epithelium. The reported prevalence is 1-2.5% with no gender predilection. BMG shows periods of exacerbation and remission with recovery in one area and appearance in other area; thus explaining the typical migratory nature of this lesion.[1-3] Various studies have found association of BMG with psoriasis, diabetes mellitus, Reiter's syndrome, Down's syndrome, pregnancy, psychological factors, genetic factors, hypersensitivity, fissured tongue[3], and consumption of oral contraceptive pills and lithium carbonate.[4] Though usually asymptomatic in nature; pain and burning sensation in the affected area of the tongue has been reported on consumption of spicy/salty food and/or alcoholic drinks. Similar lesions may also be seen in atrophic candidiasis, local chemical or mechanical trauma, drug induced reactions, psoriasis and atrophic lichen planus.[5]

A 26-year-old male patient, an Egyptian soldier performing his duties in United Nations-African Union Mission in Darfur (UNAMID), reported to Pakistani Field Hospital Darfur, Sudan; complaining of pain and burning sensation in his tongue for the last two months.

The patient was asymptomatic two months back when he developed burning sensation in his tongue on taking spicy food and hot drinks. After a few days, the severity of burning increased in response to the same stimuli. In addition to it, he developed mild continuous pain and discomfort in tongue which aggravated during speech and mastication. He took multivitamin tablets and systemic antifungal medication (capsules) on the advice of a general practitioner but was not relieved of his symptoms. He also complained of disturbed sleep and loss of appetite. The patient related his symptoms with composite filling of his upper molar tooth which had been done one month before appearance of his symptoms. Currently, he was not taking any medication and denied allergy to any medication or food item. His family history was not significant. He was non-alcoholic and remained non-smoker until two weeks ago, when he started smoking about 10 cigarettes per day, without any known reason. He was unmarried, eldest son of the family, having two sisters, two brothers and two parents; all dependent upon him. In mission area, his job was procurement and logistics management.

On clinical examination, he was a young male of average built and height. Temporomandibular joint (TMJ) examination and mouth opening were normal. Intraoral examination showed satisfactory oral hygiene with all teeth intact. There were wear facets on lower anterior teeth. Examination of tongue revealed characteristic morphological features of geographic tongue. Multiple flat, smooth, erythematous patches of depapillated mucosa with yellowish white, slightly elevated, irregular peripheral borders were visible on dorsum and lateral margins of the tongue (Figure 1). No ulceration, bleeding or pus discharge was observed. Systemic examinations was unremarkable. Investigations included blood complete picture with RBC indices, serum glucose level, serum folate, vitamin B12 and albumin level and total iron binding capacity; to distinguish from glossitis associated with anemia or other nutritional deficiencies.

Patient was reassured of the benign nature of the lesion. For symptomatic relief, he was advised topical application of triamcinolone acetonide (Kenalog in orabase ointment) and use of benzydamine mouth wash for ten days. He was also advised tablet paroxetine, 10 mg once daily for 3 months, by our psychiatrist. The patient was reviewed after 2 weeks; who reported significant reduction in severity of his symptoms with only mild discomfort during eating. On examination of the tongue, the peripheral white zones on lateral margins of the tongue had disappeared (Figure 1). Patient was advised to observe fortnightly follow-up visits for the rest of his stay in mission area.

Symptomatic treatment with topical corticosteroids, topical local aneasthetic agents, anti-inflammatory mouthwashes, antihistamines, and anxiolytic agents is documented in literature.[5] This patient responded well to a 10-day course of topical corticosteroids (triamcinolone acetonide) with significant reduction of his symptoms and disappearance of peripheral zone of the lesion, which is a sign of recovering mucosa.[3-5]

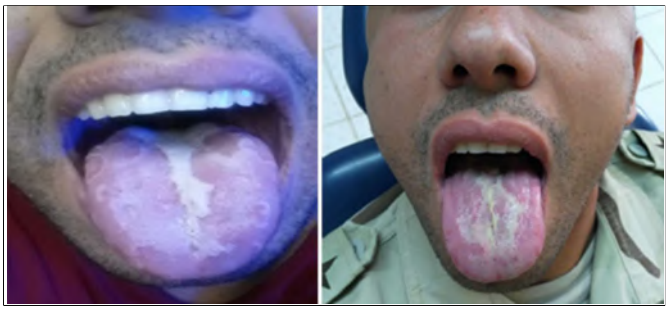

Figure 1: Initial clinical presentation (left). 14 days post-treatment (right).

BMG is a completely benign mucosal lesion that shows periods of exacerbation and remission. In asymptomatic cases, its diagnosis is important to relieve cancer anxiety of the patient. In symptomatic cases, symptomatic treatment should be provided by use of corticosteroids along with correction of the cause. Possible role of stress in development of symptomatic BMG has been observed in this case report which may be confirmed by further investigations.

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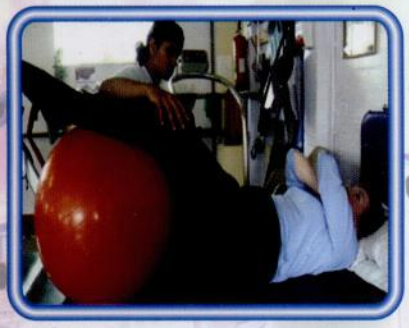

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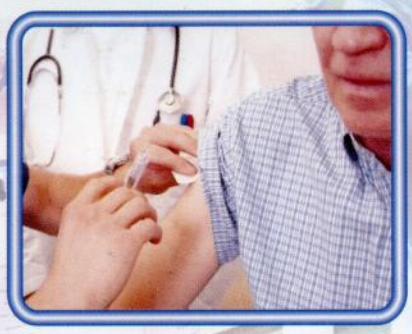

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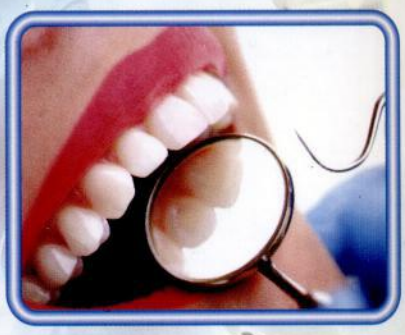

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2 King et.al. 2015. A review of the clinical utility of duloxetine in the treatment of diabetic peripheral neuropathic pain. Therapeutics and Clinical Risk Management:11 1163–1175

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