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Editorial

Current Updates on Arthroplasty in Developing Countries

Farooq Azam Khan

Osteoarthritis (OA) is one of the most common joint diseases and the most common arthritis, worldwide. Its global prevalence has doubled from 247.51 million cases in 1990 to 527.81 million cases in the last thirty years. Osteoarthritis has a female predominance and the age group most affected is the elderly between the age of 60 and 70.[1] It is predicted that by 2050, 22% of the global population will be over the age of 60 hence, there will be a significant increase in the prevalence of OA in the upcoming years.[2] Osteoarthritis can be classified into two types; primary and secondary. Primary osteoarthritis, also known as idiopathic osteoarthritis, is characterized by the degeneration of the cartilage and joint without any trauma or disease. Risk factors include advanced age, obesity, female gender, and genetics. Secondary osteoarthritis occurs in the context of preexisting joint pathology. Common causes of secondary osteoarthritis include trauma, rheumatoid arthritis, avascular necrosis, and hemoglobinopathy.[3]

The Kellgren and Lawrence classification, which uses five grades from zero to four, can be used to classify the severity of osteoarthritis. Grade one and two can be treated conservatively; however, grade three and four require joint replacement.[4] The demand for total hip replacement (THR) and total knee replacement (TKR) is constantly rising and is projected to increase by 276 percent and 208 percent, respectively, by the year 2030.[5] Non-surgical treatment options for knee osteoarthritis include analgesics ranging from acetaminophen to opioids, serotonin-norepinephrine reuptake inhibitors, vitamin D supplements, glucosamine and chondroitin sulphate supplements, and intraarticular injections. Intraarticular injections include the use of steroids, platelet-rich plasma, and hyaluronic acid.[6] These treatments and lifestyle changes are only useful in the early stages of osteoarthritis and are ineffective in advanced cases. Arthroplasty is safe and successful, but it requires state-of-the-art operating rooms and the high cost associated with such surgeries is one of the major concerns for surgeons and patients in underdeveloped nations. Hip and knee replacements are among the most costly surgeries worldwide. The costs of these surgical procedures include implants, hospitalization, anaesthesia services, and operating room fees. Among these, the cost of implants accounts for the majority of expenses. Postoperative care, which includes physiotherapy and nursing care that must be continued after hospital discharge, adds to the treatment expense. In the United States, the cost of arthroplasty (THR/TKR) ranges from 30,000 to 120,000 United States Dollars (USD), and the cost of an implant ranges from 3,000 to 10,000 USD.[7] In Australia, the cost of arthroplasty ranges between 19,000 to 30,000 Australian Dollars (AUD), and the cost of osteoarthritis therapy is expected to surpass 2.9 billion AUD over the next eight years.[5] In Canada, the mean cost of total hip arthroplasty is 10,477 Canadian Dollars (CAD).[8] Despite the cost, arthroplasty is more cost-efficient than non-surgical intervention in the management of severe osteoarthritis of the knee and hip joint.[9]

Pakistan is a third world country with per capita gross domestic product (GDP) of 1658.36 USD, which is ten times lower than the global GDP.[10] Cost of THR/TKR ranges from 300,000 to 800,000 Pakistani Rupee (PKR) and the cost of the implant alone varies from 120,000 to 250,000 PKR. In a developing country, where tuberculosis is still endemic, this elective procedure imposes a substantial financial burden on patients. The major financial challenge in implementing arthroplasty is the cost of the implant, which can be overcome by indigenous implant production. Price capping, as utilized in India, can also be used to reduce the cost of imported implants. The profit margin on implants has been reported to exceed 300 percent. Price capping, despite its downsides, might significantly cut the cost of implants.[11] In 2020, the government of Pakistan launched health insurance, the Sehat Sahulat Program, similar to Medicare in the United States. Total joint replacement indicated for traumatic cases was one of the numerous expensive surgeries covered by it.[12]

The patients' demographics and surgical indications for arthroplasty in a third world country are significantly different from those in a developed one.

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The mean age of patients undergoing THR in the United States and the United Kingdom is 60.5 years and 69.4 years, respectively, which is greater than the range of 33 to 50 years for patients from developing countries. So, the disease burden is high in the developing countries.[13,14]

Postoperative complications following arthroplasty include surgical site infection (SSI), bleeding, thromboembolic disease, neurovascular injury, dislocation, stiffness, malalignment, dislocation, and fracture. The risk of infection at the surgery site is considered the greatest concern among these various complications. In developed nations, the rate of SSI ranges from 0.50% to 1.50%, however, in developing nations such as ours, the percentage can be as high as 4.6%, which poses major risks and may require revision surgery.[15] The revision surgery is substantially more expensive than the primary arthroplasty because of the longer operating time and hospital stay. In addition to being financially burdensome, revision operations are often associated with a poor prognosis. Surgical site infections can be prevented by using preoperative antibiotics, maintaining sterility in the operating room, and proper wound care.[16]

Registries have a significant capacity to offer information regarding medical devices, especially implants, which are crucial to arthroplasty. The first arthroplasty registry was established in Sweden, and currently, Finland, Norway, Denmark, Australia, New Zealand, Canada, Romania, England, and The Netherlands have active national registries on total knee and total hip replacement.[17] Analysis of data from the registries offers an accurate assessment of implant reliability and early failure identification. Hence, allowing for earlier intervention, and lowering revision rates. Pakistan Arthroplasty Society established Pakistan National Joint Registry in 2014, and its primary objective is collecting and analyzing data on joint replacements.[18] It has members both in private and governmental hospitals throughout Pakistan.

Joint Replacement operations are generally performed in metropolitan areas, and patient dropout is common due to the long distance patients must travel for follow-up care. Other issues identified during the follow-up evaluation in our setting included a lack of access to physiotherapy and inadequate wound care. One of the primary obstacles in assessing outcomes in developing nations is a patient dropout, with some studies reporting dropout of greater than 30%.[9] In a resource-constrained area, this can be addressed by utilizing telecommunication for follow-up after a few hospital visits.

Total joint arthroplasty is a highly successful procedure, and its global demand is growing, but it places a significant financial strain on settings with limited resources. The challenges faced by the surgeon include the unavailability of the modern operating theatre, the cost of the implant, late presentation, and lack of structure for patient referral. Despite these obstacles in a low-resource setting, arthroplasty is being successfully performed, and the demand for it is increasing; however, optimal circumstances are required for ideal results. Beginning with perioperative care and extending to follow-up, implementing an arthroplasty program is fraught with challenges. The issues at hand should be addressed on a national scale in order to overcome them.

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Original Article

Determination of Estrogen Receptor, Progesterone Receptor & Human Epidermal Growth Factor Receptor 2 Status in Breast Cancer: A Single-Center Experience

Aafrinish Amanat, Sadaf Waris, Nadia Wali, Sadia Alam, Atika Masood, Maleeha Aslam

ABSTRACT

Objective: To determine the frequency of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expressions and to compare them with certain morphological parameters of breast tumors.

Methodology: It was a retrospective record-based study carried out in the Pathology Department of Akhtar Saeed Medical & Dental College, Lahore. A total of 45 cases of breast cancer from January 2021 to May 2022 were included in this study. The cases included needle core biopsies and mastectomy specimens. The sections were processed and examined under a microscope. Grading of tumors was done by the Bloom Richardson grading system and tumor characteristics for mastectomy specimens such as tumor size and lymph node status were noted. The luminal classification was done and frequencies were calculated. The immunohistochemical analysis was performed for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Scoring was done according to the breast cancer template of the College of American Pathologists. The frequencies were calculated for ER, PR, and HER2 status, Bloom grades, and tumor characteristics.

Results: In our study, the mean age of the patients was 46.31 years and most of the cases were seen in the age group of 55 to 64 years. The frequency of ER-positive cases was 29(64.4%) while the frequency of ER-negative cases was 16(35.6%), the frequency of PR-positive cases was 19(42.2%), and PR-negative cases was 26(57.8%). In contrast, the frequency of HER2-positive cases was 13(28.9%) and the frequency of HER2-negative cases was 28(62.2%). Most ER-positive cases were observed in the age group 45 to 64 years and most of the HER2-negative cases were seen in the age group 55 to 64 years. The majority of the cases (95.6%) were invasive ductal carcinoma. Most of the cases of breast cancer were of grade II (87%) and luminal A type (40%) was the most frequent one. In the mastectomy specimens, the size of the tumor in most of the cases (55.6%) was 2 to 5 cm and a majority of the cases (33.3%) had ≥4 lymph nodes positive.

Conclusion: Estrogen receptor was most frequently expressed along with loss of HER2 receptor. Invasive ductal carcinoma was the most common histological type and the majority of breast cancer cases showed grade II. The most common type of carcinoma was the luminal A type.

Keywords: *Breast carcinoma. Estrogen receptor. Progesterone receptor.*

INTRODUCTION

Breast cancer is the fifth leading cause of cancer-related deaths, globally. According to some recent statistics, almost 2.3 million cases of breast cancer and 685,000 deaths were reported in the year 2020.[1] The total number of cases may rise to 4.4 million by the year 2070.[2] In the year 2020, this deadly cancer has already taken over other malignancies among females with a reported incidence of a total of 24.5% cases and mortality of 15.5% worldwide.[1] Pakistan with 90,000 cases annually and nearly 40,000 breast cancer-related deaths, remains the leading country among other Asian countries.[3] According to the annual report of Nuclear Medicine, Oncology & Radiotherapy Institute, Islamabad, breast cancer accounts for 33% of all cancers in females admitted to the center.[4] In urban India, the incidence of breast cancer in women is about 25 to 33%.[5]

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Breast cancer was once considered a disease of elderly females. But according to several recent studies, the incidence is steadily increasing in young females, with 47.3% of total breast cancer cases in developing countries. The age group most prone to develop the disease is 45-49 years with an incidence of 45.42%.[6] Majority of such cases are attributed to loss of hormonal influence leading to abnormal expression of estrogen receptor and progesterone receptor. The hereditary factors contribute to only 10% of breast cancers while other risk factors like socio-demographic factors, reproductive health, and lifestyle constitute the most.[5,7]

There are various markers used to identify breast cancer including estrogen and progesterone receptors. Breast cancers with positive ER and PR status are associated with improved outcomes and response to therapy. On the contrary, another marker is a tyrosine kinase receptor (HER2) related to the epidermal growth factor receptor family. If HER2 is over-expressed, it is associated with relapse and resistance to therapies as compared to ER & PR-positive cases.[5] Therefore, it is imperative to evaluate ER, PR, and HER2 status of breast carcinomas diagnosed on hematoxylin and eosin (H & E) staining.[8] The purpose of this study was to analyze the frequency of ER, PR, and HER2 receptor

expression, and their correlation with established clinicopathological prognostic criteria (age, histological type, and grade) to improve the medical care provided to breast cancer patients.

METHODOLOGY

It was a retrospective record-based study carried out in the Pathology Department of Akhtar Saeed Medical & Dental College, Lahore. A total of 45 cases of breast cancer from January 2021 to May 2022 were included in this study. The cases were received from Akhtar Saeed Trust Hospital and Farooq Hospital, Lahore. The specimens included formalin-fixed needle core biopsies and mastectomies (27 needle cores and 18 mastectomies). The poorly preserved specimens were not included in this study. All the relevant information from patients was collected on a predesigned proforma. Grading of tumors was done by the Bloom Richardson grading system. The tumor characteristics for mastectomy specimens such as tumor size and lymph node status were recorded. The luminal classification was also done and frequencies were calculated. The immunohistochemical analysis was performed for ER, PR, HER2, and scoring was done according to the breast cancer template of the College of American Pathologists. The frequencies were calculated for ER, PR, and HER2 status, Bloom grades, and tumor characteristics.

STATISTICAL ANALYSIS

The collected data was analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. The mean and standard deviation was used to summarise the statistics of continuous variables. Categorical variables were reported as frequencies and percentages.

RESULTS

In our study, the mean age of the patients was 46.31 years and most of the cases were seen in the age range of 55 to 64 years. The majority of the cases (95.6%) were of invasive ductal carcinoma and 4.4% of the cases were diagnosed as invasive lobular type. According to Bloom's grade, most of the cases were of grade II (87%) (Figure 1).

The frequency of ER-positive cases was 29(64.4%) while the frequency of ER-negative cases was 16(35.6%), the frequency of PR-positive cases was 19(42.2%), and that of PR-negative cases was 26(57.8%). In contrast, the frequency of HER2-positive cases was 13(28.9%) as reported in Table 1.

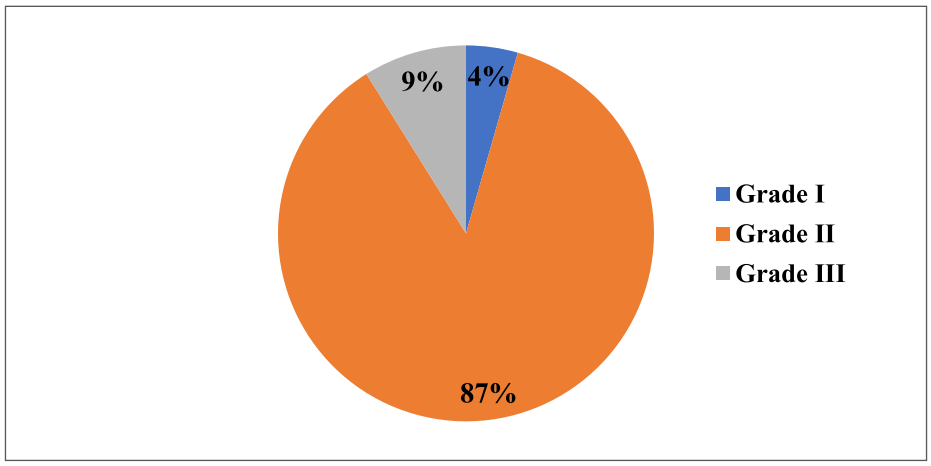

Figure 1: Distribution of Cases (Needle Core Biopsy & Mastectomy) according to Bloom's Grade

Table 1: Receptor Expression Pattern in all Age Groups

Receptor Type	Positive Frequency & Percentage	Negative Frequency & Percentage
Estrogen Receptor	29(64.4%)	16(35.6%)
Progesterone Receptor	19(42.2%)	26(57.8%)
HER2	13(28.9%)	28(62.2%)
Equivocal Frequency & Percentage	4(8.9%)	

The frequency of receptor-positive cases among various age groups was compared. Most ER-positive cases (n=18) were observed in the age group 45 to 64 years and most of the HER2-negative cases (n=9) were recorded in the age range of 55 to 64 years (Table 2). Considering the luminal classification of breast cancer, it was found that most of the cases were of luminal A type (40%) and HER2-enriched cases were of minimum frequency (8.9%). The triple negative cases comprised 22.2% (Table 3).

In the mastectomy specimens, the size of the tumor in most of the cases [10(55.6%)] was 2 to 5 cm. In 6(33.3%) cases, the size of the tumor was more than 5 cm and 2(11.1%) cases had a tumor of size less than 2 cm. The lymph node status of the mastectomy specimen revealed that most of the cases (33.3%) had ≥4 lymph nodes positive (Table 3). This study also depicted that as the tumor size increased, the frequency of HER2-negative status also increased (Table 4).

Table 2: Receptor Expression Pattern in Patients according to Age Groups

Age (Years)	ER-Positive (n)	ER-Negative (n)	PR-Positive (n)	PR-Negative (n)	HER2-Positive (n)	HER2-Negative (n)
25 to 34	5	2	5	2	2	4
35 to 44	5	6	6	5	2	8
45 to 54	7	5	3	9	6	6
55 to 64	11	3	5	9	3	9
≥65	1	0	0	1	0	1

Table 3: Distribution of Cases according to Luminal Types and Lymph Node Metastasis

Distribution of Needle Core Biopsy & Mastectomy Specimens according to Luminal Types	
Luminal Classification	**Frequency & Percentage**
Luminal A	18(40%)
Luminal B	9(20%)
HER2-Enriched	4(8.9%)
Triple Negative	10(22.2%)
Distribution of Mastectomy Specimens according to Lymph Node Metastasis	
Lymph Node Status	**Frequency & Percentage**
Not Identified	5(27.8%)
Negative (0)	2(11.1%)
Positive (1-3)	5(27.8%)
Positive (4 or more)	6(33.3%)

Table 4: Receptor Status according to Tumor Size

Tumor Size (cm)	No. of Cases	ER-Positive	ER-Negative	PR-Positive	PR-Negative	HER2-Positive	HER2-Negative
<2	2(11.1%)	2	0	2	0	1	0
2 to 5	10(55.6%)	8	2	7	3	1	9
>5	6(33.3%)	2	4	1	5	1	5

DISCUSSION

A multidisciplinary approach involving a surgeon, pathologist, and oncologist is required for the appropriate management of breast carcinomas. The hormone receptors play a critical role in the determination of a therapeutic plan, treatment response, and prognosis.[8] The patients can be categorized into two groups based on the receptor positivity status. One group of those who can benefit from adjuvant chemotherapy and the other group of

those who are less likely to respond. Therefore, the hormone receptor status should be determined in all breast carcinomas.

In a study by Singh et al., the frequency of ER-positive cases was observed to be 56% and ER-negative cases 44%, PR-positive cases 38% and PR-negative cases 62%. Human epidermal growth factor receptor 2 positivity was observed in 30% of total cases, HER2-negative in 70%, and triple-negative in 30%, respectively.[9] Another study conducted by Khalid et al., showed that the frequency of ER-positive cases was found to be 53.3%, ER-negative 46.7%, PR-positive 55.1%, PR-negative 44.9%, HER2-positive 30.8%, and HER2-negative 39.3%.[10] Some other studies also reported similar observations, showing a relatively higher percentage of ER and PR-positivity in breast cancer cases.[11,12] All these findings support the findings of the current study where ER-positive and negative cases were 64.4% & 35.6%, respectively, PR-positive in 42.2%, and negative in 57.8%, and HER2 positivity was observed in 28.9% & negativity in 62.2% cases.

Our study showed that most of the cases were of luminal A type (40%) and HER2-enriched cases were of minimum frequency (8.9%). The triple negative cases comprised 22.2%. Another study reported that more than half of the total tumor cases (51.9%) fall in the category of grade II, 42.9% in grade III, and only 5.2% in grade I. Luminal A subtype was found to be the most prevalent (38.8%). Triple-negative hormone receptors and luminal B cases were 15.5% each, however, 14.9% of cases were HER2-enriched.

In an Indian study carried by Bhardwaj et al., the mean age of development of carcinoma breast was 50.28±12.83 years. The majority of the cases (53.52%) were in the age group of 35 to 54 years. The most frequent type (76.64%) was invasive ductal carcinoma not otherwise specified (NOS) and the most frequent grade was grade II (47.88%) followed by grade III (32.39%). The results of this study showed that 38(53.52%) of the cases were ER/PR-positive, 9(12.67%) were ER-positive/PR-positive/HER2-positive, and 22(30.98%) were ER-negative/PR-negative /HER2-negative, respectively.[13]

Another study showed a majority of patients in the age group of 40 to 59 years. The most frequent type was invasive ductal carcinoma NOS (83.9%) and the most frequent grade was I (49%) followed by grade III (40%). In addition, the frequency of ER & PR expression was found to be 48.39% and 41.9%, respectively.[14]

In a Malaysian study, the median age for carcinoma breast was 55 years, and infiltrating ductal carcinoma (IDC) was found to be the most common histological type (6.8%).[15] Similarly, African researchers conducted a study on 343 breast cancer patients. It showed that the mean age for breast cancer was 49.7 years and the common histological type was invasive ductal carcinoma of no special type (89%). More than half percent of the total cases (51.9%) had tumor sizes ranging from 2-5 cm and some (39.1%) with >5 cm. A greater percentage of these tumors were of grades II and III types.[16]

A study on the population of Pakistan indicated that ER and PR-positive cases were present in 45.4% and 36.9%, respectively, of breast cancers. The most common histological type was invasive ductal carcinoma, not otherwise defined, which made up 95.4% of all cases. Age at diagnosis ranged from 24 to 78 years, with the majority of cases (53.1%) being under 50 years old. In the same study, 40(30.8%) of the cases showed positive results for both receptors (ER, PR) while 63(48.5%) of the cases showed negative results for both receptors. Among other findings 6.1% of cases had ER-negative, PR-positive while 14.6% of all cases had ER-positive, PR-negative.[17] All of these conclusions are consistent with the current research.

CONCLUSION

The present study showed that ER receptor was most frequently expressed along with the loss of HER2. Invasive ductal carcinoma was the most common histological type of breast cancer and a majority of the cases showed grade II morphology. Luminal A type of breast cancer was the most common among other subtypes in our setting.

LIMITATIONS & RECOMMENDATIONS

This was a single-centered study and the sample size was relatively small due to time constraints. Therefore, the findings of the present study cannot be applied to the general population. Further large-scale studies on a single biopsy type are recommended to validate these results.

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Original Article

Cardiovascular Risk Factors in Patients Undergoing Primary Percutaneous Coronary Intervention for ST-Elevation Myocardial Infarction and their Association with Left Anterior Descending Artery Involvement & Triple Vessel Disease

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ABSTRACT

Objective: To determine the frequency of cardiovascular risk factors in patients presenting for primary percutaneous coronary intervention (P-PCI) and evaluate their association with left anterior descending artery involvement and triple vessel disease.

Methodology: This descriptive cross-sectional study was done at the Rawalpindi Institute of Cardiology, Rawalpindi from March 2019 to January 2021 after approval from the ethical review board. A total of 3000 patients with ST-elevation myocardial infarction (STEMI), who underwent P-PCI were enrolled by non-probability convenient sampling. The patient information including age, gender, predominant vessel, number of vessels involved, smoking status, left ventricular ejection fraction (LVEF), and history of diabetes mellitus and hypertension was noted on a proforma. Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.

Results: Diabetes mellitus, hypertension, and smoking were present in 897(29.9%), 1216(40.5%), and 990(33%) of the patients, respectively. The left anterior descending artery (LAD) was involved in most of the cases (56.2%) followed by the right coronary artery (33.7%) while left circumflex was involved in 10.1% of the cases. Single vessel disease was present in 55.7% and triple vessel disease, (TVD) in 15.8% of the patients. Left anterior descending artery involvement was associated with age, gender, LVEF, triple vessel disease, and type of disease. However, there was no significant association found between the involvement of LAD and the occurrence of risk factors such as smoking, HTN, and DM. Triple vessel disease was associated with age, LVEF, predominance of vessel, and hypertension.

Conclusion: Risk factors such as diabetes, hypertension, and smoking are more common in patients presenting for P-PCI as compared to the general population. Triple vessel disease is associated with age, left ventricular ejection fraction (LVEF), the predominance of the vessel, and hypertension. Left anterior descending artery involvement is associated with age, gender, LVEF, triple vessel disease, and type of disease.

Keywords: *ST-elevation myocardial infarction. Cardiovascular risk factors. Coronary artery disease.*

INTRODUCTION

Cardiovascular diseases (CVDs) are among the major cause of death, disability, and financial burden worldwide.[1] According to the data from 2018, the American Heart Association concluded that the death rate attributed to cardiovascular diseases was 217 per 100,000 people. In the United States, every 36 seconds a person dies due to myocardial infarction. Among the deaths caused by cardiovascular diseases, 42.1% are caused by coronary artery disease (CAD).[2] The World Health Organization reported that 16.49% of deaths are accounted to CAD leading to a mortality rate of 193 per 100,000 people. Cardiovascular diseases have declined significantly in western countries due to the scaling-up of preventive measures but they are still an important public health issue in developing countries.[3]

According to a study evaluating the burden of CVDs, it was estimated that cardiovascular diseases increased in South Asia drastically from 1990 to 2019. The prevalence of CVDs increased up to 49.6% over the past 30 years. The relative increase in mortality was estimated to be about 30.3%. While ischemic heart disease was the major contributor to the mortality rate (56.51%), it was followed by stroke (29.77%) as the second most common cause.[4]

To curb the mortality rates attributed to CVDs worldwide, efforts are being put through timely intervention, medications, and public health measures in the past few years.[5] To further reduce the incidence, it is imperative to understand the role of modifiable and non-modifiable risk factors in the disease pathophysiology. According to the Framingham study, the emphasis should be to identify the risk factors and assess their role in disease causation. Some risk factors are non-modifiable risk factors such as gender, age, race, and family history. Modifiable factors such as smoking, diabetes mellitus, dyslipidemia, hypertension, and a sedentary lifestyle also have an important role in the disease pathogenesis and can be controlled.[6]

Studies done over the past three decades have associated triple vessel disease as an important risk factor for angina and myocardial infarction. A study reported that there is a significant association of TVD with major adverse cardiovascular events and mortality as compared to single or double vessel disease. The

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most common coronary artery being blocked is the left anterior descending. This artery supplies a large area of the heart muscle, including the interventricular septum, and the bundle branches. Its importance has been stressed with the acronym of 'widow-maker'; because myocardial infarction due to blockage at the start of the left anterior descending artery has fatal consequences.[7] Non-modifiable risk factors make up more than 90% of the risk factors responsible for CVDs. Lifestyle changes and prevention regimes to combat the risk factors can reduce the burden of CVDs to a significant level. Triple vessel disease and left anterior descending artery dominance are the factors that increase the chances of developing the acute coronary syndrome.[8] However, risk factors leading to their development are still not widely studied individually. This study was designed to find out various risk factors which are present in patients undergoing primary percutaneous coronary intervention. Another aim was to find the risk factors which are responsible for LAD predominance and occurrence of TVD as angiographic finding in patients undergoing P-PCI.

METHODOLOGY

After taking approval from the institution ethics committee and informed consent, the study was done at the Rawalpindi Institute of Cardiology, Rawalpindi from March 2019 to January 2021. It was a descriptive cross-sectional study, in which 3000 patients who underwent P-PCI were enrolled by non-probability convenient sampling technique. The patient information including age, gender, predominant vessel, number of vessels involved, smoking status, ejection fraction, and history of diabetes mellitus and hypertension was noted on a proforma. Blood pressure greater than 130/80 mmHg on two separate occasions within 4 hours apart was labeled as hypertension. Diabetes mellitus was defined as HbA1C greater than 6%. According to the WHO's smoking and tobacco use policy, a person who smokes any tobacco product on either a daily or occasional basis is defined as a smoker.

The patients who presented with chest pain, diaphoresis, or apprehension as their primary symptoms, underwent electrocardiography and cardiac enzymes as per protocol. Based on the results, the patients were given medical treatment as per hospital protocol. Patients with ST-elevation myocardial infarction who presented within 12 hours duration of symptoms underwent P-PCI.

Contrast injected in coronary ostia following cannulation allowed imaging and subsequent assessment of the coronary arteries which facilitated the planning of further intervention. Vessels that were categorized as unsuitable for PCI included those that had diffuse disease not amenable to PCI or severely calcified or referred for coronary artery bypass grafting. Vessels that fulfilled the criteria for further intervention were the ones with significant stenosis; major epicardial vessels or their branches with >70% stenosis or left main stem stenosis with >50% stenosis. Intervention for these cases involved PCI with percutaneous transluminal coronary angioplasty±stenting using drug eluting stents.

STATISTICAL ANALYSIS

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25 and frequency & percentages were calculated. The involvement of LAD and the occurrence of TVD formed the basis of data stratification. Post-stratification Chi-square test was performed. A significant p-value was ≤0.05.

RESULTS

Among the 3000 patients who presented with STEMI, 349(11.6%) were less than 40 years, 1803(60.1%) were between 40 to 60 years of age, while 848(28.3%) were greater than 60 years. Among the patients, 2563(85.4%) were males while 437(14.6%) were females. Diabetes mellitus, hypertension, and smoking were present in 897(29.9%), 1216(40.5%), and 990(33%) of the patients, respectively. Left anterior descending artery was involved in most of the cases (56.2%) followed by the right coronary artery (33.7%) and left circumflex artery was involved in 10.1% cases. Single vessel disease was present in 55.7% and TVD in 15.8% of the patients.

Left anterior descending artery involvement was more common among males when compared to females (57.1% versus 51%, p=0.018). Similarly, LAD involvement was 67.3% in the age group <40 years followed by 56.6% in the age group 40-60 years, and 50.8% in >60 years age group. These differences were highly significant with a p-value <0.001. The lower the left ventricular ejection fraction (LVEF), the greater was the frequency of LAD involvement. In patients with LVEF <31%, the frequency of LAD involvement was 56.2% whereas, LAD was involved in 54.9% in patients with LVEF 31-50%. In cases with LVEF ≥51% LAD was involved in 47.3% of the patients. The association of LVEF and LAD involvement was statistically significant (p=0.029). Similarly, the involvement of LAD was more common in single vessel disease while decreased with an increase in the involvement of vessels; single vessel disease (63.1%), double vessel disease (49.8%), and triple vessel disease (43.7%), p <0.001. However, there was no significant association found between the involvement of LAD and the occurrence of risk factors such as smoking, HTN, and DM.

Triple vessel disease was frequent among patients in the age group greater than 60 years, while least among the age group <40 years (>60 years:23%, 40-60

years:14.4%, <40 years:5.7%, p <0.001). Similarly, a greater percentage of participants with decreased ejection fraction had TVD (<31%:27.7%, 31-50%:12.1%, ≥51%:1.4%). Triple vessel disease was more common in patients with the predominant right coronary artery (20.5%) followed by the left circumflex artery (19.9%) and left anterior descending artery (12.3%), p <0.001. While the occurrence of TVD did not have a significant difference among gender, smoking, and DM but was significantly associated with HTN (14.1%, p=0.031) (Table 1).

Table 1: Association of Various Demographic Variables and Risk Factors with Left Anterior Descending Artery Involvement and Triple Vessel Disease

Variables		Total Numbers of Patients	Left Anterior Descending Artery Involvement			Triple Vessel Disease		
			Yes	No	p-value	Yes	No	p-value
Age Groups (Years)	<40	349(11.6%)	235(67.3%)	114(32.7%)	**<0.001***	20(5.7%)	329(94.3%)	**<0.001***
	40-60	1803(60.1%)	1021(56.6%)	782(43.4%)		259(14.4%)	1544(85.6%)	
	>60	848(28.3%)	431(50.8%)	417(49.2%)		195(23%)	653(77%)	
Gender	Male	2563(85.4%)	1464(57.1%)	1099(42.9%)	**0.018***	395(15.4%)	2168(84.6%)	0.158
	Female	437(14.6%)	223(51%)	214(49%)		79(18.1%)	358(81.9%)	
Left Ventricular Ejection Fraction	<31%	967(32.2%)	543(56.2%)	424(43.8%)	**0.029***	268(27.7%)	699(72.3%)	**<0.001***
	31-50%	1665(55.5%)	914(54.9%)	751(45.1%)		201(12.1%)	1464(87.9%)	
	≥51%	368(12.3%)	174(47.3%)	194(52.7%)		5(1.4%)	363(98.6%)	
Triple Vessel Disease	Yes	474(15.8%)	207(43.7%)	267(56.3%)	**<0.001***	474(100%)	0(0%)	-
	No	2526(84.2%)	1480(58.6%)	1046(41.4%)		0(0%)	2526(100%)	
Type of Disease	Single	1672(55.7%)	1055(63.1%)	617(36.9%)	**<0.001***	0(0%)	1672(100%)	-
	Double	854(28.5%)	425(49.8%)	429(50.2%)		0(0%)	854(100%)	
	Triple	474(15.8%)	207(43.7%)	267(56.3%)		474(100%)	0(0%)	
Predominant Vessel	Right Coronary Artery	1011(33.7%)	0(0%)	1011(100%)	-	207(20.5%)	804(79.5%)	**<0.001***
	Left Anterior Descending Artery	1687(56.2%)	1687(100%)	0(0%)		207(12.3%)	1480(87.7%)	
	Left Circumflex Artery	302(10.1%)	0(0%)	302(100%)		60(19.9%)	242(80.1%)	
Diabetes Mellitus	Yes	897(29.9%)	492(54.8%)	405(45.2%)	0.318	152(16.9%)	745(83.1%)	0.261
	No	2103(70.1%)	1195(56.8%)	908(43.2%)		322(15.3%)	1781(84.7%)	
Smoking Status	Yes	990(33%)	567(57.3%)	423(42.7%)	0.421	151(15.3%)	839(84.7%)	0.564
	No	2010(67%)	1120(55.7%)	890(44.3%)		323(16.1%)	1687(83.9%)	
Hypertension	Yes	1216(40.5%)	688(56.6%)	528(43.4%)	0.753	171(14.1%)	1045(85.9%)	**0.031***
	No	1784(59.5%)	999(56%)	785(44%)		303(17%)	1481(83%)	
DM and Smoking	Yes	280(9.3%)	156(55.7%)	124(44.3%)	0.854	47(16.8%)	233(83.2%)	0.635
	No	2720(90.7%)	1531(56.3%)	1189(43.7%)		427(15.7%)	2293(84.3%)	
Hypertension and Smoking	Yes	424(14.1%)	242(57.1%)	182(42.9%)	0.706	58(13.7%)	366(86.3%)	0.196
	No	2576(85.9%)	1445(56.1%)	1131(43.9%)		416(16.1%)	2160(83.9%)	
DM and Hypertension	Yes	139(4.6%)	78(56.1%)	61(43.9%)	0.977	17(12.2%)	122(87.8%)	0.237
	No	2861(95.4%)	1609(56.2%)	1252(43.8%)		457(16%)	2404(84%)	
All Three Present	Yes	139(4.6%)	78(56.1%)	61(43.9%)	0.977	17(12.2%)	122(87.8%)	0.237
	No	2861(95.4%)	1609(56.2%)	1252(43.8%)		457(16%)	2404(84%)	

**Significant p-value ≤ 0.05*

DISCUSSION

This study was done to determine the risk factors present in patients undergoing PCI for STEMI. The male to female ratio in our study was found to be 5.800:1.000, while according to prevalence studies in Pakistan, the male to female ratio was 1.033:1.000.[9] This shows that males are more likely to present for P-PCI as compared to women.

In our study, 15.8% of the patients suffered from triple vessel disease, 28.5% had double vessel involvement, and 55.7% had a single vessel disease. A study reported a much higher frequency of triple vessel disease in patients with non-ST-elevation MI patients (30.3%) as compared to single vessel disease (69.7%).[10] Similarly, another study conducted on 2225 patients revealed that 31.9% of patients had TVD and among them, the predominance of LAD was 58.4%.[7] This was similar to the current study as LAD predominance was reported in 56.2% of the patients. A study conducted by Sinha and colleagues reported that LAD was the dominant vessel blocked in 58.1% of the patients while TVD occurred in 6.6% of the patients.[11] Although, the prevalence of LAD blockage was comparable to our study but TVD was in higher proportion in our study.

Our study reported that diabetes mellitus, hypertension, and smoking were present in 29.9%, 40.5%, and 33% of the patients, respectively. The prevalence of smoking was 13.4%, diabetes mellitus was 30.8%, and hypertension was 26.34% in the general population in Pakistan.[12-14] This analysis shows that the patients who presented for PCI had these risk factors in a greater proportion than the general population. Shang et al. reported that hypertension was the most common risk factor (71.1%), followed by smoking (63.8%) and overweight/obesity (63%). Other risk factors were dyslipidemia (39%), diabetes mellitus (35.8%), and a history of previous CAD (14.2%).[15] The frequency of risk factors is quite high as compared to our study. An Indian study also reported the incidence of smoking at 78.5%, hypertension at 20.5%, and diabetes at 17.2%. These were not comparable to our study and this is attributable to the different age groups of the sample populations.[16]

In addition to determining the prevalence of risk factors and angiographic findings among the patients in this study, the association of various factors was seen with TVD and LAD involvement. Triple vessel disease was significantly associated with age, LVEF, the predominance of the vessel, and hypertension ($p < 0.05$). Although the current study did not find significant differences with diabetes, another study conducted on non-ST-elevation MI patients reported that patients with triple vessel disease had a higher incidence of diabetes (90.4% vs 33%, $p < 0.001$). The study did not find any significant association with age, gender, smoking, and hypertension.[10] A study conducted in Mexico reported a prevalence of 18.2% of patients with TVD and it had an association with gender, hypertension, diabetes mellitus, previous episodes of myocardial infarction, and heart failure.[17]

Our study showed that left artery involvement was associated with age, gender, LVEF, TVD, and type of disease. Left anterior descending predominance was more common in younger patients (67.3%) as compared to older patients (50.8%). This difference was significant. It was more common among males (57.1% vs 51%, $p < 0.001$) and less common among patients with triple vessel disease (43.7%). Adding further, it was more common among patients with a single vessel disease as compared to the double vessel or triple vessel disease ($p < 0.001$). Another study showed a strong relationship between LAD blockage and subsequent development of myocardial infarction but still was not been able to isolate factors that determine the increased probability of LAD involvement.[18] Hence, the current study adds important aspects to the present literature.

CONCLUSION

Risk factors such as diabetes, hypertension, and smoking are more common in patients presenting for P-PCI as compared to the general population. Triple vessel disease is associated with age, left ventricular ejection fraction, the predominance of the vessel, and hypertension. Left anterior descending artery involvement is associated with age, gender, left ventricular ejection fraction, triple vessel disease, and type of disease.

LIMITATIONS & RECOMMENDATIONS

The study determined the frequency of cardiovascular risk factors, triple vessel disease, and LAD involvement in patients presenting for P-PCI for STEMI but it did not evaluate the association of these factors with major adverse cardiovascular events and mortality. Future studies are required at a multicenter level which not only determine the prevalence of risk factors but also evaluate their association with adverse outcomes.

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Original Article

Serum Vitamin D and Calcium Levels in Preeclampsia

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ABSTRACT

Objective: To measure and compare serum vitamin D and calcium levels in primigravida women with preeclampsia (PE) and normotensive primigravida.

Methodology: This cross-sectional comparative study was conducted at the Department of Biochemistry, University of Health Sciences after ethical approval. Vitamin D and calcium levels were measured and compared from the venous blood of 45 pregnant females with preeclampsia (group A) and 45 pregnant normotensive females (group B) taken as controls. Vitamin D & calcium levels were measured by enzyme-linked immunosorbent assay (ELISA) and atomic absorption spectrometer, respectively.

Results: The mean serum calcium level of the controls was 9.25±0.98 mg/dL and that of preeclamptic females was 8.92±0.84 mg/dL. The difference between the two groups was not statistically significant (p-value=0.073). The mean serum vitamin D level of normotensive pregnant females was 19.25±9.25 ng/mL whereas it was 14.88±6.57 ng/mL in the preeclamptic pregnant females. The difference in these findings was significant between the two groups (p-value=0.038). A significant negative correlation between weight and BMI was observed with vitamin D in the preeclamptic group while no such correlation was found in the control group.

Conclusion: Preeclampsia is associated with lower levels of vitamin D. The pathophysiology of PE also involves altered metabolism of calcium however, a strong correlation was not found between altered levels of calcium and PE in the present study.

Keywords: *Preeclampsia. Gestational age. Calcium. Vitamin D. Hypertension.*

INTRODUCTION

Hypertensive disorders of pregnancy are important public health challenges and research efforts are yet to unravel the exact aetiology and pathogenesis of these disorders. Preeclampsia (PE) is a condition that is characterized by increased blood pressure (>140/90 mmHg) measured on two separate occasions at least 6 hours apart and proteinuria (>100 mg/dL) on urine analysis or >300 mg/24 hour urine collection, after 20 weeks of gestation in previously normotensive women.[1,2] The two mechanisms which play a major role in the pathogenesis are insufficient trophoblastic invasion and endothelial dysfunction leading to inappropriate growth of the placenta and reduced placental perfusion. In addition, exaggerated inflammatory and immune responses also contribute to PE.[3]

Vitamin D, also known as calcitriol plays a key role in the pathophysiology of PE. It binds to its nuclear vitamin D receptors, which are present in almost all the tissues of the body including the placenta.[4] Preeclampsia is thought to occur due to an abnormal maternal immune response that prevents a maternal placental invasion. Its deficiency leads to immune dysfunction, placental inflammation, and release of the placental-derived vasoconstrictive substance causing maternal hypertension and proteinuria.[5] Vitamin D suppresses renin synthesis in the renin-angiotensin system. Therefore, if the vitamin D level would be optimum in the body it will prevent hypertension by preventing the formation of renin and its deficiency can cause preeclampsia. In addition to its effects on the renin-angiotensin system, it also has a direct effect in suppressing the proliferation of vascular smooth muscle cells, improving endothelial cell-dependent vasodilation, and inhibiting anticoagulant activity.[6] In vascular smooth muscle cells, expression of vascular endothelial growth factor is also regulated by vitamin D. Vascular endothelial growth factors are essential for regulating the early changes which occur in the vasculature of placenta; improving angiogenesis of placenta and remodelling of maternal spiral arteries.[7]

Calcium plays important role in maintaining blood pressure. How calcium is involved in the pathogenesis of preeclampsia is nowadays receiving growing interest. A link between low serum calcium levels and increased incidence of preeclampsia and supplementation with calcium has been found to prevent PE and its related complications.[8] Several physiological events lead to a decline in maternal serum levels of calcium such as plasma volume expansion, decrease in the level of albumin, increase in glomerular filtration rate, increased calciuria, and transfer of calcium from the maternal system to the growing fetus.[9] The decrease in serum calcium levels causes an increase in parathyroid hormone (PTH) levels which helps to maintain constant serum calcium levels by increasing the absorption from the gastrointestinal

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tract. If calcium supplementation is provided, particularly late in pregnancy, this hyper-parathyroidism state may be reduced. The elevation of parathyroid hormone causes an increase in free intracellular ionized calcium, which results in vasoconstriction and increased vascular resistance causing high blood pressure.[10]

The association between the pathophysiology of PE and calcium is still controversial and further studies are required to establish this fact. This study was planned to measure vitamin D and calcium in preeclampsia and normotensive women during 30-34 weeks of pregnancy and see whether these parameters give some insight into the role of these metabolites in PE.

METHODOLOGY

This was a cross-sectional comparative study conducted at the Department of Biochemistry, University of Health Sciences, Lahore. After approval from the ethical review board and Advanced Studies & Research Board of the University. Subjects were recruited from the Department of Gynecological, Government Kot Khawaja Saeed Teaching Hospital, Lahore. The study was conducted from July 2018 to July 2019.

Informed consent was taken and all the data information was recorded on a subject datasheet. Ninety subjects were included in this study and were divided into two groups. Group A included 45 preeclamptic patients and 45 normotensive pregnant females were included in group B. The two groups were matched in maternal age, gestational age, and socioeconomic status. Inclusion criteria for group A were gestational age between 30-34 weeks, primigravida females with a single pregnancy, blood pressure >140/90 mmHg, and proteinuria of >300 mg/24 hours on urine sample analysis after 20 weeks of pregnancy. Inclusion criteria for group B were pregnant females of gestational age between 30-34 weeks, primigravida females with a single pregnancy, and normotensive. Exclusion criteria for both groups were multiple pregnancies, females taking vitamin D and calcium supplements, diabetes, renal disease, infections, obesity, and family history of hypertension. Blood samples were taken by a convenient sampling technique. Blood pressure was recorded by a digital sphygmomanometer device. Serum vitamin D level was determined by competitive enzyme-linked immunosorbent assay by using 25-OH vitamin D3 human ELISA kit (ORGENTEC Diagnostika GmbH, Germany). Normal serum vitamin D level was taken as 20-40 ng/mL. Serum calcium levels were measured by colourimetric method using a spectrophotometer (Hitachi Z2000) and its normal value was considered as 8.5-10.2 mg/dL.

STATISTICAL ANALYSIS

The data was analyzed by using Statistical Package for the Social Sciences version 22.0. Mean & standard deviation (SD) was calculated for normally distributed quantitative variables and median and interquartile range (IQR) were given for non-normally distributed quantitative variables. Student t-test was used to compare means in both groups for normally distributed quantitative variables. Mann-Whitney U test was used to compare non-normally quantitative variables between study groups. Pearson's correlation (r) was used to see a correlation between normally distributed quantitative variables and Spearman's rho correlation (rho) was used to find a correlation between non-normally distributed quantitative variables. A p-value of ≤ 0.05 was taken as statistically significant.

RESULTS

In the control group, the mean age was 22.4±2.85 years while it was 23.5±3.97 years for the preeclamptic women. No significant difference was seen in the age of the healthy primigravida females and primigravida preeclamptic patients (p-value=0.148). The median (IQR) gestational age of normotensive pregnant females was 32(31-33) weeks while the median (IQR) gestational age of preeclamptic females was also 32(30.5-33) weeks. No significant difference was found between the gestational age of both groups (p-value=0.179). The mean BMI of the normal pregnant females was 27.5±1.48 kg/m^2 while the BMI of the preeclamptic patients was 28.8±1.42 kg/m^2. This difference in values was significant (p-value=0.001). Mean systolic blood pressure in group A & B was 156.86±8.27 mmHg and 112.8±10.62 mmHg, respectively. Mann-Whitney U test was used to compare the results of the two groups and a significant difference was seen (p-value=0.001). The mean value of diastolic blood pressure of the controls was 74.0±10.27 mmHg while it was 106.9±11.66 mmHg for the females with preeclampsia. The mean serum calcium level of the controls was 9.25±0.98 mg/dL and that of preeclamptic females was 8.92±0.84 mg/dL. No statistically significant difference was found between the two groups (p-value=0.073). The mean serum vitamin D level of normotensive pregnant females was 19.25±9.25 ng/mL whereas it was 14.88±6.57 ng/mL for the preeclamptic pregnant females. The difference in these findings was significant between the two groups (p-value=0.038) (Table 1).

A positive correlation between systolic and diastolic blood pressure was seen in the control group and the patient group. The correlation between weight, height, and BMI was also positive in both groups. A significant positive correlation between vitamin D with calcium was seen in the control group (Table 2).

The Spearman's rho correlation for this association was 0.408 and the p-value was 0.005. A significant positive correlation between vitamin D with calcium was also found in the preeclamptic group (Spearman's rho=0.368; p-value=0.013). A negative correlation between weight and BMI was observed with vitamin D in the preeclamptic group. However, no such correlation was found in the control group.

Table 1: Comparison of Parameters in the Preeclamptic and Control Groups

Parameters	Group A (Preeclamptic Patients)		Group B (Control)		p-value
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
Age (Years)	23.5±3.97	23(20.5-26)	22.4±2.85	22(20-25)	0.148$^{\alpha}$
Gestational Age (Weeks)	31.68±1.32	32(30.5-33)	32±1.44	32(31-33)	0.179$^{\alpha}$
Systolic Blood Pressure (mmHg)	156.8±8.27	156(150-164)	112.8±10.62	112(102-123)	0.001*$^{\alpha}$
Diastolic Blood Pressure (mmHg	106.9±11.6	106(96-118)	74±10.27	72(65-83)	0.001*$^{\alpha}$
BMI (kg/m^2)	28.8±1.42	28.76(27.7-29.76)	27.5±1.48	27.6(26.45-28.1)	0.001*$^{\beta}$
Vitamin D (ng/mL)	14.88±6.57	13.29(10.63-16.5)	19.25±9.25	17.25(11.09-24.8)	0.038*$^{\alpha}$
Calcium (mg/dL)	8.92±0.84	8.6(8.4-9.5)	9.25±0.98	8.9(8.5-10.25)	0.073*$^{\alpha}$

$^{\alpha}$p-value calculated by Mann-Whitney U Test
$^{\beta}$p-value calculated by Independent Sample t-test
**Significant p-value ≤0.05*

Table 2: Correlation Matrix of Clinical and Biochemical Variables of Study Groups

Correlation Matrix of Clinical and Biochemical Variables of Preeclamptic Patients						
Parameters	Correlation	Systolic Blood Pressure	Weight	BMI	Vitamin D	Calcium
Diastolic Blood Pressure	r/rho	0.801$^{\beta}$				
	p-value	0.001*				
Height	r/rho	-0.070$^{\beta}$	0.773$^{\beta}$			
	p-value	0.649	0.001*			
BMI	r/rho	-0.193$^{\beta}$	0.704$^{\alpha}$			
	p-value	0.205	0.001*			
Vitamin D	r/rho	-0.036$^{\beta}$	-0.343$^{\beta}$	-0.319$^{\beta}$		
	p-value	0.814	0.021	0.033*		
Calcium	r/rho	0.055$^{\alpha}$	0.056$^{\beta}$	0.196$^{\beta}$	0.368$^{\beta}$	
	p-value	0.719	0.713	0.202	0.013*	
Correlation Matrix of Clinical and Biochemical Variables of Healthy Controls						
Diastolic Blood Pressure	r/rho	0.570$^{\beta}$				
	p-value	0.001*				
Height	r/rho	-0.267$^{\beta}$	0.651$^{\beta}$			
	p-value	0.077	0.001*			
BMI	r/rho	-0.101$^{\beta}$	0.557$^{\alpha}$			
	p-value	0.507	0.001*			
Calcium	r/rho	-0.192$^{\beta}$	0.040$^{\beta}$	0.140$^{\beta}$	0.408$^{\beta}$	
	p-value	0.207	0.794	0.359	0.005*	

$^{\alpha}$Correlation coefficient (r) & p-values are calculated by Pearson's Correlation coefficient
$^{\beta}$Correlation coefficient (rho) & p-values are calculated by Spearman's Rho Correlation coefficient
**Significant p-value ≤0.05*

DISCUSSION

Establishing an exact relationship between vitamin D, calcium, and preeclampsia is complicated. We found a remarkable difference in the level of vitamin D between the control and patient groups. Although vitamin D in both groups had deficiency but it was more severe in the preeclamptic group and this difference in the serum vitamin D levels among the two groups was statistically significant (p=0.046). Similarly, many studies have supported the hypothesis on the role of vitamin D in the aetiology of preeclampsia.[11-14]

For an adequate synthesis of vitamin D (3000 IU), a minimum exposure of at least 10 minutes to direct sunlight in the wavelength between 290-315 nm is required. One of the reasons for vitamin D deficiency in both the pregnant female groups included in this study was maybe due to the reason that most of the Pakistani females cover their whole body to observe purdah while going outside and many of the females wear burqa (a black colored garment worn by Muslim females). It has been documented that clothing preference affects vitamin D synthesis by decreasing the absorbance of ultraviolet (UV) rays by the skin.[15]

Another observation in this study was that most of the females in the preeclamptic group were housewives and spent most of their time indoors, thus they had minimum sun exposure during the times when the sun is the brightest accounting for more severe vitamin D deficiency in preeclamptic group. The finding is in contrast with the findings of some other studies which did not report any association between vitamin D deficiency and preeclampsia.[16] There are several possible explanations for the inconsistent and equivocal findings about the association of vitamin D & preeclampsia. It can be related to the differences in lifestyle, differences in dietary intake of vitamin D, skin color, genetic differences in the metabolism of vitamin D, duration of exposure to sunlight, climate changes, and method of measurement of vitamin D.

We found out that the PE women were heavier (p=0.018) and had higher (p <0.001) BMI than the women with a healthy pregnancy. Also, a negative correlation between vitamin D was observed with weight and BMI in the preeclamptic group of this study (Spearman's rho=-0.319, p=0.033). This finding is similar to the study of Delle Monache et al., who reported that an increase in BMI (obesity) can proportionally reduce the vitamin D level in women regardless of their ages.[17] It is hypothesized that the metabolic clearance of vitamin D might increase in obesity possibly due to the increased sequestration of vitamin D in adipocytes and consequently alteration of its release into the circulation. Another explanation for the correlation between an increase in BMI and low vitamin D levels can be that overweight patients usually have a sedentary lifestyle and spend more time indoors. This also deprives them of vitamin D since UV rays are essential for its synthesis.[18]

Calcium is an important bivalent ion which not only maintains good bone health but also acts as a cofactor for many enzymes. The maternal serum calcium levels directly affect the fetal serum calcium levels. Calcium is very essential for the development of fetal bones, muscles, and nerves. As a result of the increasing demand for calcium in the mother and fetus during pregnancy, calcium deficiency is a commonly encountered issue, especially in developing countries. It also maintains the smooth muscle vascular tone hence, it is involved in the regulation of blood pressure.[19]

Our results showed that the mean serum calcium levels in the preeclamptic females (8.92±0.84 mg/dL) were lower as compared to controls (9.25±0.98 mg/dL). The difference between the two groups was not statistically significant (p-value=0.073). This is in agreement with the findings of other studies which observed no significant difference in the serum calcium levels between preeclamptic and normotensive pregnant females.[20,21] The finding of the present study and the previous studies can be explained by the fact that these studies reported decreased serum vitamin D levels with the consequent increase in parathyroid hormone levels. This rise in PTH levels tries to maintain the serum calcium levels within the normal range by increasing its reabsorption from the intestines and the kidney tubules.[22]

On contrary, Winarno et al., & Al-Jameil et al., have reported a statistically significant decrease in serum calcium levels in PE.[23,24] It is possible that this difference is due to the different methods of assay, sample size, genetics, dietary habits, and lifestyle of the population studied.

CONCLUSION

Preeclampsia is associated with lower levels of vitamin D. The pathophysiology of PE also involves altered metabolism of calcium however, a strong correlation was not found between altered levels of calcium and PE in the present study.

LIMITATIONS & RECOMMENDATIONS

The sample size was taken smaller due to the financial constraints, however, further prospective studies with large sample sizes should be conducted to confirm or refute these findings. In addition to vitamin D and calcium, parathyroid hormone levels and bone-related parameters should also be investigated. The current study utilized the ELISA technique for measuring vitamin D levels. Other modern techniques such as high-performance liquid chromatography should also be explored. This study recommends that improvement in nutrition can have a beneficial effect on the overall

health of pregnant females and it can prevent pregnancy-related complications such as preeclampsia.

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Original Article

A Comparative Analysis of Students' Level of Satisfaction Regarding Online versus On-Campus Learning in the Subject of Anatomy

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ABSTRACT

Objective: To compare the perceptions of students about online versus on-campus teaching in the subject of Anatomy and to analyze the difference in the performance of students in assessments after on-campus and online teaching.

Methodology: A cross-sectional survey was carried out with the help of a closed-ended validated questionnaire provided to first and second year MBBS students. The on-campus assessment was taken at end of face to face and online teaching to compare the performance of students after the use of both teaching modalities. Data collected was analyzed by using Statistical Package for the Social Sciences (SPSS) version 26.

Results: A total of 208 MBBS students participated in the study. Among these students, 103 were first year MBBS and 105 were second year MBBS students. Sixty nine (67%) first year students were females and 34(33%) first year students were males. Among second year, 71(67%) were females and 34(32%) were male students. Regarding online teaching, 52% students of 1st year MBBS were satisfied, 26.4% were dissatisfied, and 21.6% had neutral responses. While 50.3% students of 2nd year MBBS were satisfied, 30.1% were dissatisfied, and 19.6% had neutral responses. The pass percentage of first year MBBS class after online teaching was 58% and after on-campus teaching, it was 67%. The pass percentage of second year MBBS after online teaching was 70% and after on-campus teaching, it was 75%.

Conclusion: Most of the students were not satisfied with online teaching. The subject of Anatomy is difficult to grasp through online teaching but effective use of videos, digital photographs, and synchronous teaching can facilitate students in achieving the desired concepts.

Keywords: *Online learning. On-campus learning. Anatomy.*

INTRODUCTION

The COVID-19 pandemic has disrupted the education system and forced us to find new ways to address the teaching and learning crisis. A change occurred from conventional teaching and learning to online educational activities to ensure the safety of students, teachers, and related staff. These online activities were required for the sustainability of teaching, and learning processes at different levels of education including medical colleges.[1] In medical education, online learning can be a more effective and easier way, especially in uncertain global situations such as pandemics.[2] The success of online learning depends on many factors including availability and accessibility of appropriate equipment, methods of delivering, the course content, and assessment criteria. Online learning, like other methods of teaching, has benefits as well as shortcomings for both students and teachers.[2]

A face to face teaching set-up provides immediate feedback to faculty members and students about the understanding of the lesson and ways of its delivery. Moreover, questions and comments from fellow students can assist in learning. Direct observation of the student's response allows the faculty member to immediately adjust the teaching method and its pace. Online education does not provide these same clues and the teacher and students must count on written responses or feedback.[3]

In Pakistan, online learning has never been considered as a part of formal education by a majority of medical institutions until the spread of COVID-19.[4] The administrators and teachers of medical colleges took all necessary measures to conduct effective online learning via lectures and small group discussions like demonstration, practical, and case-based learning. Also, various software for online teaching are being explored by teachers to meet this challenge and to deliver the teaching material.[5] Anatomy is a labor-intensive subject in which students have to memorize anatomical terms and hardcore facts. The concepts are acquired by studying bones, 3-D models, histological slides, and cadaveric specimens during face to face sessions which enhance and facilitate understanding and learning of the subject.[6]

As online teaching methodology is newly adapted, both teachers and students are still in the process of getting accustomed to this system so it is essential to find out students' views regarding this method of teaching and learning in the subject of Anatomy.[7] It will be of interest to analyze whether the students are adjusted to learning Anatomy through online classes or if they want any modifications. Therefore, the purpose of this study was to achieve a comparative analysis of students' perceptions regarding online versus on-campus

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learning in the subject of Anatomy and to measure the performance of students after the use of both online and on-campus teaching modalities. The study will be helpful in identifying & addressing the weak points so that online teaching can be more effective and meaningful.

METHODOLOGY

It was a cross-sectional study performed at Fazaia Medical College, Air University, Islamabad from July to November 2021. A total of 208 MBBS students participated in the study. Among these students, 103 were first year MBBS and 105 were second year MBBS students. The identity of students was not revealed. Students who were absent at the time of data collection were excluded from the study. The sampling technique used was probability sampling. All the participants were briefed about the basic concept behind the study before providing the questionnaire. Written informed consent was taken from every participant. The study was approved by the institutional ethical review board. For this study, a self-constructed questionnaire was used having a total of 25 close-ended questions. A pilot study was conducted to validate the questionnaire. Nine questions were concerned with on-campus and 15 were related to online teaching. Percentages and frequencies were calculated for the answers given against each question as per the Likert scale. One & 2 were taken as highly dissatisfied (strongly disagree & disagree), 4 & 5 were taken as satisfied (strongly agree & agree) and 3 was considered as uncertain. Another variable of the study was the assessment of students after online and on-campus teaching to compare the difference in performance among students after using two different teaching modalities.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 26. Frequency & percentage were calculated for categorical data like gender & satisfaction of students. Chi-square was applied to analyze the difference between the performances after online and on-campus teaching. A p-value ≤ 0.05 was considered significant.

RESULTS

Out of 103 first year MBBS students, 69(67%) were females and 34(33%) were males. Among second year, 71(67%) were females and 34(32%) were male students. Regarding on-campus teaching, 82.3% students of 1st year MBBS were satisfied, 8.4% were dissatisfied, and 9.3% had a neutral response. Whereas 81.7% students of 2nd year MBBS were satisfied, 9.4% were dissatisfied, and 8.9% had a neutral response (Table 1).

Regarding online teaching, 52% students of 1st year MBBS were satisfied, 26.4% were dissatisfied, and 21.6% had neutral responses. While 50.3% students of 2nd year MBBS were satisfied, 30.1% were dissatisfied, and 19.6% had neutral responses (Table 2).

To assess and compare the performance after on-campus and online teaching in the subject of Anatomy, the results of two end of block examinations were analyzed. The pass percentage of first year MBBS class after online teaching was 58% and after on-campus teaching, it was 67%. The pass percentage of second year MBBS after online teaching was 70% and after on-campus teaching, it was 75% (Table 3).

DISCUSSION

This study was primarily focused on face to face versus online teaching and learning of students of Fazaia Medical College in the subject of Anatomy. This study showed that 92.2% of 1st year and 94.3% of 2nd year MBBS students agreed that on-campus face to face teaching is more beneficial, as it is easy for them to understand and retain the bones and models during face to face sessions. Student-teacher interaction was found more effective during on-campus classes as compared to online sessions which is another reason students (89.3% 1st year and 85.7% 2nd year MBBS students) preferred this system over online learning. Baczek et al. also showed less effective interaction between students and facilitators during e-learning. The attention span and concentration towards lectures are better in on-campus teaching as students find it easier to ask questions there and then clear all the queries and confusions about the topic.[8] Our results are comparable with the research conducted by Barrot et al., in 2021 which raised concerns that lack of face to face study leads to unfavorable effects on students' learning along with communication and socializing skills.[9] One of the reasons for this response could be that there is more distraction while studying online from home along with a lack of knowledge and training in technology. The cause of distraction could also be due to the lack of college academic environment, eye to eye contact with instructors, and effective interaction with peers during online classes.[10,11] Subsequently, 76.7% of 1st year and 69.5% of 2nd year students felt less motivated in online teaching in the current study. The lack of extra-curricular activities like sports, dramatics, and other cultural events may also be a reason for demotivation and lack of interest.

In our study, it was identified that our students were not satisfied with their computer knowledge and IT skills which is a basic necessity for online learning. Although 61.2% of 1st year and 79% of 2nd year MBBS students accepted the availability of sufficient equipment and facilities required for e-learning, lack of digital literacy and technical expertise impeded the educational

Table 1: Level of Satisfaction of First & Second Year MBBS Students Regarding On-Campus Teaching

Sr. No.	Questions Regarding On-Campus Teaching	Student Response	First Year MBBS n=103	Second Year MBBS n=105
1	Traditional on-campus face to face teaching is more beneficial than online teaching for the subject of Anatomy.	Agree	95(92.2%)	99(94.3%)
		Neutral	5(4.9%)	4(3.8%)
		Disagree	3(2.9%)	2(1.9%)
2	Teaching and demonstration of bones and Anatomy models were difficult to grasp in online sessions compared to face to face sessions.	Agree	100(97.1%)	93(88.6%)
		Neutral	3(2.9%)	8(7.6%)
		Disagree	0(0%)	4(3.8%)
3	On-campus lectures are more interesting and innovative than online lectures for the subject of Anatomy.	Agree	86(83.5%)	94(89.5%)
		Neutral	15(14.6%)	7(6.7%)
		Disagree	2(1.9%)	4(3.8%)
4	On-campus teaching created more effective interaction with teachers compared to online teaching.	Agree	92(89.3%)	90(85.7%)
		Neutral	8(7.8%)	10(9.5%)
		Disagree	3(2.9%)	5(4.8%)
5	The facility to ask questions or clear doubts during on-campus face to face lectures is much more convenient.	Agree	81(78.6%)	86(81.9%)
		Neutral	16(15.6%)	16(15.2%)
		Disagree	6(5.8%)	3(2.9%)
6	Attention span and concentration towards the lecture and teacher are markedly reduced during on-campus classes.	Agree	33(32%)	32(30.5%)
		Neutral	16(15.5%)	17(16.2%)
		Disagree	54(52.5%)	56(53.3%)
7	The study of Anatomy is much more interesting with the models and bones in the hand.	Agree	95(92.2%)	98(93.3%)
		Neutral	6(5.9%)	4(3.8%)
		Disagree	2(1.9%)	3(2.9%)
8	Some very basic terminology and concepts are easy to explain in face to face learning.	Agree	92(89.3%)	91(86.7%)
		Neutral	9(8.8%)	9(8.5%)
		Disagree	2(1.9%)	5(4.8%)
9	This academic year was a hybrid of on-campus and online learning methods. On-campus learning of the subject was easy to grasp and long-lasting.	Agree	89(86.4%)	89(84.8%)
		Neutral	8(7.8%)	9(8.7%)
		Disagree	6(5.8%)	7(6.7%)

capabilities of many students. If we are unable to come up with students' expectations regarding online studies, it will have a negative effect on students' learning.[12] During online learning two modalities were used. Initially, prerecorded Anatomy lectures were shared with students and later on synchronous teaching was also performed. Students showed a positive response towards synchronous teaching as compared to prerecorded sessions since synchronous teaching provides an opportunity to interact with the teacher at the time of learning to clarify and grasp the concepts of Anatomy. In contrast to our findings, a research conducted at Ottawa university showed that though medical students had a slight preference for asynchronous as compared to synchronous Anatomy learning, they were generally satisfied with both modalities for various Anatomy courses.[13] A research conducted in the year 2021 in the UK found that students preferred a hybrid method of teaching, including live interactive lectures as well as prerecorded sessions, but on-campus lectures were preferred over any teaching modality.[14] In another study, when two forms of instructional strategies (both prerecorded and online sessions) were compared, students not only showed strong agreement for prerecorded lectures for the flexibility and convenience to study from audio/video recordings but they also showed a lack of motivation in learning from video lectures and excessive workload accumulation specially before exams.[15] In a question, regarding the

Table 2: Level of Satisfaction of First & Second Year MBBS Students Regarding Online Teaching

Sr. No.	Questions Regarding Online Teaching	Student Response	First Year MBBS n=103	Second Year MBBS n=105
1	Do you have sufficient computer knowledge and IT skills to manage your online learning?	Agree	56(54.4%)	80(76.2%)
		Neutral	32(31.1%)	18(17.1%)
		Disagree	15(14.5%)	7(6.7%)
2	Do you have sufficient equipment and facilities (computer/laptop/internet/software) to participate in online lectures?	Agree	63(61.2%)	83(79%)
		Neutral	29(28.2%)	12(11.5%)
		Disagree	11(10.6%)	10(9.5%)
3	Online lectures are more interesting and innovative than traditional/live classroom lectures for the subject of Anatomy.	Agree	13(12.6%)	12(11.4%)
		Neutral	20(19.4%)	25(23.8%)
		Disagree	70(68%)	68(64.8%)
4	Online teaching created more effective interaction with teachers compared to on-campus face to face teaching.	Agree	14(13.6%)	13(12.4%)
		Neutral	26(25.2%)	18(17.1%)
		Disagree	63(61.2%)	74(70.5%)
5	The facility to ask questions or clear doubts during online lectures is much more convenient.	Agree	23(22.3%)	13(12.4%)
		Neutral	36(35%)	31(29.5%)
		Disagree	44(42.7%)	61(58.1%)
6	Studying through e-learning mode provides flexibility to study at a time convenient to the learner.	Agree	50(48.5%)	44(41.9%)
		Neutral	25(24.3%)	26(24.8%)
		Disagree	28(27.2%)	35(33.3%)
7	Attempting an assessment after online teaching is convenient as compared to an assessment taken after on-campus face to face learning.	Agree	20(19.4%)	20(19%)
		Neutral	22(21.4%)	26(24.8%)
		Disagree	61(59.2%)	59(56.2%)
8	Do you feel the deficiency of online resource material?	Agree	34(33%)	41(39.1%)
		Neutral	38(36.9%)	23(21.9%)
		Disagree	31(30.1%)	41(39%)
9	You missed live models and histology laboratory sessions.	Agree	72(69.9%)	69(65.7%)
		Neutral	13(12.6%)	14(13.3%)
		Disagree	18(17.5%)	22(21%)
10	Learning embryology without models is more difficult.	Agree	89(86.4%)	73(69.5%)
		Neutral	8(7.8%)	17(16.2%)
		Disagree	6(5.8%)	15(14.3%)
11	Live online classes are more beneficial compared to prerecorded lectures.	Agree	50(48.5%)	57(54.3%)
		Neutral	22(21.4%)	12(11.4%)
		Disagree	31(30.1%)	36(34.3%)
12	There is always an element of distraction.	Agree	76(73.8%)	71(67.6%)
		Neutral	22(21.3%)	25(23.8%)
		Disagree	5(4.9%)	9(8.6%)
13	I miss the college environment, companions, cultural and sporting events.	Agree	89(86.4%)	76(72.4%)
		Neutral	9(8.7%)	16(15.2%)
		Disagree	5(4.9%)	13(12.4%)
14	There is a lack of self-motivation.	Agree	79(76.7%)	73(69.5%)
		Neutral	16(15.5%)	21(20%)
		Disagree	8(7.8%)	11(10.5%)
15	I feel uncertain about concepts in the current academic year.	Agree	75(72.8%)	67(63.8%)
		Neutral	16(15.5%)	24(22.9%)
		Disagree	12(11.7%)	14(13.3%)

Table 3: Performance of First & Second Year MBBS Students in Assessment

Class	End of Block Examination	Pass	Fail	Total	p-value
First Year MBBS	EOB 1 (After On-Campus Teaching)	70	32	102	0.123
	EOB 2 (After Online Teaching)	60	43	103	
Second Year MBBS	EOB 1 (After On-Campus Teaching)	71	34	105	0.226
	EOB 2 (After Online Teaching)	76	25	103	

availability of study material during e-learning, 36.9% 1st year and 21.9% 2nd year students gave neutral responses whereas 30.1% 1st year and 39% 2nd year students didn't perceive any deficiency of resource material. One of the reasons could be that students were provided sufficient study material in the form of pictures/videos of models, cadaveric specimens, and histology slides by uploading the content on their Google classrooms. Besides the availability of sufficient resource material, students preferred the hands-on study of models, bones, cadaveric specimen, and histology slides under microscopes which shows that understanding and retention is better by the use of one's psychomotor skills during learning. Our results are comparable with a research conducted on two groups of students where one group was taught in the dissection hall and the other group was taught by showing Anatomy videos. Students who learnt Anatomy through hands-on practice were more satisfied and also showed higher grades.[16]

Our results showed that 82.3% students of 1st year MBBS and 81.7% students of 2nd year MBBS were satisfied with on-campus teaching. Whereas, only 52% students of 1st year MBBS and 50.3% students of 2nd year MBBS were satisfied with online teaching. Similar results were shown in another study conducted by Ahsan et al., which showed that 61.9% students were not satisfied with online teaching.[17] Another study reported that 89.62% of medical students believe that traditional classroom teaching is better than online teaching.[18]

In the current study, assessments were taken after both online and on-campus teaching and both results were compared which showed an insignificant difference between the two teaching modalities. Though results after on-campus teaching were slightly better, but the difference between the two was statistically insignificant which showed that besides apprehensions and perceptions regarding hindrances and difficulties faced during e-learning, it had minimal effect on the results of students showing that synchronous teaching and sufficient resource material in the form of videos provided by the faculty bridged the gaps in online teaching. Another study reported that there is no significant difference in the performance of students after online and on-campus teaching.[19]

CONCLUSION

Most of the students were not satisfied with online teaching. The subject of Anatomy is difficult to grasp through online teaching but effective use of videos, digital photographs, and synchronous teaching can facilitate students in achieving the desired concepts.

LIMITATIONS & RECOMMENDATIONS

The study was conducted in one particular college, so the results cannot be generalized. In this study, only two modules were compared. Comparison among multiple modules could have given more reliable results. For future recommendations, data from multiple colleges conducting online as well as face to face studies in the subject of Anatomy can be taken for comparison. Online teaching sessions should be conducted during on-campus teaching to train faculty and students.

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Original Article

Diagnostic Accuracy of Mean Platelet Volume in Neonatal Sepsis taking Blood Culture as Gold Standard

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ABSTRACT

Objective: To find out the diagnostic accuracy of mean platelet volume (MPV) in neonatal sepsis by taking blood culture as a gold standard.

Methodology: This cross-sectional study was carried out at Hematology Department, Combined Military Hospital, Lahore from May 2019 to August 2020. Two hundred and five neonates of either gender aged 0 to 28 days with suspected neonatal sepsis were included. Peripheral venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA) and blood culture bottles. Two separate Sysmex KX-21 hematology analyzers were used to evaluate MPV. The mean of each was used as the final reading. On culture, neonatal sepsis was considered as positive for samples that yielded bacterial pathogens. The diagnostic accuracy of MPV was calculated utilizing blood culture as a gold standard.

Results: The mean age of patients was 14.52±8.31 days. There were 99(48.3%) male and 106(51.7%) female cases with a higher female to male ratio. The cutoff value of MPV was 10.2 fL. Fifty three (26%) cases were diagnosed positive for MPV and 47(23%) cases had positive blood cultures. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of MPV were 82.98%, 91.14%, 73.58%, 94.74%, and 89.27%, respectively.

Conclusion: Mean platelet volume has a high diagnostic accuracy and hence in future, this biomarker can be utilized as a diagnostic tool for rapid diagnosis of neonatal sepsis.

Keywords: *Neonatal sepsis. Blood culture. Mean platelet volume. Sensitivity. Specificity.*

INTRODUCTION

Neonatal sepsis (NS), which can occur with or without bacteremia, is a disease characterized by infection-related symptoms and signs in the first month after birth.[1] It is a serious condition in neonatal intensive care units, and it is one of the leading causes of neonatal deaths with a prevalence rate of 20%.[2] It is further estimated that 17.6% of all neonates die globally with sepsis.[3] It is caused by bacterial, viral, fungal, or protozoal pathogens.[4] The clinical picture of neonatal sepsis is subtle and non-specific which renders detection challenging for clinicians. Blood culture has been the standard criteria for diagnosis of septicemia, but it requires a lot of time and is mostly not easily accessible. This delay in diagnosis can add a risk of mortality due to delayed treatment.

Platelet indices have also gained popularity as inflammation and infection indicators.[5] Platelets are essential in primary hemostasis and are often involved in secondary hemostasis. Platelets play a function in inflammation, and their antimicrobial efficacy has been shown without a shadow of a doubt in a number of acute and chronic infections.[6] Infectious diseases have been related to shifts in platelet volume indices. Patients with sepsis had higher MPV and platelet distribution width values than patients who did not have sepsis. Platelet indices are cheap and easily available routinely performed tests so these can be used for the diagnosis of neonatal sepsis.[7]

Prompt diagnosis and treatment of neonatal sepsis is an important factor in reducing morbidity and mortality. No local study is available and international data published yet has inconsistent sensitivity and specificity. Hence, this study can help to know the diagnostic accuracy of MPV in our population. In future, this biomarker can be utilized as a diagnostic tool as there is an urgent need for rapid diagnosis of neonatal sepsis.

METHODOLOGY

A cross-sectional study was carried out at the Hematology Department, Combined Military Hospital, Lahore from May 2019 to August 2020. A consecutive sampling technique was utilized to draw the blood sample of neonates with clinical suspicion of sepsis. A sample size of 205 was calculated at a 5% significance level and taking the expected prevalence of neonatal sepsis as 19.3%.

All neonates of either & gender aged 0 to 28 days with suspected neonatal sepsis were included. Neonates who had suffered birth asphyxia (assessed on clinical presentation or medical record), low birth weight of less than 1500 grams (as defined by their birth card/record), gestational age at birth of less than 32 weeks (as determined by ultrasound at the time of birth) and serious congenital malformations such as meningomyelocele (as determined by clinical presentation) were excluded.

After taking approval from the hospital ethical review

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committee 205 patients reporting to the Pediatric Emergency Department fulfilling the study criteria were included in the study. Informed written consent from the parents was taken. Peripheral venous blood samples, 3 mL in EDTA were taken along with blood culture samples (5 mL) in blood culture bottle as advised by the Clinical Microbiologist of Combined Military Hospital, Lahore. The samples were deposited at the Pathology Department of Combined Military Hospital, Lahore within half an hour time-lapse. Two separate Sysmex KX-21 hematology analyzers were used to evaluate MPV and platelet count. The mean of each was used as the final reading. For the risk assessment and management, thrombocytopenia was classified according to different severity levels (severe thrombocytopenia <50,000/µL, moderate thrombocytopenia 50,000-1,00,000/µL, mild thrombocytopenia 1,00,000-1,50,000/µL).[8] On culture, neonatal sepsis was considered as positive for samples that yielded bacterial pathogens. Mean platelet volume was measured in fL and a cutoff was determined by the receiver operating characteristic curve. On the proforma, the results of blood cultures and MPV were reported. Specificity, sensitivity, negative predictive value, positive predictive value, and diagnostic accuracy were calculated.

STATISTICAL ANALYSIS

All the collected data was entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 22. Mean±SD was calculated for quantitative data (age in days). Frequency and percentages were used for qualitative data like the gender of the neonate, diagnosis of neonatal sepsis on blood culture, and MPV. A 2x2 table was made to calculate sensitivity, specificity, positive, and negative predictive values of MPV taking blood culture as a gold standard.

RESULTS

The mean age of patients was 14.52±8.31 days with minimum and maximum age of 1 and 28 days, respectively. There were 95(46.34%) neonates who were 1-13 days old and 110(53.66%) cases were 14-28 days old. There were 99(48.3%) male and 106(51.7%) female cases with a higher female to male ratio. There were 30(14.6%) patients with normal platelet count, 100(48.8%) with mild thrombocytopenia, 55(26.8%) with moderate thrombocytopenia, and 20(9.8%) with severe thrombocytopenia (Table 1).

The cutoff value of MPV was 10.2 fL. There were 53(26%) patients diagnosed positive for MPV and 47(23%) cases had positive findings on culture. There were 39(74%) cases that had positive findings on both culture and MPV and 144(95%) cases had negative findings on both culture and MPV. There were 14(26%) cases that had negative findings on culture but had positive findings on MPV while 8(5%) cases had positive findings on culture but had negative findings on MPV (Table 2). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of MPV were 82.98%, 91.14%, 73.58%, 94.74%, and 89.27%, respectively.

Table 1: Degree of Thrombocytopenia in Study Subjects

Thrombocytopenia	Frequency & Percentage
No Thrombocytopenia	30(14.6%)
Mild (1,00,000-1,50,000/µL)	100(48.8%)
Moderate (50,000-1,00,000/µL)	55(26.8%)
Severe (<50,000/µL)	20(9.8%)

Table 2: Comparison of Diagnosis of Neonatal Sepsis on Blood Culture & MPV

Diagnosis on MPV	Diagnosis on Blood Culture		Total
	Positive	Negative	
Positive	39(74%)	14(26%)	53(26%)
Negative	8(5%)	144(95%)	152(74%)
Total	**47(23%)**	**158(77%)**	**205(100%)**

DISCUSSION

Neonatal sepsis is a widespread and significant cause of morbidity and mortality, responsible for one-quarter of all neonatal deaths.[8] Since the clinical signs and symptoms of sepsis in newborns may be subtle, diagnosing it involves a strong level of skepticism. There is no single laboratory test that is both sensitive and accurate.[9] It is impossible to render an early diagnosis of NS, as it is often identified late owing to the wide variety of clinical symptoms. The gold standard diagnostic technique for NS is positive blood culture but it results in a delay in the initiation of

antibiotic therapy, resulting in high mortality.[10] Mean platelet volume is a simple platelet parameter that can be used for the diagnosis of sepsis.[11]

In the current study, the mean age of patients was 14.52±8.31 days with minimum and maximum age of 1 and 28 days, respectively. There were 99(48.3%) males and 106(51.7%) females with a higher female to male ratio. Thrombocytopenia was found in 85.4% of patients and 14.6% of cases had normal platelet levels. Similar results were found in another study conducted by Sri Laxmi et al. They reported thrombocytopenia in 80% of cases.[8]

Fifty three (26%) were diagnosed positive for MPV and 47(23%) cases had positive findings on culture. In this study, the sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of MPV were 82.98%, 91.14%, 73.58%, 94.74%, and 89.27%, respectively. A case-control study conducted by Meabed et al., included 50 patients with neonatal sepsis and 50 healthy neonates as a control. They found a significantly higher level of MPV in patients as compared to controls. They concluded that MPV can be utilized as an early diagnostic marker in NS.[12] A study was conducted in Bali to assess the diagnostic accuracy of MPV in neonatal sepsis. They concluded that a cutoff point of 7.44 fL of MPV is used for the diagnosis of neonatal sepsis with a specificity of 84.2% and sensitivity of 80%.[13] Another prospective case-control analysis found 80% specificity and sensitivity of MPV at a cutoff point of 10.2 fL.[5]

On contrary, a study was conducted in Iran to evaluate the diagnostic role of MPV in neonatal sepsis. They included 72 diagnosed cases of newborns with sepsis and 50 healthy infants as a control. Mean platelet volume, white blood cell count, and C-reactive protein (CRP) were compared in both groups. Mean platelet volume was high in the neonatal sepsis group but its sensitivity and specificity were inadequate. The study concluded that MPV cannot be used as a diagnostic test. C-reactive protein is a better diagnostic marker and MPV can be used in combination with CRP.[14]

A study was conducted by Hanaganahalli et al., to assess the predictive role of MPV in the diagnosis of neonatal sepsis. In NS, the sensitivity and accuracy of MPV were both 100%. Its sensitivity and accuracy were 96% and 100%, respectively when paired with CRP. As a consequence, the use of CRP and MPV in conjunction should be regarded in the early diagnosis of NS and uric acid levels should only be used as a secondary method to validate the diagnosis. In the management of neonatal sepsis, MPV is more responsive and precise than CRP and uric acid. They concluded that in patients with culture-proven sepsis, MPV was significantly high as compared to controls. So, MPV can be utilized as a specific economical marker of neonatal sepsis.[15] A study was conducted to evaluate the efficacy of MPV in the initial diagnosis and follow-up of patients with sepsis. They also compared its efficacy with CRP and interleukin-6 levels in sepsis. The study showed that at the MPV cutoff value of 10.35 fL, the sensitivity was 97.8% and the specificity was 78.7%. The mean platelet volume can be used for diagnosis and follow-up of sepsis.[16] Another study reported that MPV and platelet distribution width have a diagnostic role in neonatal sepsis.[17] All these studies effectively correlate with the results of the present study.

CONCLUSION

Mean platelet volume has high diagnostic accuracy and hence in future, this biomarker can be utilized as a diagnostic tool for rapid diagnosis of NS. By early detection of the condition, the treatment can be planned timely which can surely reduce the risk of mortality.

LIMITATIONS & RECOMMENDATIONS

The limitation of this study, required to be conceded, is that it was a single-centered study including a small sample size as compared to the affected population. Secondly, the media utilized for blood culture was only helpful for the isolation of bacteria and fungi. Other etiologies of neonatal sepsis such as viruses could not be detected. Quantitative CRP can also be performed along with MPV and blood culture for more accurate results. Large-scale studies using diagnostic strategies for the determination of other pathogens are required to establish the diagnostic role of MPV in neonatal sepsis.

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Original Article

Arginase-1 Immunohistochemical Staining in Hepatocellular Carcinoma

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ABSTRACT

Objective: To determine the immunohistochemical expression of arginase-1 in different grades of hepatocellular carcinoma (HCC).

Methodology: It was a cross-sectional study conducted at the Pathology Department of Shifa International Hospital, Islamabad, commencing from September 2018 to July 2019. Arginase-1 immunohistochemical stain was performed on 70 cases of hepatocellular carcinoma. Information regarding patient age, gender, histological grade of tumor, and expression of arginase-1 on hepatocellular carcinoma cases were recorded on a proforma sheet.

Results: A total of 70 cases were included in the study. The arginase biomarker was positive in 65(92.9%) cases and negative in 5(7.1%) cases. Out of these, 7(87.5%) out of 8 cases were well differentiated, 47(95.9%) out of 49 cases were moderately differentiated, and 11(84.6%) out of 13 cases were poorly differentiated HCCs. Out of a total of five cases which did not show arginase reactivity, 1 was well differentiated, 2 were moderately differentiated, and 2 were poorly differentiated HCCs. The distribution of arginase immunostaining among different histological grades showed no statistical significance (p=0.458).

Conclusion: Arginase-1 has a high sensitivity for hepatocellular carcinoma. It has a high sensitivity for well and moderately differentiated HCCs as compared with poorly differentiated HCCs.

Keywords: *Arginase. Hepatocellular carcinoma. Immunohistochemical markers.*

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy.[1] It is the fourth-leading cause of cancer-related deaths worldwide.[2] Hepatitis B virus and hepatitis C virus remain the most important risk factors for HCC.[3] The epidemiological trend of HCC is shifting away from viral hepatitis due to hepatitis B vaccination and hepatitis C treatment programs, globally. An increasing proportion of cases are now due to non-alcoholic steatohepatitis.[4] The diagnosis of hepatocellular carcinoma often requires the use of immunohistochemistry, especially in small biopsy specimens to differentiate primary vs metastatic vs benign disease. Several immunohistochemical stains are available for the diagnosis of hepatocellular differentiation. These include hepatocyte paraffin antigen-1 (HepPar-1), polyclonal carcinoembryonic antigen (pCEA), CD10, alpha-fetoprotein (AFP), and glypican-3. The utility of these immunomarkers is limited by interpretational difficulty and suboptimal sensitivity.[5]

Arginase, a binuclear manganese metalloenzyme, belongs to the ureohydrolase family of enzymes. It acts as a catalyst in the urea cycle. It hydrolyzes L-arginine to L-ornithine and urea, thus detoxifying ammonia.[6]

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Arginase-1 is a cytosolic enzyme. It is primarily expressed in hepatocytes of normal liver. It is concentrated in the periportal hepatocytes which are highlighted by immunohistochemistry. It is not expressed in vascular endothelial cells, Kupffer cells, and bile duct epithelial cells. It has been reported to be expressed in macrophages and neutrophils.[7] Arginase-1 immunomarker stains normal and neoplastic lesions of the liver and helps in differentiating hepatocellular carcinoma from metastatic tumors.[5,7]

Arginase-1 is a relatively new marker and studies on arginase-1 expression in HCC are scarce. This study was designed to observe the expression of arginase-1 immunostain in all grades of hepatocellular carcinoma to observe if it can help in the diagnosis of HCC, especially poorly differentiated HCC.

METHODOLOGY

This cross-sectional study was carried out at the Pathology Department of Shifa International Hospital, Islamabad from September 2018 to July 2019. Seventy patients, irrespective of age or gender, diagnosed with hepatocellular carcinoma based on radiological, morphological, and immunohistochemical analysis were included in the study.

The cases included trucut biopsies, hepatectomy or partial hepatectomy specimens. Immunostains CK7/CK 20 negative, glypican, and HepPar-1 positive were selected as per inclusion criteria. Patients who had received transarterial chemoembolization therapy with no viable tumor and poorly fixed samples were excluded. Non-probability, consecutive sampling was applied. The study was conducted after approval by the Institutional Review Board & Ethics Committee of

Shifa International Hospital, Islamabad. All specimens were formalin-fixed and grossed as per techniques mentioned in the College of American Pathologists (CAP) protocols 2011. Sections were embedded using paraffin and tissue blocks were cut into 3-5 microns sections. Slides were stained by Hematoxylin & Eosin for light microscopy. Histological grading of hepatocellular carcinoma was done using guidelines of College of American Pathologists protocols 2011. Blocks best representative of the lesion were taken for immunohistochemistry (IHC). Sections for IHC were cut at 4 microns from paraffin-embedded blocks, de-paraffinized, and rehydrated. The immuno-histochemical staining was performed using IHC kits. Anti arginase-1, rabbit monoclonal primary antibody (SP156) was used. The staining was performed using a Ventana autostainer. The results were interpreted on a light microscope using a high-power field objective. Cytoplasmic and nuclear reactivity or only cytoplasmic reactivity was considered as positive staining for arginase-1.[8,9] Normal liver tissues were used as a positive control. Results were verified by a consultant histopathologist to minimize bias. A data collection proforma of every patient was filled enlisting personal, morphological, and IHC parameters.

STATISTICAL ANALYSIS

Statistical analysis was done using Statistical Package for the Social Sciences (SSPS) version 26.0. Qualitative variables i.e., tumor grades were presented as percentages. Quantitative variables like age and gender were presented as mean±standard deviation (SD). The frequency and percentage of arginase-1 in each tumor grade were measured. Effect modifiers like age and gender were controlled through stratification by non-probability consecutive sampling.

RESULTS

A total of 70 cases were included in the study. The age of patients ranged from 12 to 80 years. The mean age was 57±11.1 years. However, decade-wise distribution of hepatocellular carcinoma patients showed an increased frequency of cases in the 5th decade i.e., 51 to 60 years (n=23). It was followed by the 4th decade i.e, 41-50 years (n=19) and 6th decade i.e., 61 to 70 years (n=19) whereas 9 patients were less than 40 years of age. There were 59(84.3%) males and 11(15.7%) female patients. The arginase biomarker was positive in 65(92.9%) cases and negative in 5(7.1%) cases. Out of these, 7(87.5%) out of 8 cases were well differentiated, 47(95.9%) out of 49 cases were moderately differentiated, and 11(84.6%) out of 13 cases were poorly differentiated HCCs. In well differentiated tumors, arginase was diffusely positive in 7(87.5%) out of 8 cases. In moderately differentiated tumors, arginase was diffusely positive in 40(81.6%) out of 49 cases, and focally positive in 7(14.3%) out of 49 cases. In poorly differentiated tumors, arginase was diffusely positive in 9(69.2%) out of 13 cases and focally positive in 2(15.4%) out of 13 cases (Figure 1a & 1b). Thus, a total of 56(80%) cases of HCC were diffuse positive, 9(12.9%) cases were focal positive, and 5(7.1%) cases were negative for arginase immunostain. Of the five cases, which did not show arginase reactivity, 1 was well differentiated, 2 were moderately differentiated, and 2 were poorly differentiated HCCs. The overall sensitivity of arginase-1 was found to be 92.9%. The distribution of arginase immunostaining among different histological grades showed no statistical significance (p=0.458) (Table 1).

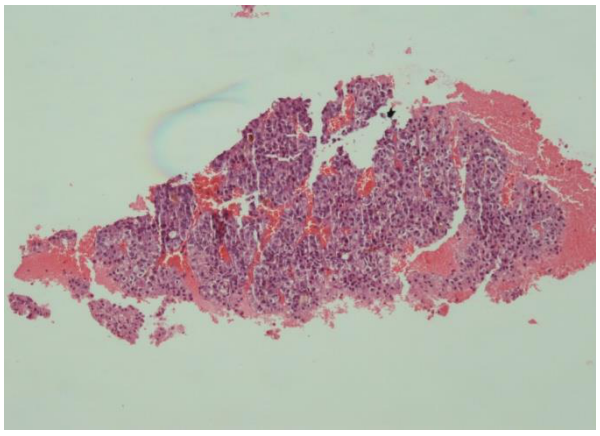

Figure 1a: Core Biopsy Showing Poorly Differentiated HCC (H & E stain, 100x magnification)

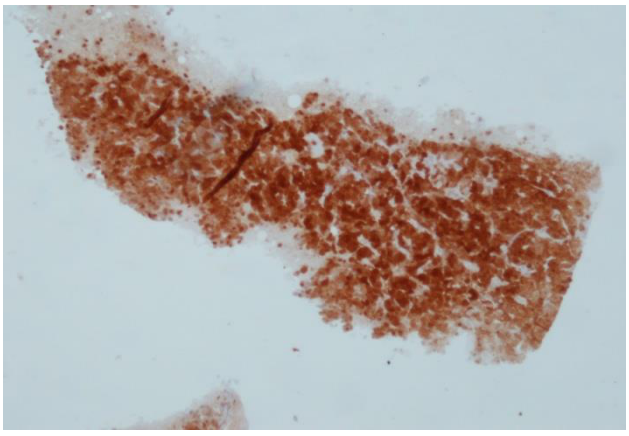

Figure 1b: Core Biopsy Showing Poorly Differentiated HCC Diffuse Staining by Arginase-1 (H & E stain, 100x magnification)

Table 1: Frequency of Arginase-1 Positivity in Different Histological Grades of Hepatocellular Carcinoma

Histological Grade		Arginase-1			Total
		Negative	Focal Positive	Diffuse Positive	
Well Differentiated	Count (n)	1	0	7	8
	% within Grade	12.5%	0%	87.5%	100%
Moderately Differentiated	Count (n)	2	7	40	49
	% within Grade	4.1%	14.3%	81.6%	100%
Poorly Differentiated	Count (n)	2	2	9	13
	% within Grade	15.4%	15.4%	69.2%	100%
Total	Count (n)	5	9	56	70
	% within Grade	7.1%	12.9%	80%	100%

DISCUSSION

Hepatocellular carcinoma (HCC) is the most common primary liver cancer.[1] The diagnosis of HCC requires clinical, radiological, serological, and histo-morphological correlation. Histopathology remains the gold standard. The diagnosis is challenging in atypical lesions and indeterminate cases. Immuno-histochemistry is an important adjuvant to morphology and its use is important in small biopsy or fine needle biopsy specimens where morphology alone is insufficient for a definite diagnosis. Many immunostains have been used for the diagnosis of HCC over the past two decades, namely CD10, polyclonal CEA, bile salt export pump (BSEP), alpha-fetoprotein, glypican-3, and HepPar-1. All these markers have low sensitivity and/or specificity.[5,10] A relatively new marker, arginase-1 has been studied as a potential marker of hepatocyte differentiation. A small number of studies have been published on the expression of arginase-1 in hepatocellular and non-hepatocellular tumors. The published studies indicate the high sensitivity and specificity of this marker for hepatocellular differentiation.[5,11]

In the current study, arginase-1 had an overall sensitivity of 92.8% for HCC. The sensitivities for well, moderate, and poorly differentiated tumors were 87.5%, 95.9%, and 84.6%, respectively.

A study by Labib et al., in 2020 concluded that the sensitivity and specificity of arginase-1 expression in diagnosing HCC was 93.3% and arginase-1 helped in diagnosing most cases of HCC.[12] The findings of another study in Iran suggested that arginase-1 shows 100% sensitivity and 82.6% specificity for the diagnosis of HCC.[13] The results of these studies potentiate the present study findings.

A study was performed on 79 formalin-fixed, paraffin-embedded HCC cases. The intensity and extent of staining were recorded. The immunostain intensity was scaled into four groups as negative, mild, moderate, and strong. The extent of immunostaining was scaled based on the percentage of positive malignant cells staining as focal (5%), patchy (5-50%), and diffuse (>50%). Arginase-1 had an overall sensitivity of 100% in well differentiated, 100% in moderately differentiated, and 97% in poorly differentiated HCC cases when 5% of tumor cells staining was considered positive. With 50% staining considered as positive, the sensitivity was 100%, 98%, and 88% in well, moderate, and poorly differentiated HCC cases, respectively.[14]

Atta et al. conducted a study on formalin-fixed, paraffin-embedded blocks of hepatocellular carcinoma. Arginase-1 positivity was seen in 55 out of 62 cases of HCC. All cases of well differentiation (27 cases), 24 out of 28 cases of moderate differentiation, and 3 out of 7 cases of poor differentiation showed positivity for arginase-1. Thus, arginase-1 had an overall sensitivity of 88.7% in hepatocellular carcinoma with 100% sensitivity seen in well differentiated, 85.7% in moderately differentiated, and 42.85% in poorly differentiated cases.[15] Another study showed that a subset of well differentiated HCCs was negative for arginase-1. The study demonstrated that lack of this information can lead to missing a diagnosis of hepatocellular carcinoma.[16]

A study by Obiorah et al. was performed on formalin-fixed, paraffin-embedded tissue microarray (TMA) blocks of surgical specimens. A total of 40 well differentiated HCCs were evaluated for arginase-1 expression using a monoclonal antibody. In this study, 36 out of 40 HCCs were positive for arginase-1 with a sensitivity of 90%. Four cases were completely negative for arginase-1. The study highlighted that arginase-1 can be negative in some cases of well differentiated HCCs.[17]

In China, research on formalin-fixed, paraffin-embedded tissue blocks was conducted in 2020. Arginase-1 positivity was demonstrated in 36 out of 47 cases of HCC. Fourteen out of 19 cases of well

differentiated HCCs, 5 out of 20 cases of moderately differentiated, and 3 out of 8 cases of poorly differentiated HCCs showed positivity for arginase-1. The study concluded 73.68% sensitivity for well differentiated, 85.0% for moderately differentiated, and 62.5% for poorly differentiated cases. An overall sensitivity of 76.6% was found for all cases of hepatocellular carcinoma.[18] All of the above-mentioned studies support the results of the present study by highlighting the fact that a subset of well differentiated hepatocellular carcinoma cases is negative for arginase-1. In our study, only one well differentiated HCC was completely negative for arginase-1.

CONCLUSION

Arginase-1 has a high sensitivity for hepatocellular carcinoma. Its sensitivity is more for well and moderately differentiated HCCs than poorly differentiated HCCs.

LIMITATIONS & RECOMMENDATIONS

Although the results obtained in this research are similar to previous studies, our study cohort has less number of well and poorly differentiated HCC cases as compared to moderately differentiated HCC cases. It is recommended to use a panel of other immunomarkers for confirming the hepatocellular origin of a tumor as arginase-1 can be negative in any grade of hepatocellular carcinoma.

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Original Article

Efficacy and Safety of Optilume; A Drug-Coated Balloon Treatment for Anterior Urethral Stricture

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ABSTRACT

Objective: To determine the efficacy and safety of Optilume; a drug-coated balloon (DCB) treatment for anterior urethral strictures.

Methodology: This multi-centered descriptive case series study was carried out in the Department of Urology and Renal Transplant, Sharif Medical City Hospital, Lahore and the Department of Urology, National Hospital & Medical Center, Lahore on thirty cases presenting with ≤3 cm strictures in the anterior urethra. This study was conducted from November 2021 to October 2022. Follow-up was done at two weeks, 3 months, 6 months, and one year. The primary efficacy endpoint was an improvement in International Prostate Symptom Score (IPSS), secondary outcomes included post micturition residual urine (PMRU), maximum urinary flow rate (Qmax), the International Index of Erectile Function (IIEF), and quality of life. The variables were analyzed by computer-based software and descriptive statistics were used to present the outcomes.

Results: A total of 30 patients were treated with Optilume DCB, 13(43%) patients completed one year follow-up and 17(56%) completed 6 months follow-up. Eighteen (60%) patients had a history of endoscopic treatment. There were no treatment-related side effects after one year of treatment. Success was achieved in 100% at a mean follow-up of 8.4 months and the baseline mean change in IPSS score was 18 at one year. Quality of life, Qmax flow rate, and PMRU improved significantly from the baseline.

Conclusion: One year treatment data shows that Optilume DCB is safe and effective for the treatment of recurrent strictures of the anterior urethra as it showed a significant improvement in the present study.

Keywords: *Anterior urethral strictures. Quality of life International Prostate Symptom Score.*

INTRODUCTION

Urethral stricture is a morbidity that could result due to inflammation, ischemia or any traumatic process. All these issues lead to the formation of scar tissue and consequently become the reason for low urine flow rate.[1] The annual cases that have been presented with this condition range from 200 to 1200 cases per 100 thousand which multiplies as the age of the population increases. In industrialized countries, its prevalence is less than 10%.[2] In addition to difficulty in treating urethral strictures, stricture recurrences are common.[3]

The treatment of urethral stricture disease has evolved over time from minimal invasive endoscopic procedures to urethroplasty. But recurrence rate of stricture is high for endoscopic treatments. Endoscopic management of anterior urethral strictures is made easier with Optilume DCB.[4] Optilume DCB is an alternative to repeated urethroplasty and dilatations. Optilume DCB is an ideal option for patients undergoing repeated endoscopic urethral procedures and for patients who are not willing for urethroplasty or are unfit for surgery.[5]

Optilume DCB coating has paclitaxel that has hydrophobic and lipophilic properties making it an ideal anti-proliferative agent for uptake and residency in urothelial tissue.[6] It inhibits the post-procedure inflammatory response and provides sustained symptom improvement. Paclitaxel is anti-mitotic, anti-microtubule, anti-fibrotic, and anti-proliferative drug, thus stopping new tissue growth and prevents fibrotic scarring that leads to stricture recurrence.[6,7]

Optilume DCB is a dynamic compliant balloon that expands at high pressure, expanding the lumen and creating microfissures in the urethral tissue. Upon inflation, the coating releases paclitaxel directly into the fissured tissue. Paclitaxel is absorbed by the urothelium where it resides for more than 30 days, preventing cell proliferation and fibrotic scar tissue generation.[8]

The present study was conducted to determine the efficacy and safety of Optilume, a drug-coated balloon treatment for anterior urethral strictures. Very less literature is available on the efficacy of Optilume DCB. So, the study will help in the evaluation of Optilume DCB in improving the overall quality of life in men with urethral strictures.

METHODOLOGY

This was a multi-centered descriptive case series study. This study included 30 male patients after the hospital's ethical committee approved the protocol. The sample

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elements were collected by non-probability consecutive sampling method. The study was carried out for a duration of one year starting from November 2021 to October 2022 in the Department of Urology and Renal Transplant, Sharif Medical City Hospital, Lahore and the Department of Urology, National Hospital & Medical Center, Lahore. Patients with single anterior urethral stricture measuring ≤3 cm in length on urethrogram, IPSS >14, a maximum flow rate of 10 mL/sec, and at least 0-5 prior endoscopic stricture treatments were included in this study. Patients with prior urethroplasty, radical prostatectomy, penile prosthesis, artificial urethral sphincters, a neurogenic bladder, or pelvic radiation were excluded from this study.

Optilume DCB was performed on all patients. Optilume DCB 30 French (Fr) and 5 cm in length was used and balloon was inflated at a pressure of 10-12 atm for 7-10 minutes. Stricture dilatation was confirmed visually on urethroscopy. Patients were followed-up at 14 days, three months, six months, and 12 months. Each follow-up included IPSS, maximum flow rate, PMRU, and IIEF.

A key safety endpoint was urethral fistulas, urinary retention >14 days' posttreatment, stress incontinence, or urethral rupture associated with treatment. International Prostate Symptom Score improvement of 50% relative to a baseline without retreatment was our primary efficacy endpoint. If the IPSS improvement at the last follow-up did not reach 50% or retreatment was required, the patient was considered to have failed treatment. In addition to sexual function and overall satisfaction, Qmax, and PMRU were secondary endpoints.

STATISTICAL ANALYSIS

Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23. Quantitative data was presented with the use of mean & standard deviation. Qualitative data was presented with frequency & percentage. Paired t-test & repeated measures ANOVA was used to determine the mean difference in the significant difference of the mean value changes for baseline to the follow-up visit. A p-value of ≤0.05 was taken as significant.

RESULTS

The mean age of the study patients was 50±14.34 years and its range was 20-80 years. The suprapubic catheter was noted in 5(16.67%) cases. On clinical investigation of the stricture aetiology, it was observed that 13(43%) cases were iatrogenic, 11(36%) cases were idiopathic, and 6(20%) cases were traumatic. The majority of the cases [10(33%)] had only one previous endoscopic treatment (Table 1).

There were no treatment-related side effects like urethral rupture, stress incontinence, and urinary retention. Among 10 encountered adverse events in our study participants, 16% had urinary tract infections, 6% had fevers and 6% had dysurias. At a mean follow-up of 8.4 months, 30/30 (100%) of men achieved functional treatment success.

The mean change in the IPSS score from baseline to 12th month follow-up was 18±0.04, IPSS quality of life score was 4±0.19, overall satisfaction score 1.9±0.26,

Table 1: Patient Demographics and Urological Variables

Variables		Values
Age (Years)	**Mean±SD**	50±14.34
	Range	20-80
Gender Frequency & Percentage	**Male**	30(100%)
Suprapubic Catheter at Baseline Frequency & Percentage		5(16.67%)
Stricture Aetiology Frequency & Percentage	**Iatrogenic**	13(43.3%)
	Idiopathic	11(36.67%)
	Traumatic	6(20%)
Stricture Length in mm Mean±SD (n)		15±4.2(30)
Pretreatment Direct Vision Internal Urethrotomy		1/30(3.3%)
Number of Previous Endoscopic Treatments Frequency & Percentage	**0**	3(10%)
	1	10(33.3%)
	2	8(26.7%)
	3	6(20%)
	4	2(6.7%)
	5	1(3.3%)

Table 2: Mean Changes in the Study Variables from Baseline to the Follow-up Visits

Category	Baseline Mean±SD (n)	3 Months Mean±SD (n)	6 Months Mean±SD (n)	12 Months Mean±SD (n)	p-value
IPSS	25±3.56(30)	9.1±6.3(30)	8.6±5.18(30)	7±3.6(13)	0.00*
IPSS Quality of Life	4.6±0.76(30)	0.9±1.52(30)	0.8±0.89(30)	0.6±0.95(13)	0.00*
IIEF: Overall Satisfaction	6.3±2.67(30)	7.8±2.53(30)	7.4±2.87(30)	8.2±2.41(13)	0.00*
Qmax (mL/sec)	5.3±2.56(30)	22±12.54(30)	21.6±10.78(30)	18±10(13)	0.00*
PMRU (mL)	150.4±104.6(30)	41.5±36.56(30)	35.2±42.84(30)	26.4±31.08(13)	0.00*

**Significant p-value ≤ 0.05*

Qmax score was 12.7±7.44 & PMRU score was 124±73.52. All the mean changes were significantly different when compared with the baseline (Table 2).

DISCUSSION

Urethral strictures are nowadays a common problem among the men above 40 years and cause a substantial economic burden. The epidemiology of urethral strictures varies as it depends on multiple factors that enhance its chances to occur in a population. Moreover, the incidence of this disease is widely due to the underlying risk factors that need to be addressed.[6]

Our results showed that at a mean follow-up of 8.4 months, 30/30 (100%) of men achieved functional treatment success. The mean change in the IPSS score from baseline to 12th month follow-up was 18±0.04, IPSS quality of life score was 4±0.19, overall satisfaction score 1.9±0.26, Qmax score was 12.7±7.44 & PMRU score was 124±73.52. All the mean changes were significantly different when compared to the baseline. Another multi-centered study included 53 patients with recurrent strictures with 1 to 4 prior endoscopic treatments. These patients were treated with Optilume and forty six patients completed their follow-up after 24 months. Thirty two patients had at least 50% improvement in IPSS without retreatment. The mean baseline IPSS was improved from 25.2 to 6.9 after 24 months ($p < 0.0001$). Flow rate, post void residual urinary volume, and quality of life significantly improved from baseline. They concluded that Optilume is safe for the treatment of urethral strictures and it has good efficacy.[9]

A single-blind randomized controlled trial was conducted on 127 patients. The trial reported that Optilume is a safe treatment option and it is superior to direct vision internal urethrotomy.[10] In our study, there were no treatment-related side effects like urethral rupture, stress incontinence, and urinary retention. Among 10 encountered adverse events in our study participants, 16% had urinary tract infections, 6% had fevers, and 6% had dysurias.

A study conducted by Virasoro et al. showed that there were no serious adverse events reported within 90 days of the treatment. There were seven cases of cystoscopic recurrence, five retreatments, and two patients left the study early due to symptom recurrences.[8] Another study reported no significant device-related side effects. They found fever in 8%, headache in 6%, urinary tract infection in 17%, dysuria in 7%, and acute urinary retention in 6%.[9]

The results of this study are in line with the findings of the ROBUST I study with 1 year, 2 years, and 3 years follow-up. However, further ROBUST I study findings after 2nd and 3rd year follow-ups showed that Optilume paclitaxel-coated balloons were effective in treating recurrent strictures.[11,12] According to literature reports, 50% to 0% of those with 2 and 3 prior interventions experienced success following endoscopic treatment of recurrent urethral strictures.[12,13] After three years of treatment with Optilume DCBs, 77% of patients remain free from repeated interventions. With the Optilume drug-coated balloon, paclitaxel is circumferentially delivered while dilation is taking place. In minimally invasive vascular applications, paclitaxel serves as an anti-fibrotic & anti-proliferative coating to prevent restenosis.[14]

In the management of urethral strictures, the Optilume drug-coated balloon represents as a significant advancement. Before urethral dilatations and visual internal urethrotomies were available treatment modalities.[2,15] When these techniques failed, men had no choice but to undergo urethroplasty. With the Optilume drug-coated balloon, one can avoid repeated dilatations and urethroplasty for the rest of the life. Patients who are poor surgical candidates or decline urethroplasty may benefit from this treatment as an alternative to current endoscopic management. Paclitaxel's anti-proliferative properties reduce collagen reformation when applied locally to scar tissue.[16,17]

Radiation therapy, penile urethral strictures, bladder neck contractures, or lichen sclerosis may have adversely affected the DCB.[18] The results of this study are consistent with the ROBUST study except a minor deflection in the adverse reactions. Resultantly, it is not wrong to conclude that the introduction of Optilume DCB is a good addition to available treatment options for urethral strictures.

CONCLUSION

Optilume DCB is a safe procedure with low symptomatic recurrence rates one year after the procedure. It has not only successfully addressed the issue, but also caused an improvement in the quality of the life. We recommend that Optilume DCB should be opted by the urologists for the management of urethral strictures.

LIMITATIONS & RECOMMENDATIONS

Like every clinical research, this study also has limitations. Due to financial constraints, the study could only be conducted up to a follow-up of one year with the selection of cases who already had urethral stricture disease. Due to the absence of real aetiology & cause of this condition, the results are a little less precise. Moreover, the smaller sample size and the corresponding results may be less important for a wider population. Hence, considering this weakness of the study, we recommend that more such studies are needed to be carried out on the same analogy on a larger sample size with ample financial assistance. Future studies should also address the wider geographical population for a better understanding of the results, treatment options, and effectiveness of Optilume DCB.

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Original Article

Analysis of the Outcomes of Transarterial Chemoembolization as A Bridge Therapy to Liver Transplant for Hepatocellular Carcinomas: A Single-Centered Retrospective Study

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ABSTRACT

Objective: To determine the outcomes of transarterial chemoembolization (TACE) as a bridging therapy to liver transplant for Barcelona clinic liver cancer (BCLC) stage A hepatocellular carcinoma.

Methodology: This retrospective study comprised data of 40 patients with BCLC stage A hepatocellular carcinoma (HCC) who received TACE as a bridge therapy prior to liver transplant from January 2022 to June 2022. Tumor response to TACE was assessed on imaging based on modified response evaluation criteria in solid tumors (mRECIST). All the descriptive statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.

Results: In this study, the mean age of included patients was 56.68±7.17 years. The mean pre-TACE tumor size was calculated as 3.56±10.69 cm. Post-TACE imaging response showed complete response in 13 patients, partial response in 11 patients, and progressive disease in 7 patients. Of the 40 patients, 4(10%) patients were dropouts from waiting list, 6(15%) individuals underwent liver transplant (LT) after bridging TACE, 1 (2.5%) patient opted for hepatic wedge resection for HCC instead of LT, and 7(17.5%) patients were lost to follow-up. Twenty two (55%) patients were still in waiting list (mean waiting time=273±30 days) for LT.

Conclusion: Transarterial chemoembolization as a bridge therapy prior to LT for early stage HCC has beneficial results and reduces dropout rates during waiting time.

Keywords: *Liver transplant. Hepatocellular carcinoma. Transarterial chemoembolization.*

INTRODUCTION

Hepatocellular carcinoma is the worldwide fastest growing cause of cancer-related deaths, with patients having a 5 year survival rate of less than 12%.[1] Globally, HCC is the fifth most common cancer and second most common cause of cancer-related deaths.[2] Liver transplant has emerged as the preferred treatment option for early-stage HCC patients with advanced liver disease. Through the removal of the cirrhotic liver, the primary risk factor for HCC, liver transplantation provides a chance to both treat and prevent de novo HCC.[3] The accepted standard indication for LT is HCC which meets the Milan criteria introduced in 1996.[4] Milan criteria include patients with one tumor of <5 cm diameter or up to three tumor foci, each having a diameter of <3 cm with no vascular invasion and no extrahepatic metastasis.[4] These individuals have a 70% five year survival rate and a recurrence rate of less than 20%.[5]

However, due to the scarcity of liver donors, not all HCC patients can receive transplantation immediately, which results in a long waiting list and subsequently a high dropout rate of 30-40% per year due to tumor progression.[6] If HCC is left untreated, dropout rates at 6 months and 1 year are estimated to be as high as 12% and 15-30%, respectively.[5] In order to lower the dropout rate, most centers have adopted the practice of treating HCC patients with locoregional therapies as a bridge to slow tumor growth prior to LT while they await transplantation if the anticipated waiting period is more than six months.[7] In patients with HCC within Milan criteria, bridging therapy is estimated to decrease the dropout rate to 0-10%.[5] The main bridging treatment options for HCC patients prior to LT include transarterial chemoembolization, radiofrequency ablation, liver resection, and stereotactic body radiation therapy (SBRT).[8] Among all the candidates with end-stage liver disease, HCC patients have the highest rates of waiting time for LT.[7] Transarterial chemoembolization and radioembolization are the two main intraarterial techniques used in the treatment of HCC. So far, no recommendation can be made for preferring one type of intraarterial locoregional treatment over another prior to LT.[7]

For patients who do not initially meet the criteria for LT, another goal of locoregional therapy is to reduce the tumor load until it is within the acceptable criteria, allowing them to be added to the waiting list for LT.[9]

This retrospective study aimed to analyze the outcomes of TACE as a bridging therapy to liver transplant for early-stage Barcelona clinic liver cancer hepatocellular carcinomas.

METHODOLOGY

This cross-sectional study was conducted at the

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Radiology Department of Pakistan Kidney and Liver Institute and Research Centre, Lahore. This study was approved by the institutional review board of the institute. All the TACE procedures were performed after informed written consent by a consultant interventional radiologist with more than five years of experience in the field. This study comprised data of 40 consecutive patients with HCC who received TACE as a bridge therapy prior to liver transplant from January 2022 to June 2022. All the patients were followed-up for at least four months after the last bridging TACE received by the patient.

Inclusion criteria were all adults with early stage BCLC HCC within Milan criteria. Patients with angioinvasion, extrahepatic disease, and Eastern Cooperative Oncology Group performance status >2 were excluded from the study. Only patients with HCC whose anticipated waiting time for LT was longer than three months were considered for bridging TACE.

Prior to TACE, each patient had a triphasic computed tomography (CT) examination including the arterial, portal-venous, and delayed phases using multidetector scanners as part of the preoperative work-up. The prerequisites for TACE were an appropriate blood clotting profile with a partial thromboplastin time <38 seconds, an international normalized ratio <1.5, and a platelet count of $150\text{-}400x10^9$/L.

After obtaining written informed consent from all the patients, super-selective TACE was performed under fluoroscopic guidance by injecting chemotherapy emulsion (50 mg doxorubicin-10 mL Lipiodol emulsion) to chemo-embolize the tumor followed by postprocedure embolization of the feeding vessel with polyvinyl alcohol particles to obtain adequate stasis in the tumor-feeding vessels.

All post-TACE patients were followed after 6 weeks with triphasic CT abdomen or dynamic contrast-enhanced magnetic resonance imaging (MRI) liver and then discussed in the liver transplant multidisciplinary meeting. Tumor response to TACE was assessed on imaging based on modified response evaluation criteria in solid tumors. According to mRECIST criteria, complete response was defined as the disappearance of arterially enhanced areas in the targeted lesion; partial response (PR) was defined as at least a 30% reduction in the sum of viable tumor diameter taking the baseline sum of the target lesions diameter as a reference; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of viable tumor; and stable disease was defined as any case that is ineligible for PD or PR.[10] For patients with residual or recurrent tumors, re-TACE treatment was done after re-assessment in multidisciplinary team meetings. Patients who had tumor progression beyond Milan criteria after TACE were dropped out, whereas those who had minimal progression (progression that did not meet the mRECIST criteria), stable disease or tumor regression proceeded for LT.

STATISTICAL ANALYSIS

Data was recorded on an Excel sheet and subsequently exported to the Statistical Package for the Social Sciences (SPSS) version 25 for statistical analysis. Numerical variables were measured as mean and standard deviations, whereas categorical variables were expressed as frequencies and percentages.

RESULTS

In this study, 40 patients selected for bridging therapy prior to LT had a mean age of 56.68±7.17 years. Among them, 30(75%) were males and 10(25%) were females. All patients received TACE as a bridge therapy. The etiologies of end stage liver disease were hepatitis C virus (HCV) infection in 33(82.5%) patients, hepatitis B virus (HBV) infection in 1(2.5%) patient, HBV and HCV co-infection in 3(7.5%) patients, and non-alcoholic fatty liver disease (NAFLD) in 1(2.5%) patient (Table 1).

Based on mRECIST criteria, post-TACE imaging response was evaluated after six weeks which showed complete response in 13 patients, partial response in 11 patients, and progressive disease in 7 patients (Figure 1).

All patients had early-stage HCC (BCLC stage A), according to the BCLC staging. The mean pre-TACE tumor size was 3.56±10.69 cm. Among 40 patients, 28(70%) patients had solitary HCC nodule, 8(20%) patients had two HCC nodules, and 4(10%) patients had three HCC nodules. All patients fulfilled the Milan criteria prior to the bridging TACE therapy. Child-Pugh A cirrhosis was observed in 31(77.5%) patients and Child-Pugh B cirrhosis in 9(22.5%) patients. Of 40 patients, 29(72.50%) patients received TACE as bridging therapy once, 8(20%) patients received twice, and 3(7.50%) patients received thrice. Mean pre-TACE alpha-fetoprotein (AFP) was 147.85±308.38 ng/mL and it decreased to 83.56±274.36 ng/mL at 6 weeks follow-up. Aspartate aminotransferase (AST) and alanine transaminase (ALT) were also recorded before TACE (pre-TACE) and one day after TACE (post-TACE) as shown in Table 2.

In this study, 7(17.50%) patients were lost to follow-up. Among the remaining 33 patients, 4(10%) patients had tumor progression beyond Milan criteria despite bridging therapy and were dropped out of the waiting list, 6(15%) patients underwent LT after bridging TACE (mean time=126±56.17 days on the waiting list before LT), and 1(2.50%) patient opted for hepatic wedge resection for HCC (mean time=77 days) instead of LT as his liver was non-cirrhotic. The remaining 22(55%) patients were still in waiting list for LT (mean waiting time=273±30 days) without any evidence of

tumor progression. Of these 22 patients, 10 individuals had complete tumor response to TACE as assessed on CT/MRI imaging, 9 individuals had a partial response, and 3 individuals had progressive disease according to mRECIST criteria but within the Milan criteria. In the transplantation group (n=6), 2 patients had shortened interval to LT post-TACE, so post-TACE imaging could not be done to see tumor response to TACE. In the remaining 4 patients, 3(7.5%) patients had a complete response and 1(2.5%) had a partial response to TACE as assessed on imaging. While in the explanted liver specimen, complete necrosis was reported in 3(7.5%) patients and partial necrosis in the remaining 3(7.5%) patients on histopathological examination. One (2.5%) patient with complete imaging response to TACE had a viable tumor in its explant.

Figures 2 and 3 show complete and partial post-TACE response in the patients having solitary HCC in segment VII.

Table 1: Demographics of the Patients Included in the Study

Characteristic		Frequency & Percentage
Age (Years)	**Mean±SD**	56.68±7.17
Gender	**Male**	30(75%)
	Female	10(25%)
Aetiology of Liver Disease	**HCV**	33(82.5%)
	HBV/HCV Co-Infection	3(7.5%)
	HBV	1(2.5%)
	NAFLD	1(2.5%)
	Cryptogenic	2(5%)
Child-Pugh Cirrhosis	**A**	31(77.5%)
	B	9(22.5%)

Table 2: Study Variables of the Participants

Study Variables		Frequency & Percentage
Number of HCC Nodules	**Solitary**	28(70%)
	Two	8(20%)
	Three	4(10%)
Frequency of TACE as a Bridging Therapy	**Once**	29(72.5%)
	Twice	8(20%)
	Thrice	3(7.5%)
Mean Pre-TACE Tumor Size (cm)		3.56±10.69
Lobar Involvement	**Unilobar**	40(100%)
	Bilobar	0(0%)
Mean Pre-TACE AFP[a] (ng/mL)		147.85±308.38
Mean Post-TACE AFP[b] (ng/mL)		83.56±274.36
Mean Pre-TACE AST[a] (IU/L)		62.13±49.32
Mean Post-TACE AST[a] (IU/L)		115.55±101.87
Mean Pre-TACE ALT[a] (IU/L)		44.52±30.37
Mean Post-TACE ALT[a] (IU/L)		74.48±58.43

[a]of 31 patients
[b]of 25 patients

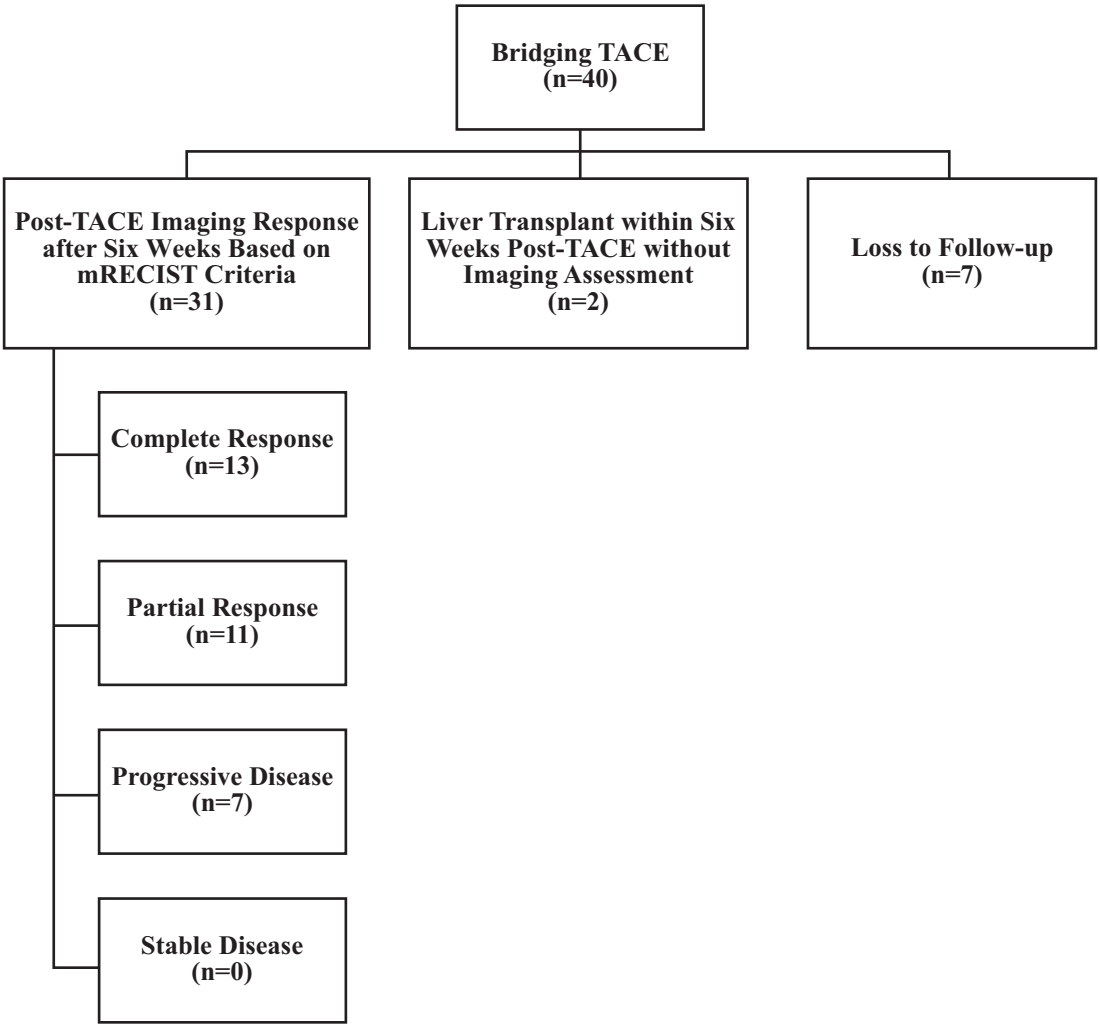
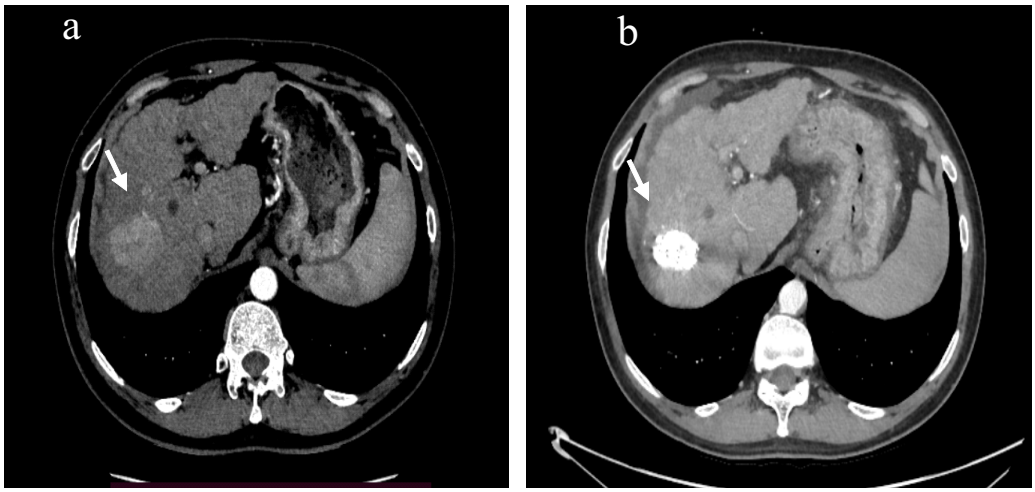


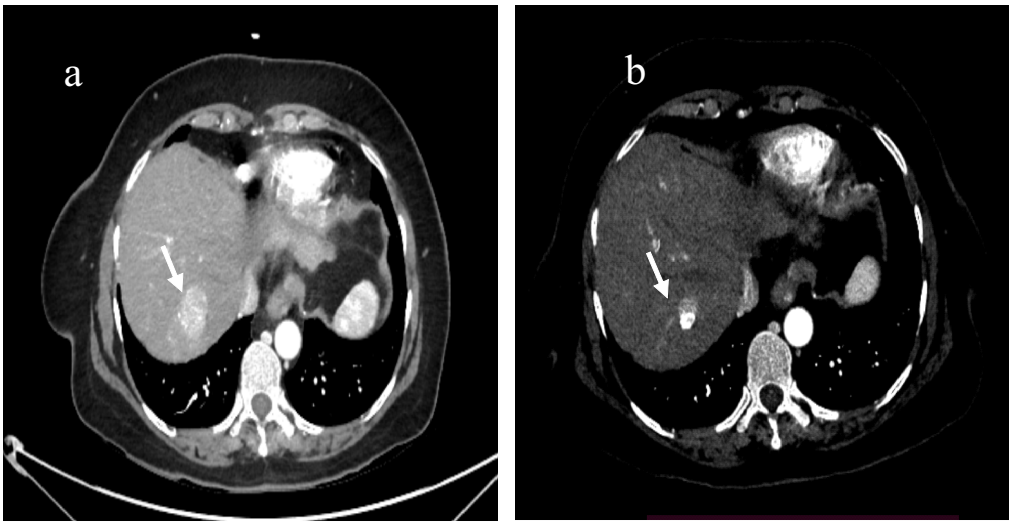
Figure 1: Post-TACE Imaging Response of the Study Participants



(a) Axial contrast-enhanced CT image of the abdomen at the level of the liver in the arterial phase shows an arterially enhancing focal lesion in keeping with HCC in segment VII (white arrow) on a background of the cirrhotic liver.

(b) Axial contrast-enhanced CT image of the abdomen at the level of liver performed 6 weeks after TACE showed the treated lesion is lipiodol packed (white arrow) without any residual disease suggesting complete response.

Figure 2: Complete Post-TACE Response in a 50 Year Old Man with a Solitary HCC



(a) Axial contrast-enhanced CT image of the abdomen at the level of the liver in the keeping with HCC in segment VII.

(b) Axial contrast-enhanced CT image of the abdomen at the level of liver performed 6 weeks after TACE showed the treated lesion is lipiodol packed posteriorly with a residual arterialized component anteriorly (white arrow) suggesting partial response.

Figure 3: Partial Post-TACE Treatment Response in a 55 Year Old Female with a Solitary HCC

DISCUSSION

Liver transplantation has become a widely acknowledged treatment option for patients with HCC who meet the Milan criteria or the University of California, San Francisco criteria.[11] Only transplantation offers a cure for both HCC and the underlying liver cirrhosis.[3] Studies have shown that it is particularly effective in treating early-stage (BCLC stage A) HCCs.[3] As the availability of liver donors (both deceased and living) is limited, not all patients diagnosed with HCC can receive LT immediately. This ultimately leads to a long waiting list and subsequently a high dropout rate of more than 30% per year due to tumor progression beyond the acceptance criteria.[6]

While on the waiting list, the use of locoregional therapies has emerged as a temporary measure to slow tumor progression before transplant. Given the unpredictable waiting time for a liver transplant, nearly all waitlisted HCC patients are treated with locoregional bridging therapy in clinical practice.[12] Many locoregional therapy options are available but currently, there is no guideline for favoring any specific locoregional therapy.[13] Transarterial chemoembolization is the most frequently used intra-arterial bridging therapy globally.[8]

In literature, variable outcomes and dropout rates following bridging treatment have been described but due to the heterogeneity of patient cohorts and wide range of bridging therapy options, it is challenging to determine the exact contribution of bridging therapy to the reduction of dropout rates. In a study, the dropout rate was 8.7% in HCC patients receiving direct-acting antiviral therapy.[14] It is comparable to our study with a 10% dropout rate in HCC patients receiving bridging TACE. It has been shown that the dropout rate decreases when TACE is performed as a bridging therapy prior to LT.[15] Transarterial chemoembolization pretreatment was associated with improved posttransplant survival, with patients experiencing a 44% reduction in posttransplant mortality.[16]

Another study showed that in HCC patients with a high risk of dropping out from waiting list for LT, bridging therapy combining SBRT and TACE may be more beneficial than TACE alone.[1] In 2018, Tan et al. observed that there was no difference between the bridging therapy (BT) group and the non-bridging therapy group in terms of the frequency of waiting list dropouts, although there was a tendency for the waiting duration to be longer in the BT group. As a result, it was concluded that BT might allow the LT candidate with HCC to wait longer.[17]

Till date, there is debate over performing locoregional therapies as a bridge for patients awaiting LT. The reported outcomes of bridging TACE are controversial and its efficacy in lowering dropout rates hasn't been proven by any prospective randomized controlled studies until now. But still, there is general agreement that if the waiting duration is six months or more, locoregional treatment for HCC should be performed to minimize waitlist drop-out.[15]

Imaging (CT/MRI) may also underestimate the presence of persistent or recurrent disease after locoregional therapy.[1] Our results were also consistent with this finding. One of our patients who had a

complete imaging response to TACE still had a viable tumor in its explant on histopathological examination. In this study, 10 out of 22(45.5%) patients showed complete radiological response to TACE. Rubinstein et al. reported similar results showing that 64% of nodules in his study had a complete imaging response to treatment but only 30% had complete tumor necrosis in their explants.[1] Hence, data shows that patients who have had a complete response to treatment on imaging may have a viable tumor in their explants, indicating that better treatment options are required to improve patient outcomes.

In this study, the mean pre-TACE alpha-fetoprotein was 147.85±308.38 ng/mL and it decreased to 83.56±274.36 ng/mL at 6 weeks follow-up. Another study reported that patients with AFP levels of ≥66 ng/mL prior to the LT have poor outcomes after LT independent of Milan criteria. An AFP value of more than 1000 ng/mL was found to be associated with a worse outcome after liver transplantation in HCC that met the Milan criteria.[7]

CONCLUSION

Overall, TACE as a bridge therapy prior to LT for early stage HCCs, has beneficial results and is effective in reducing dropout rates during waiting time. A future extension of this study five years from today may be helpful in determining the survival rate in the transplantation group and the HCC recurrence rate in transplanted livers after bridging TACE.

LIMITATIONS & RECOMMENDATIONS

We acknowledge that our study has few limitations. It was a retrospective and single centered study, hence inherent bias to all such studies could not be excluded. It may carry the risk of bias for the treatment. Our sample size was small and follow-up information was dependent on data available in the hospital record system. Nevertheless, it is the first such study from Pakistan and may provide additional context for prospective studies and randomised controlled trials in comparing different locoregional therapies prior to LT.

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Original Article

Aberrant Expression of CD Markers on Flow Cytometric Analysis in Suspected Patients of Leukemias and Lymphomas: A Single-Centered Study

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ABSTRACT

Objective: To determine the frequencies and pattern of aberrant CD markers expression in Pakistani patients of leukemias and lymphomas.

Methodology: It was a cross-sectional study conducted from April to September 2020 at the Department of Hematology and Transfusion Medicine, Aga Khan University Hospital, Karachi. Peripheral blood/bone marrow samples from patients of suspected hematological malignancies subjected to flow cytometric evaluation were included. Samples were lysed, tested for viability, and analyzed through 5-colour flow cytometer (FC500). Data was entered in Statistical Package for the Social Sciences (SPSS) version 22.

Results: A total of 262 patients were enrolled. Male to female ratio in the study was determined to be 1.5:1. Further categorization showed acute leukemias comprised 75.78% of the total cases whereas chronic lymphoproliferative disorders were 22.26%. The overall presence of aberrant markers was 22.8% among all cases. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients.

Conclusion: Aberrant expression of CD markers is a common entity. Their possible presence should be considered and evaluated while determining the lineage for acute leukemias specifically according to the latest WHO criteria through flow cytometry.

Keywords: *Leukemias. Lymphomas. Flow cytometry.*

INTRODUCTION

Leukemias and lymphomas pose a major burden among all malignancies.[1] Their diagnosis relied upon morphology and cytochemical stains in the past.[2] Now after advancement in diagnostic tools, above mentioned malignancies are diagnosed by immunophenotyping, cytogenetic, and molecular genetics in addition to morphology and cytochemical examination of blast cells.[3] The paradigm shifted in 2008 after the revision of the WHO classification of tumors.[4]

Flow cytometry has become increasingly important in the field of hematology for clinical purpose.[5] It is an excellent tool for the diagnosis, monitoring, and evaluation of malignancies.[6] It does a commendable job by identification of all CD markers expressed by clonal cells. The presence or absence of aberrant markers may also be associated with poor or favorable prognosis.[7] This integrated evaluation utilizing flow cytometry and molecular studies is a basic requirement in diagnostic essentials. Flow cytometric analysis of peripheral blood as well as bone marrow aspirate samples has become a basic diagnostic tool for suspected patients of hematological malignancies since then.[6] Clonal cells in addition to lineage-specific markers, can also express markers of other lineages, so are called "aberrant" markers. Expression of aberrant markers brings about diagnostic difficulties and a sound knowledge about their pattern in our population can be helpful considering the application of targeted antibodies.[4]

Aberrant expression of CD markers has been a concern. A few ambiguities were laid down while the diagnosis was being established. It has been suggested to have prognostic values.[8] Pakistani data has not been widely studied for the presence of aberrant markers and further study on targeted therapies is also missing. The objective of the current study was to overview the frequency and pattern of expression of aberrant CD markers in suspected patients of leukemia and lymphoma on flow cytometric analysis. Aga Khan University Hospital receives a large number of samples across Pakistan. This study will help to predict the said pattern in our population at large as a first step.

METHODOLOGY

It was a cross-sectional study conducted from April to September 2020 at the Department of Hematology and Transfusion Medicine, Aga Khan University Hospital, Karachi. Peripheral blood/bone marrow samples from patients of suspected hematological malignancies subjected to flow cytometric evaluation were included. All patients of suspected leukemias and lymphomas

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whose samples were received from April to September 2020 at the Department of Hematology and Transfusion Medicine were enrolled in the study. Previously diagnosed patients, relapsed cases, and patients for minimal residual disease evaluation were excluded from the study. Samples from peripheral blood/bone marrow aspirates were lysed, tested for viability index, and incubated with monoclonal antibodies in 5-colour flow cytometer (FC500).

STATISTICAL ANALYSIS

The data was entered and analyzed by using Statistical Package for the Social Sciences (SPSS) version 22. Mean±standard deviation (SD) was given for normally distributed quantitative variables while the median and interquartile range was given for non-normally distributed quantitative variables. Qualitative variables were expressed in the form of frequencies and percentages.

RESULTS

A total of 262 patients were enrolled in the study. The mean age was found to be 38.8±21 years ranging from 8 months to 102 years. Seventeen percent of the study population was less than 15 years. Further stratification of patients in different age groups showed that a maximum number of patients (81/262) were between 15-35 years. There were 158(60.3%) males and 104(39.7%) female patients. Male to female ratio was 1.5:1. Majority of patients suffered from acute myeloid leukemia (AML) (35.55%) whereas the least number were diagnosed as suffering from T-cell lymphoproliferative disease (T-LPD) (0.39%) (Figure 1).

Categorization of the study population according to the type of malignancy showed acute leukemias [AML, B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), acute promyelocytic leukaemia (APML), mixed phenotype acute leukemia (MPAL)] composed of 75.78% of the total cases, chronic lymphoproliferative disorders [chronic lymphocytic leukemia (CLL), B-cell lymphoproliferative disease (B-LPD), T-LPD] were 22.26% and hairy cell leukemia (HCL) & monoclonal B-cell lymphocytosis (MBL) were 1.17% and 0.78%, respectively.

The overall presence of aberrant markers was 22.8%. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients (Figure 2).

The commonest expression was seen for CD7 constituting 11.9% of the total expressed aberrant marker. Patients suffering from AML had the maximum aberrant expression whereas T-lymphoproliferative disorders did not show any aberrant markers. Table 1 shows the detailed expression of aberrant markers in all the diseases studied.

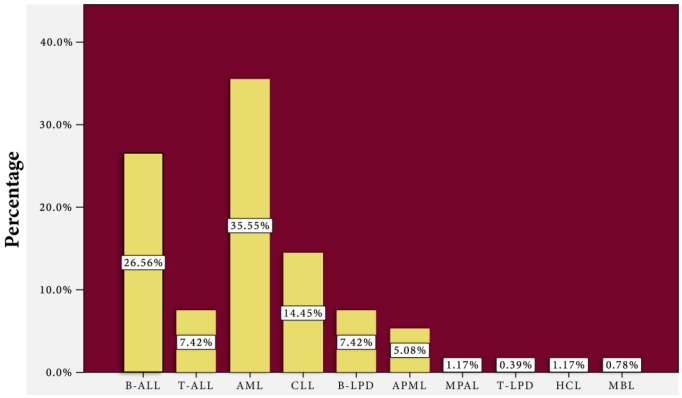

Figure 1: Disease Spectrum of the Study Population

Table 1: Pattern of Expression of Aberrant Markers in Patients of Leukemias and Lymphom as (n=262)

Sr. No.	Disease	Aberrant Markers (%)	CD3%	CD5%	CD7%	CD10%	CD19%	CD22%	CD79a%	CD13%	CD33%	MPO%
1	AML	14.12	0	8	70	5.4	16.2	0	0	NA	NA	NA
2	B-ALL	4.6	0	8.3	25	NA	8.3	NA	NA	0	58.3	0
3	T-ALL	3.43	NA	NA	NA	66	11.1	0	11.1	0	11.1	0
4	CLL	1.14	2.7	NA	5.5	NA	NA	NA	NA	0	0	0
5	B-LPD (MCL**, HCL, PCM***)	0.38	0	0	0	NA	NA	NA	NA	0	3.3	0
6	T-LPD	0	NA	NA	NA	0	0	0	0	0	0	0

**NA: Not applicable as aberrant maker for the disease*
***MCL: Mantle cell lymphoma*
***PCM: Plasma cell myeloma*

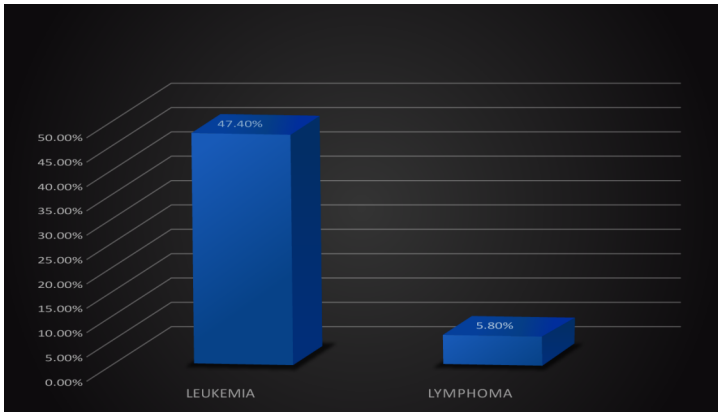

Figure 2: Expression of Aberrant Markers in Leukemias and Lymphomas

DISCUSSION

Flow cytometry is an excellent tool in addition to morphology and cytochemistry for reliable diagnosis of hematological malignancies.[9] It has a role both in early diagnosis and follow-up, with increasing importance in the detection of very small residual disease populations (minimal residual disease).[10] Due to financial constraints in our population, flow cytometry has not been extensively studied as yet for the expression of aberrant markers. In this study, we took a large number of patients (262) with a wide age range considering the occurrence of lymphoproliferative disorders. In the present study, the mean age of the patients was 38.8 years and there were 158(60.3%) males and 104(39.7%) females. Similar results were found in another study conducted by Shahni et al. They reported that the mean age of the patients was 32 years and male to female ratio as 1.5:1.[11]

Our results showed that acute leukemias were composed of 75.78% of the total cases whereas chronic lymphoproliferative disorders were 22.26%. Another study conducted in India reported that 66.8% of patients had acute leukemia and 33.2% suffered from chronic leukemia.[12]

In our study, the overall presence of aberrant markers was 22.8%. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients. The presence of aberrant markers showed both differences and similarities to available data. Comparable results were reported by Shahni et al., and Tipu et al. These studies reported aberrant marker expression in 21.2% and 19%, respectively.[11,13] Another study concluded that 20-24% of the study population expressed aberrant markers.[14] A Pakistani study revealed that 38% of the patients showed aberrant expression which is higher than the international and other local data.[15]

About 70% of AML patients under study having aberrant markers showed the presence of CD7. This was followed by CD19 and CD10. Comparable results were found in another study conducted in Lahore, Pakistan. They reported that the expression of CD7 aberrant marker is high in AML.[16] Another study conducted in Army Medical College, Rawalpindi found aberrant expression of CD7 and CD 19 in 26.4% and 1.1% of AML cases.[17]

In the current study, among lymphoid leukemias, CD33 was the commonest aberrant marker. In contrast, Shahni et al., and Tipu et al., reported CD13 & CD117, respectively as the commonest markers in lymphoid leukemias.[11,13] Another study reported CD33 and 13 as common aberrant markers.[14] Racial differences can be responsible for these differences.

Among the chronic lymphoproliferative disorders, chronic lymphocytic leukemia expressed CD7 as the most prevalent aberrant marker. Shahni et al. has reported CD11c in chronic lymphoid leukemia.[11] This entity has not been widely studied in the Pakistani population. Other B-lymphoproliferative disorders showed CD33 expression as an aberrant marker. On contrary, another study reported CD8 as the aberrant marker of B-lymphoproliferative disorders.[11] T-lymphoproliferative disorders have not been studied in all the other studies compared with our data. However, no aberrant expression was found in our study in T-LPD.

CONCLUSION

Aberrant expression of CD markers is a common entity. Their possible presence should be considered and evaluated while determining the lineage for acute leukemias specifically according to the latest WHO criteria through flow cytometry. Utilizing flow cytometry as a basic tool should always be considered in suspected cases of hematological malignancies. It provides an opportunity for the determination of aberrant markers which can be missed on immunohistochemistry only. Adequate knowledge of lineage-specific markers & cross-lineage expression is mandatory for diagnosis.

LIMITATIONS & RECOMMENDATIONS

The correlation of the expression of aberrant markers with molecular testing and impact on response to medication needs further study. The extensive population-based study may also help us in evaluating genetic makeup linked expressions in our population as well as organizing customized panels for patients reducing the financial impact on the healthcare system and forming a basis for further targeted therapies.

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Original Article

Morphometric Analysis of the Infraorbital Foramen in Dry Human Adult Skulls in a Local Population

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ABSTRACT

Objective: To evaluate morphometric parameters and precise anatomical position of infraorbital foramina in reference to its surrounding important anatomical landmarks in adult human skulls.

Methodology: A cross-sectional study was done on sixty adult dry skulls of unknown age and sex collected from the Anatomy Department of Sharif Medical and Dental College, Lahore. The skulls were assessed to determine the shape, vertical, and transverse diameter of infraorbital foramina, the distances between infraorbital foramina and the infraorbital margins, nasion, anterior nasal spine, and superior orbital foramen with the help of vernier calipers. Data was analyzed using Statistical Package for the Social Sciences (SPSS) version 26.

Results: The mean vertical diameter of the infraorbital foramen (IOF) was 4.14±0.83 mm, the mean horizontal diameter of IOF was 4.88±1.11 mm, the mean distance of IOF from the infraorbital margin (IOM) was 6.32±1.29 mm, from nasion was 44.61±2.84 mm, from supraorbital foramen was 41.98±2.78 mm, and from the anterior nasal spine was 34.14±3.6 mm. One-way ANOVA showed a statistically significant difference (p-value=0.001) in the mean vertical diameter of the IOF on the right and left sides of the skull. The shape of IOF was predominantly oval.

Conclusion: The mean vertical diameter of IOF on the left side was higher as compared to the right side of the skulls and the difference was statistically significant. The predominant shape of IOF was oval. The knowledge of the diameters and distance of IOF from various landmarks on the skull can aid the surgeons in careful preoperative assessment of patients who are candidates for maxillofacial surgeries and regional block anesthesia.

Keywords: *Human skull. Infraorbital foramen. Infraorbital nerve.*

INTRODUCTION

The infraorbital foramen is a small, rounded opening located on the upper portion of the maxilla near the infraorbital margin. It is the anterior continuation of the infraorbital groove through which the infraorbital nerve, infraorbital vein, and artery pass.[1] The nerve and vessels course through the floor of the orbit and supply upper teeth and maxillary sinuses through its smaller branches, posterior superior alveolar nerve, and anterior superior alveolar nerve.[2] It may vary in shape and size and is a very important reference point in various anesthetic and surgical procedures including oral and maxillofacial surgeries. The knowledge of its positional variations may help to decrease the risk in various orbital surgeries. For example, in paranasal and facial surgeries, an infraorbital nerve block is given in trigeminal neuralgia (an unexplained peripheral nerve pain). A neurectomy is done on the infraorbital nerve through the infraorbital foramen approach.[3] Infraorbital plexuses are present near infraorbital foramina and are more prone to injury in orbital surgeries. So, the infraorbital foramen is an important landmark to avoid damage to important nerve plexuses during orbital surgeries. The infraorbital foramen forms a communication between the cranial cavity and orbit, nose, and paranasal sinuses.[4] Sometimes an accessory foramen is seen in the area around the normal infraorbital foramina which is connected to the infraorbital canal and transmits various smaller branches of internal nasal, external nasal, inferior palpebral, and superior labial nerves to supply the surface of the face. An accessory infraorbital foramen could be a cause of many complications in certain surgeries for example in Le fort osteotomy because it causes disturbances in the neurosensory supply of infraorbital nerve as few branches pass through this accessory foramen.[5] A study revealed an accessory infraorbital foramen to be present in 2.2% of males and 4.8% of females of the English population.[6] The relation of the morphology of infraorbital foramina in parallel to accessory infraorbital foramina is important for the surgeon to proceed with the surgery.[7] So, the morphometric analysis of IOF provides important information to avoid traumatic surgeries that may lead to paresthesia or hypoesthesia of the face by iatrogenic injury to the infraorbital nerve and vessels. Hence, the present study was conducted to evaluate morphometric parameters and precise anatomical position of infraorbital foramina in reference to anatomical landmarks in the adult human skulls among the local population of Pakistan.

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METHODOLOGY

This cross-sectional study was conducted on 60 adult

a. Vertical Diameter of IOF

b. Horizontal Diameter of IOF

c. Distance between Infraorbital Foramen and Infraorbital Margin

d. Distance between Infraorbital Foramen and Nasion

e. Distance between Infraorbital Foramen and Supraorbital Foramen

f. Distance between Infraorbital Foramen and Anterior Nasal Spine

Figure 1: Morphometric Measurements of Infraorbital Foramen (a-f)

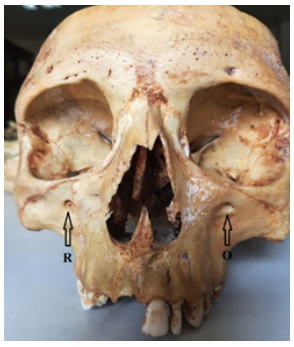

Figure 2: Shape of Infraorbital Foramen [Round (R), Oval (O)]

dry skulls collected from the Department of Anatomy, Sharif Medical & Dental College, Lahore after approval from the ethical committee of the institute. The skulls of unknown gender and age with no visible gross deformity or pathology were included while the skulls with alveolar bone resorption or damaged orbital and nasal regions were excluded from the study. The right and left sides of the adult skulls were divided into two groups. The maximum diameter (vertical and horizontal) of the IOF, the distances between the IOF and the infraorbital margin, anterior nasal spine (ANS), nasion (Na), and supraorbital foramina (SOF) of both groups (right and left) were measured by using a vernier caliper (Figure 1). The shape of the infraorbital foramen was also noted (Figure 2). To minimize the observer's error, all measurements were recorded by two investigators and the mean was taken for final analysis.[8]

STATISTICAL ANALYSIS

Data analysis was done using Statistical Package for the Social Sciences (SPSS) version 26. Mean and standard deviation was calculated for quantitative parameters. One-way ANOVA was applied for quantitative parameters and a p-value ≤0.05 was considered statistically significant.

RESULTS

The minimum, maximum, mean vertical & horizontal diameters of IOF, and the distance of IOF from the infraorbital margin, nasion, supraorbital foramen, and anterior nasal spine on the right side, left side, and both sides together (total) in millimeters is shown in Table 1.

On analyzing with ANOVA, a statistically significant difference (p-value=0.001) was observed in the mean vertical diameter of the IOF between the right and left sides. However, no other statistically significant difference was observed in the rest of the parameters (Table 1). The shape of infraorbital foramen was recorded as round and oval with a predominantly oval shape (69%). On the right side 62 % whereas, 72% on the left side had an oval shape (Table 2).

The means of various parameters of IOF on the right and left sides of the skull are displayed in the bar chart in Figure 3.

Table 1: Morphometric Measurements on Right, Left, and Total IOF

Sr. No.	Parameters	Side of Skull	Total No.	Minimum (mm)	Maximum (mm)	Mean±SD (mm)	p-value
1	Vertical Diameter of Infraorbital Foramen	Right	60	2.6	5.2	3.9±0.66	0.001*
		Left	60	3.1	7.2	4.38±0.91	
		Total	120	2.6	7.2	4.14±0.83	
2	Horizontal Diameter of Infraorbital Foramen	Right	60	2.4	7.6	4.81±1.18	0.485
		Left	60	2.7	8.2	4.95±1.03	
		Total	120	2.4	8.2	4.88±1.11	
3	Distance between Infraorbital Foramen and Infraorbital Margin	Right	60	4	9.8	6.32±1.32	0.978
		Left	60	4	10.3	6.32±1.28	
		Total	120	4	10.3	6.32±1.29	
4	Distance between Infraorbital Foramen and Nasion	Right	60	38.9	52	44.65±2.94	0.868
		Left	60	38.1	53	44.56±2.76	
		Total	120	38.1	53	44.61±2.84	
5	Distance between Infraorbital Foramen and Supraorbital Foramen	Right	60	37	47.4	41.82±2.46	0.515
		Left	60	35	49.6	42.15±3.07	
		Total	120	35	49.6	41.98±2.78	
6	Distance between Infraorbital Foramen and Anterior Nasal Spine	Right	60	27	49.2	34.28±4.28	0.676
		Left	60	28.9	45.1	34.01±2.81	
		Total	120	27	49.2	34.14±3.6	

**Significant p-value ≤0.05*

Table 2: Comparison of Shape of Infraorbital Foramen among Study Groups

Shape	Round	Oval	Total
Right	**23(38%)**	**37(62%)**	**60**
Left	**17(28%)**	**43(72%)**	**60**
Total	**33%**	**67%**	**100%**

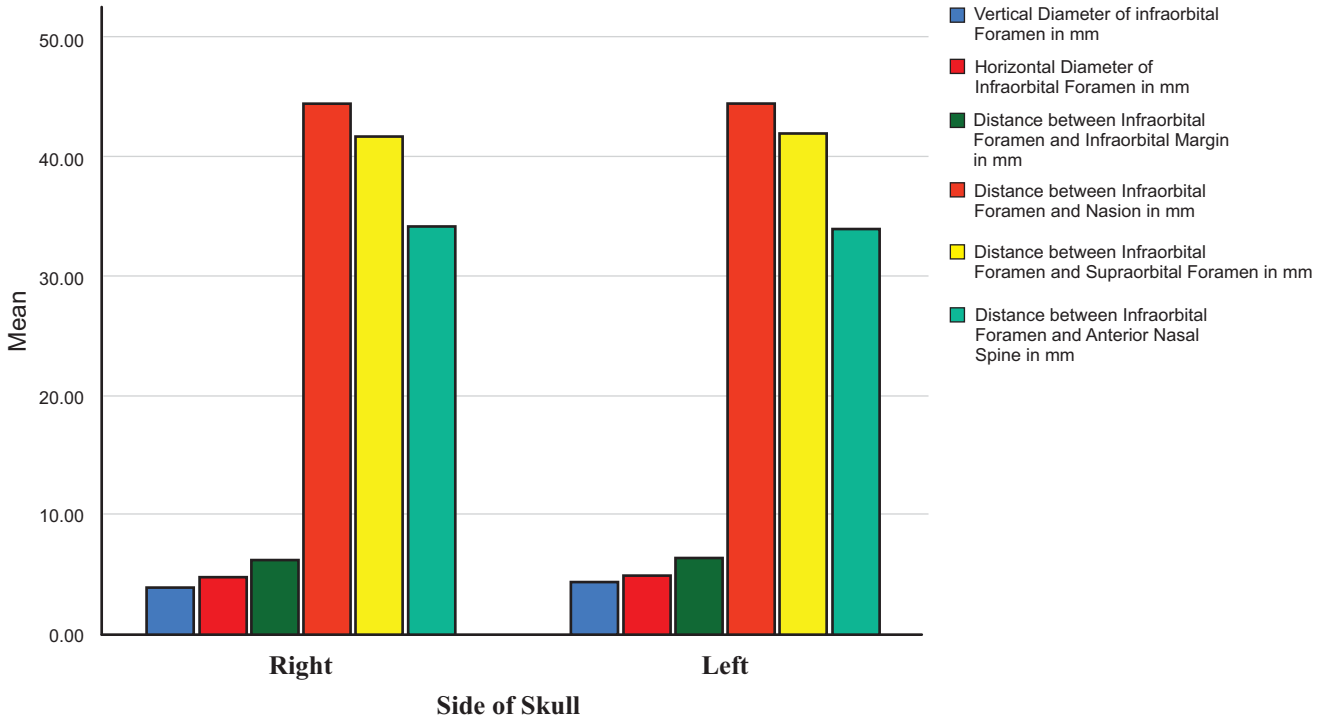

Figure 3: Mean of Morphometric Parameters of Right and Left Infraorbital Foramen

DISCUSSION

Infraorbital foramen is an important landmark for various surgeries including maxillofacial surgeries. Any damage to the neurovasculature during infraorbital nerve block might cause numbness of the area (lower eyelid, lateral wall of the nose and upper lip) supplied by the nerve.[2] So identification of the precise position of IOF is helpful in various diagnoses and clinical procedures like management of pain in trigeminal neuralgias and various aesthetic procedures.[9]

The current study showed the presence of bilateral infraorbital foramina in all sixty skulls which corresponds with the study conducted on 52 dry human skulls in the department of Anatomy, Coimbatore Medical College, India. The study revealed that the infraorbital foramen was single in 50 skulls on both sides.[10] The shape of an infraorbital foramen in the current study was found to be predominantly oval (67%). Our study was in accordance with the results of another study done by Nanaya Kara et al. They found the oval shape in 38.6% on the right side and 36.3% on the left side.[2] Furthermore, no accessory foramina were observed in any skull which is not in line with the observation of a similar study where 18.2% accessory foramina were observed.[11] An Indian study also showed that the accessory foramina were present in 2 out of 52 skulls, one skull had unilateral accessory foramen while the other skull had bilateral accessory foramina. This contradicts the present study findings.[10]

This study also showed a significant difference in the vertical diameter of the right and left infraorbital foramen. The vertical diameter of the left IOF (4.38±0.91 mm) was higher than the right IOF (3.9±0.66 mm). Similar results were reported in another study by Tewari et al.[12] Another study by Veeramuthu et al., on 105 adult dry skulls found the mean values of vertical diameters of IOF as 3.88±1.17 mm on the right side and 3.68±0.89 mm on the left side.[13] The slight difference in vertical diameters of these studies might be due to the different populations under study.

In the present study vertical diameter of IOF showed a significant difference between the right and left side (p=0.001) which is contrary to the previous study where non-significant results were observed.[4] This might be due to the different populations under study. While the distances between IOF and supraorbital foramina, anterior nasal spine, nasion, and superior orbital margin showed non-significant results that are in accordance with another study.[4] In the current study the transverse diameter of IOF was statistically non-significant which is contradictory to the previous research where significant results were observed.[14] This might be due to racial differences, larger sample size, and use of 3-D cone beam computed tomography. In our study, the mean distance between the IOF and IOM was found to be 6.32±1.29 mm which is parallel with a study done earlier where the same was 6.6±1.65 mm.[15] In another study by Karpagam et al., the mean distance between the IOF and IOM was found to be 3.69±1.01 mm which is in contrast to our study.[16]

CONCLUSION

The mean vertical diameter of IOF on the left side was higher as compared to the right side of the skulls and the difference was statistically significant. The predominant shape of IOF was oval. The knowledge of the diameters and distance of IOF from various landmarks on the skull can aid the surgeons in careful preoperative assessment of patients who are candidates for maxillofacial surgeries and regional block anesthesia.

LIMITAIONS & RECOMMENDATIONS

The study needs to be seen in the context of its limitations. Small sample size and data from a single-center remains the major limiting factor. However, similarities of the findings with international research suggest the generalizability of these results. The knowledge of the diameters and distances of IOF from various landmarks on the skull among the local population can aid the surgeons in careful preoperative assessment of patients presenting for maxillofacial surgeries and regional block anesthesia.

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