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Editorial

The Emerging Rise in *Candida auris*: A Global Threat

Aqsa Aslam

The emerging rise in *Candida auris* infections is a potential threat worldwide and one of the greatest challenges in the treatment & prevention of infectious diseases. This is attributed to the multidrug resistance of *Candida auris*, difficulty in laboratory identification of the organism and outbreaks of the infection in healthcare settings.[1] *Candida auris* is rapidly transmitted from person to person. The mortality rate of candidemia caused by *Candida auris* is 60%.[2]

The fungus was first isolated in Japan in 2009 from the ear of a patient.[3] After that *Candida auris* was identified in 15 patients with otitis media in South Korea.[4] Bloodstream infection caused by *Candida auris* was reported in South Korea in 2011.[5] *Candida auris* has also been reported from United States, Canada, Germany, India, South Korea, Israel, Japan, Kenya, Pakistan, Norway, Spain, South Africa, the United Kingdom, Kuwait, Venezuela and Oman.[6] However, only six countries of the Middle East have documented *Candida auris* infections. These include Oman, Kuwait, KSA, Israel, UAE and Iran.[7]

Recently, the Centers for Disease Control & Prevention (CDC) and the European Centre for Disease Prevention & Control (ECDC) have warned about the rising burden of *Candida auris* infections. By September 2017, 127 cases were reported to the CDC from 10 states. These strains were similar on whole-genome sequencing (WGS) analysis.[9]

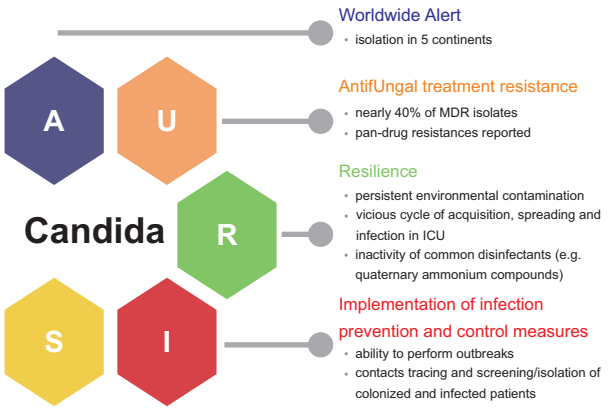

Figure 1: "Major Issues Related to *Candida auris*"[8]

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Candida auris causes invasive disease in hospitals across the globe.[10] The patients usually have undergone surgical procedures, vascular catheterization, mechanical ventilation and gastrostomy tube placement. Like other candida species, *Candida auris* causes infections by producing biofilm on indwelling medical devices.[11] *Candida auris* causes several invasive fungal infections, the most important of which is candidemia. It also causes pericarditis and respiratory tract & urinary tract infections.[12] This yeast can be recovered from several human specimens including sterile body fluids, ears, wounds and mucocutaneous swabs.[8] *Candida auris* can colonize many sites of the body such as nares, axilla, groin and rectum. The eradication is difficult even after treatment.[13] Individuals having contact with patients harboring *Candida auris* or their environment are at risk of colonization.[14] The time required for acquiring *Candida auris* is only ≥4 hours.[13] It is markedly resistant to a variety of disinfectants which explains the nosocomial transmission of the organism.[15] It survives on the surface of medical devices and hospital rooms.[11]

The rise in the prevalence of *Candida auris* is due to the increasing use of prophylactic antifungal drugs. Previously, the majority of the cases of invasive candidiasis were caused by *Candida albicans* and fluconazole was given for treatment. With the emergence of multidrug-resistant non-albican species, fluconazole cannot be the drug of choice.[16]

Neutrophils are an important defense mechanism in invasive candidiasis. They phagocytose the fungi, form neutrophil extracellular trap (NET) and deliver the antimicrobial contents. But neutrophils fail to phagocytose *Candida auris*.[11]

Candida auris produces white or cream-colored colonies on Sabouraud dextrose agar (SDA) and pink or beige colonies on CHROMagar. It forms oval to elongated budding yeast cells without pseudohyphae on cornmeal or rice Tween 80 agar. It can grow both at 37°C and 42°C. It can assimilate gluconate, succinate and N-acetylglucosamine.[17] However, it cannot grow in 0.01% cycloheximide.[12] In comparison, *Candida haemulonii* and *Candida duobushaemulonii* form

pseudohyphae, cannot grow at 42°C and do not assimilate the same sugars.[18] Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is the diagnostic method for detecting *Candida auris*, provided that it is included in the reference profile database.[19]

The outbreaks of infections are difficult to control due to several reasons. It may be misdiagnosed as *Candida haemulonii* in laboratories where molecular biology or MALDI-TOF techniques are not performed. Secondly, the eradication of the outbreaks from the affected areas is difficult. The organism has a rapid patient to patient transmission. The multidrug resistance of the organism limits the treatment options. Most of the strains of *Candida auris* exhibit intrinsic resistance to fluconazole.[20] Initial therapy with echinocandins is recommended. Patients should be monitored for antifungal resistance. Treatment should not be considered in cases of colonization without active disease. The patients with *Candida auris* infection or colonization had a history of broad-spectrum antibiotics and/or antifungals use. The antimicrobial stewardship policies should be implemented to reduce the unnecessary use of antibiotics. These policies can also help in proper diagnosis and management of these infections & their prevention.[19]

The impact of epidemics or pandemics of *Candida auris* in the future is difficult to determine but it depends on the measures taken at this point of time to manage this situation.[1] There is a high likelihood of transmission of *Candida auris* from the patient to the healthcare workers. The organism colonizes the skin or mucous membranes of the patients. The organism has also been isolated from mattresses, furniture, sinks and medical equipment.[9] The CDC emphasizes that adequate infection control measures should be reinforced in hospital settings to prevent these infections. These measures include adherence to good hand hygiene, contact precautions, isolation of infected patients, proper cleaning and disinfection of the room. Hydrogen peroxide and disinfectants containing chlorine can kill *Candida auris*. However, quaternary ammonium compounds are not active against *Candida auris*. Chlorhexidine and iodine compounds are also effective against *Candida auris*.[21] A study conducted by Schelenz et al. reported that despite the daily use of chlorhexidine washes in an outbreak, the transmission of *Candida auris* continued.[13]

In an experimental study, a vaccine developed against *Candida albicans* (NDV-3A) protected mice from *Candida auris* infection. Since the safety and effectiveness of the vaccine in humans against *Candida albicans* is proven, the vaccine should be tested against *Candida auris* in future clinical trials.[22]

In Pakistan, *Candida auris* outbreak occurred in 2014 in Aga Khan University Hospital, Karachi. The fungus was identified as *Saccharomyces cerevisiae.* But it had an unusual antifungal susceptibility pattern. The strains were reported as *Candida auris* by the CDC.[2]

A retrospective study was conducted by Sayeed et al. at Aga Khan University Hospital from September 2014 to March 2017 in which *Candida auris* was isolated from

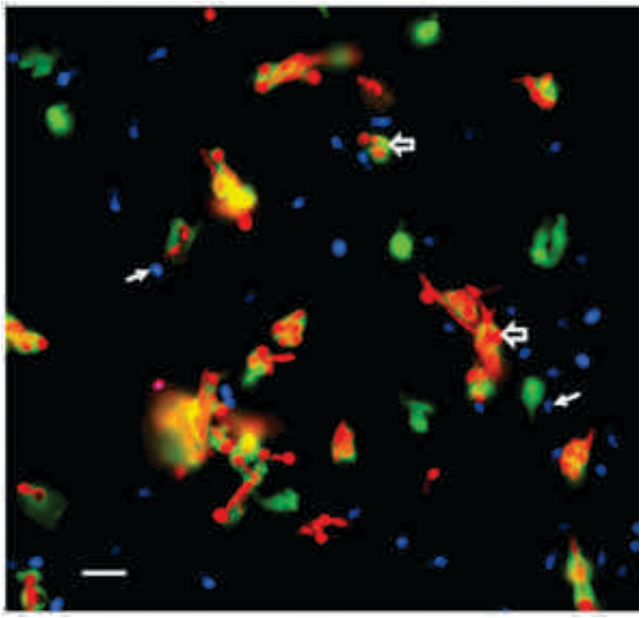

Figure 2: "Human Neutrophils Fail to Engage *Candida auris* Calcein acetoxymethyl (AM) labeled human neutrophils (green) were cocultured with red fluorescent protein-tagged *Candida albicans* (red) and calcofluor white-stained *Candida auris* (blue) for 30 min. Neutrophils preferentially engaged *Candida albicans*, ignoring *Candida auris*. Open arrows point to neutrophils phagocytosing *Candida albicans*. Closed arrows show *Candida auris* cells. Few neutrophils engage *Candida auris* in the presence or absence of *Candida albicans*. Measurement bar represents 10 µm"[11]

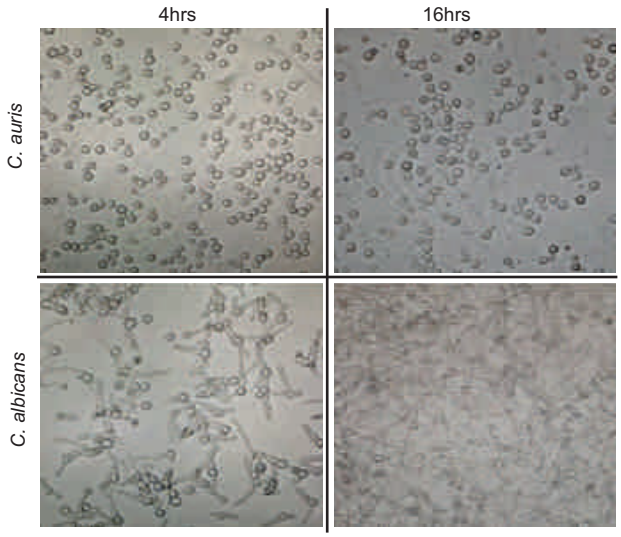

Figure 3: "Growth of *Candida auris* in Comparison to *Candida albicans* at Biofilm-Forming Condition at 4 and 16 Hours. *Candida* Isolates were Cultured at 37°C in 125 ml Corning Culture Flasks"[6]

Table 1: "Key Points for *Candida auris* Prevention and Control by the European Centre for Diseases Prevention and Control (ECDC) and Centers for Disease Control and Prevention (CDC)"[23]

ECDC	CDC
Correct identification (MALDI-TOF; DNA sequencing of the D1/D2 domain): Clinicians and microbiologists alertness; Notification and retrospective case-finding	Correct identification (MALDI-TOF; molecular methods) Confirmed isolates of *C. auris* should be reported to local and state public health officials and to CDC
Good standard infection control measures (including environmental cleaning, reprocessing of medical devices and patient isolation) and prompt notification	Infection control measures: • Placing the patient with *C. auris* in a single-patient room and using contact precautions • Emphasizing adherence to hand hygiene • Cleaning and disinfecting the patient care environment (daily and terminal clearing) with recommended products • Screening contacts of newly identified case patients to identify *C. auris* colorization
Early identification of carriers by using active surveillance cultures (sites considered for sampling include nose/throat, axilla, groin, rectum, insertion sites of venous catheters; clinical samples such as urine, faeces, wound drain fluid, and respiratory specimens)	Screening should be performed to identify colonization among potentially epidemiologically linked patients, including: • Current roommates • Roommates at the current or other facilities in the prior month (even if they have been discharged from the facility) Screening for *C. auris* should be done using a composite swab of the patient's axilla and groin (sites of consistent colonization). Patients have also been found to be colorized with *C. auris* in nose, external ear canals, oropharynx, urine, wounds, and rectum.
Establish the source of the outbreak (epidemiological investigation, cross-sectional patient screening and environmental sampling); prevention of inter-hospital and cross-border transmission Enhanced control measures to contain outbreaks (such as contact precautions, single room isolation or patient cohorting, and dedicated nursing staff for colorized or infected patients)	All laboratories, especially laboratories serving healthcare facilities where cases of *C. auris* have been detected, should: • Review past microbiology records to identify cases of confirmed or suspected *C. auris* • Conduct prospective surveillance to identify *C. auris* cases in the future •Consider screening close contacts of patients with *C. auris* for presence of colonization
Education and practice audits (for healthcare workers and contacts)	Education of all healthcare personnel, including staff working with environmental cleaning services about *C. auris* and need for appropriate precautions; Monitor adherence to infection control practices
Antifungal stewardship	Antibiotic and antifungal stewardship

92 hospitalized patients. Of the 92 patients, 65(70.7%) had an infection while 27(29.3%) had colonization with *Candida auris*. Out of 65 infected patients, 38 had bloodstream infections while 27 had other infections. All the strains showed fluconazole resistance. Voriconazole and amphotericin resistance was seen in 28.5% and 7.9% patients, respectively. The mortality rate was 42.4%, with higher mortality in candidemia patients.[24] It is the need of time to raise awareness in healthcare facilities regarding *Candida auris*. So that they establish protocols for its laboratory diagnosis and implement infection control measures.[25]

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Original Article

Does Unrealistic Optimism Contribute to Low Hepatitis B Vaccination Rates among Healthcare Workers and Medical Students?

Osama Habib, Abdul Rauf, Fahmina Ashfaq, Aniqa Anser, Muhammad Atif Qureshi, Taj Jamshad, Muhammad Akbar Chaudhary

ABSTRACT

Objective: : This study aims to determine the role of unrealistic optimism in the development of attitudes towards hepatitis B vaccination among healthcare workers (HCWs) and medical students.

Methodology: This was a cross-sectional, survey-based descriptive study conducted at the Medicine Department of Azra Naheed Medical College, Lahore. An anonymous self-administered questionnaire was developed that enquired from the participants their vaccination status and the main reason(s) for not getting vaccinated. Informed consent was received from all the study participants and the confidentiality of the data was ensured. Formal approval for the study was granted by the Ethical Review Board of Azra Naheed Medical College, Lahore. The questionnaire was completed by 325 HCWs and medical students working and studying at Azra Naheed Medical College and its affiliated hospital, Chaudhry M. Akram Teaching & Research Hospital. Five options that signaled an attitude of unrealistic optimism were included in the questionnaire and a participant was considered to exhibit unrealistic optimism if they chose one of these five reasons for not getting vaccinated. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 23.

Results: Out of 325 respondents, 143(44%) had received hepatitis B vaccination, 155(46.7%) were not vaccinated for hepatitis B while 27(8.3%) individuals were not sure whether they were vaccinated or not. The three most common reasons for not getting vaccinated were never felt the need to be vaccinated (44.9%), never thought about getting vaccinated (44%) and lack of motivation (40.5%). Nearly three fourth of the respondents, 115(74.2%) out of 155 exhibited unrealistic optimism for non-vaccination whereas the number of respondents who did not show an unrealistic optimism attitude was 40/155 (25.8%).

Conclusion: Unrealistic optimism among healthcare workers and medical students appears to be one of the main psychological factors responsible for low rates of vaccination against hepatitis B. Medical students and HCWs should be made aware of the high risk and prevalence of needlestick injuries and the urgent need for vaccination against hepatitis B. They should be educated about the various self-serving biases, including unrealistic optimism in the curriculum.

Keywords: *Unrealistic optimism. Hepatitis B vaccination. Healthcare workers. Medical students.*

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major causes of morbidity and mortality in the world, particularly in developing countries. Hepatitis B virus infection and its related complications are among the top ten leading causes of death worldwide.[1] In 2015, nearly 257 million people globally were suffering from hepatitis B virus infection according to the World Health Organization (WHO). A majority of these infected individuals were not receiving treatment and hence were prone to the complications associated with HBV infection. Viral hepatitis was responsible for 1.34 million deaths in 2015 and the mortality is worryingly on the rise. Hepatitis B and C virus infections account for 96% of these deaths.[2] Up to 40% of the patients having chronic HBV infection will develop complications like cirrhosis, liver failure or hepatocellular carcinoma.[3,4] Longitudinal studies have reported that 20-40% of males and 15% of females who are infected with HBV develop hepatocellular carcinoma later on in their life. In individuals who remain hepatitis B surface antigen (HBsAg) carriers, the risk of development of hepatocellular carcinoma is extremely high.[5]

The WHO first recommended universal immunization against HBV in 1991. By May 2002, 154 countries in the world included hepatitis B vaccination as a part of their infant immunization programs.[1] The protection from HBV vaccination lasts for at least 20 years. The WHO thus currently does not recommend booster vaccinations for individuals who have completed three mandatory doses of hepatitis B. If not vaccinated as children, the WHO recommends HBV vaccination for healthcare workers and all others who are exposed to blood or its products through their work.[6]

The Centers for Disease Control & Prevention (CDC) and the Advisory Committee on Immunization Practices (ACIP) also strongly recommend healthcare workers (HCWs) to be vaccinated against HBV infection.[7] There has been a significant reduction in the prevalence of chronic HBV infection in the most developed and developing countries of the world from 1990 to 2005. This is thought to be mainly related to

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expanded immunization. Despite this reduction, the total number of persons infected with HBV increased from 223 million in 1990 to 240 million in 2005.[8] The World Health Assembly, in May 2016, adopted and announced its first strategy to combat viral hepatitis. It aims an ambitious reduction in the number of new viral hepatitis cases by 90% and the overall mortality by 65% by the year 2030.[6]

Unrealistic optimism is the tendency of people to wrongly predict that their own personal outcomes would be more favorable than the outcome of their peers. Unrealistic optimism has been documented in over a thousand studies to affect people's judgment related to disease, unwanted pregnancies, chances of getting a divorce or having sexually transmitted diseases.[9] A qualitative study from Australia looking at parents' attitudes towards vaccinating their children found unrealistic optimism as one of the factors affecting their decision to vaccinate their children.[8] Relatively fewer studies, however, have previously reported the actual negative consequences of unrealistic optimism.[10]

Brewer and colleagues reported that individuals who considered themselves at high risk of getting influenza were 24 times more likely to get vaccinated for influenza as compared to individuals who did not consider themselves at risk.[11] A study conducted on male homosexuals found that 24.8% of the study participants cited not being in the at-risk group as the main reason for not getting vaccinated for HBV.[12] A meta-analysis suggested that risk perception was significantly associated with vaccination behavior. The authors concluded that raising the risk perception from low to high would have a major effect on vaccination behavior.[13] A large scale study of parents (n=1763) found that a high perceived vulnerability for contracting meningococcal infection resulted in a more positive evaluation of the vaccination campaign.[14] A systemic review of studies that explored the factors associated with hepatitis B vaccination in gay men found a positive association between increased perceived vulnerability and increased uptake of hepatitis B vaccination.[15]

The prevalence of HBV infection in the Pakistani general population is thought to be between 2-3% nationally.[16] The low rates of hepatitis B vaccination among HCWs and medical students is a major concern as the prevalence of needlestick injuries among HCWs is quite high. Previous studies have reported needlestick injury rates of 41% in Nigeria, 68.2% in India, 71.9% in Pakistan and 58% in Ireland.[17-20] Other studies have reported that less than a third of HCWs know reasonably well what steps they need to take immediately after receiving a needlestick injury.[21] The main purpose of the current study was to investigate the role of unrealistic optimism in hepatitis B non-vaccination among medical students and HCWs in Pakistan. Only a few studies in the past have tried to address this issue.

METHODOLOGY

This was a cross-sectional, survey-based descriptive study conducted at the Medicine Department of Azra Naheed Medical College, Lahore. An anonymous self-administered questionnaire was developed that enquired from the participants their vaccination status and the main reason(s) for not getting vaccinated. This list was based on an extensive review of the literature available in the field. Participants were able to choose one or more of the reasons for non-vaccination. The participants were asked to answer either yes or no to the following reasons for not receiving the hepatitis B vaccination. Out of all the options, a participant was deemed to exhibit an attitude of unrealistic optimism if he/she chose one of the following five options for not getting vaccinated:

- Never felt the need to be vaccinated
- Never thought about getting vaccinated
- Lack of motivation
- Not in the risk group
- Lack of awareness

The questionnaire was validated on 20 medical students and the results were found satisfactory. Participation was on a voluntary basis. Informed consent was received from all the study participants and the confidentiality of the data was ensured. Formal approval for the study was granted by the Ethical Review Board of Azra Naheed Medical College, Lahore. Data collection commenced from March to August 2018 with sampling through nonprobability convenient technique. The questionnaire was completed by 325 HCWs and medical students working and studying at and Azra Naheed Medical College and its affiliated hospital, Chaudhry M. Akram Teaching & Research Hospital.

STATISTICAL ANALYSIS

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 23 and expressed in frequency and percentage.

RESULTS

Most of the study participants (86.8%) were in their 20's. The majority of the participants were single (84.6%). There were 187(57.5%) females and 138(42.5%) males. The study sample consisted of 222(68.3%) medical students, 74(22.8%) medical doctors and 29(8.9%) allied health professionals.

Out of the 325 respondents, 143(44%) had received hepatitis B vaccination in the past. Nearly half of the respondents,155(47.7%) out of 325 were not

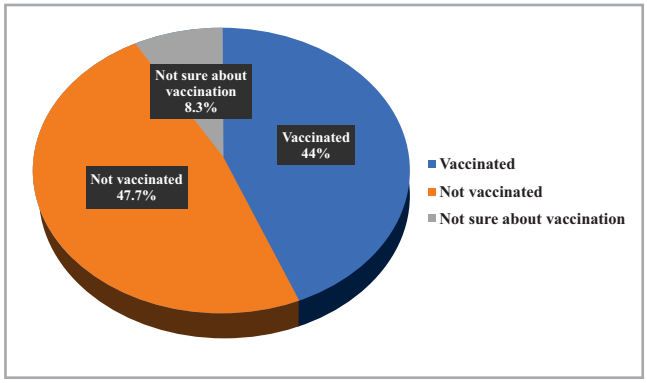

Figure 1: Vaccination Status of the Study Participants

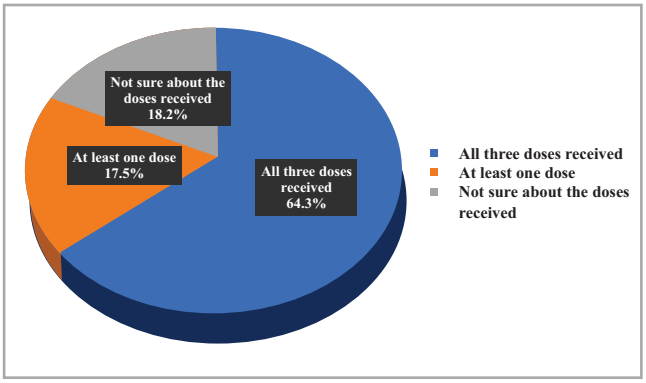

Figure 2: Number of Doses Received in Study Participants who were Vaccinated for HBV

vaccinated for hepatitis B while 27(8.3%) individuals were not sure whether they were vaccinated or not (Figure 1). Almost half of them (47.6%) received the vaccination in the preceding one year, 15.1% were vaccinated 2-5 years back, 18.9% 5-10 years back and 18.4% were vaccinated more than 10 years ago. Out of 143 individuals who were vaccinated, 92(64.3%) of the total participants received all the three doses of hepatitis B vaccination. Twenty five individuals (17.5%) received at least one dose while 26(18.2%) were not sure about the number of doses administered as shown in figure 2.

The majority of the individuals (61.6%) not previously vaccinated for HBV were willing to be vaccinated if provided with the vaccine and 57.8% were willing to be screened for the presence of the viral infection. The previous history of HBV infection was positive in 11.1% of the respondents and hepatitis C virus (HCV) infection in 9.2% of the respondents. More than a quarter of the respondents (28.9%) had a family member suffering from HBV infection.

Most of the participants, 118(76.1%) out of 155 chose more than one option for not getting vaccinated. The three most common reasons for non-vaccination were never felt the need to be vaccinated (44.9%), never thought about getting vaccinated (44%) and lack of motivation (40.5%). Other reasons for not getting vaccinated were as follows: fears about the quality of the vaccine (38.6%), fear of having an infection (38.4%), lack of awareness (35.3%), not being in the risk group (31.4%), non-availability of the vaccine (29.4%), fear of needles (28.6%), not being able to afford the vaccine (18.4%), having an allergy to the vaccine (14.1%), having a chronic illness (13.1%) and having a religious reason for not getting vaccinated (11.4%).

Nearly three fourth of the respondents, 115(74.2%) out of 155 exhibited unrealistic optimism for non-vaccination whereas the number of respondents who did not show an unrealistic optimism attitude was 40/155 (25.8%). These results are shown in table 1.

DISCUSSION

Despite the easy availability, safety and effectiveness of the hepatitis B vaccine, the number of HCWs who receive the vaccination especially in the developing countries is not satisfactory.[16] In our study, only 44% of the participants were vaccinated against HBV

Table 1: Reasons for Not Getting Vaccinated among Healthcare Workers and Medical Students

Reason for Not Getting Vaccinated	Percentage of Study Participants Choosing the Option
Never felt the need to be vaccinated	44.9%
Never thought about getting vaccinated	44%
Lack of motivation	40.5%
Quality of the product	38.6%
Fear of infection	38.4%
Lack of awareness	35.3%
Not in the risk group	31.4%
Non-availability of the vaccine	29.2%
Fear of needle	28.6%
Not being able to afford the vaccine	18.4%
Allergy with the vaccine	14.1%
Any chronic illness	13.1%
Not getting vaccinated for religious reasons	11.4%

infection. A cross-sectional study done in Karachi, Pakistan among the adults visiting family medicine clinic at a tertiary care hospital reported that only 36.4% of the respondents were vaccinated for hepatitis B.[22] In a study conducted at Allama Iqbal Medical College Lahore, 49% of HCWs and 42.2% of medical students were vaccinated against hepatitis B virus.[23] Another study from the Pakistan Institute of Medical Sciences (PIMS), Islamabad reported that 57.6% of the HCWs were fully vaccinated while 18.3% were partially vaccinated against hepatitis B virus infection.[24] One study done at 7 medical colleges/ universities in Karachi reported a higher rate of vaccination (79%) among medical students as compared to our study.[25] A study from North India reported hepatitis B vaccination rates of 59.5% among HCWs.[26]

Our results showed that unrealistic optimism was one of the major factors responsible for low vaccination rates among HCWs and medical students. Nearly three-fourths of the study participants (74.1%) did not get vaccinated against hepatitis B due to unrealistic optimism. Many studies conducted in Pakistan determined the factors associated with hepatitis B non-vaccination. Their results showed unrealistic optimism as the major cause of non-vaccination but have not used this term when reporting their findings.[23,24,27] Nasir et al. conducted a study on 205 HCWs and 327 medical students at Allama Iqbal Medical College, Lahore. The main reason for non-vaccination was the high cost of vaccination (47.7%) among HCWs and the belief that they were not at risk of getting infected.[23] A study done at the Pakistan Institute of Medical Sciences, Islamabad concluded that awareness and attitude problems were the major cause of participants not getting vaccinated against hepatitis B.[24] A study from Mohammad Medical College, Mirpurkhas enrolled 375 medical students. According to this study, lack of motivation was the most common cause (29.2%) of hepatitis B non-vaccination followed by not feeling the need to get vaccinated (24.8%), never thought of getting vaccinated (21.7%), injection fear (10.5%) and lacking belief in vaccination (8.07%).[27]

The finding that unrealistic optimism is one of the major factors that affect the decision to get vaccinated is significant for many reasons. If replicated in the future, preferably from longitudinal prospective studies, this would mean that a change of strategy is needed to achieve the goal of universal immunization of HCWs and medical students. Newer strategies that focus on the clear communication of the risks associated with non-vaccination and the actual likelihood of getting infected in case of non-vaccination would need to be developed. Medical students should be informed about these risks in their curriculum. Also, Behavioral Sciences curriculum needs to be updated to include education about various self-serving biases, in particular, unrealistic optimism. For HCWs, the hospitals should provide the information about risks of not getting vaccinated in a clear and concise manner in their initial orientation at induction. Also, awareness campaigns that target the general population should focus on communicating the risk of non-vaccination and the actual vulnerability of being infected. This is true not only for hepatitis B vaccination but also applies to other vaccination programs such as poliomyelitis and influenza vaccination campaigns.

CONCLUSION

Unrealistic optimism among healthcare workers and medical students appears to be one of the main psychological factors responsible for low rates of vaccination against hepatitis B. Medical students and healthcare workers should be made aware of the high risk and prevalence of needlestick injuries and the urgent need for vaccination against HBV infection. They should be educated about the various self-serving biases, including unrealistic optimism in the curriculum.

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Original Article

Comparison of ADAMTS13 Levels in Patients of Acquired Thrombotic Thrombocytopenic Purpura and Disseminated Intravascular Coagulation

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ABSTRACT

Objective: To compare ADAMTS13 levels in patients of acquired thrombotic thrombocytopenic purpura (TTP) and disseminated intravascular coagulation (DIC).

Methodology: This cross-sectional comparative study was carried out in the Haematology Department of Shaikh Zayed Hospital, Lahore after approval from the ethical review board. A total of 64 diagnosed cases of acquired TTP and DIC of both genders and age ≥15 years were included in the study by consecutive sampling technique. The included patients were allocated into two groups. Group A comprised of 32 diagnosed patients of TTP and group B included 32 diagnosed patients of DIC. After taking written informed consent and using aseptic measures, 5 ml of venous blood was drawn from each study participant. The ADAMTS13 levels were estimated by enzyme-linked immunosorbent assay technique (ELISA). The data was entered and analyzed by using the Statistical Package for the Social Sciences (SPSS) version 23.0.

Results: In group A, there were 5(15.6%) males and 27(84.4%) females. In group B, 14(43.8%) patients were males and 18(56.3%) were females. The levels of ADAMTS13 were low in 17(53.12%) patients in group A and 6(18.75%) patients in group B. This difference was statistically significant ($p<0.004$).

Conclusion: The ADAMTS13 levels were low in 53.12% patients of TTP and 18.75% patients of DIC. There exists a remarkable difference in the levels of ADAMTS13 in patients of acquired TTP and DIC.

Keywords: *Thrombotic thrombocytopenic purpura. Disseminated intravascular coagulation. ADAMTS13.*

INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) is characterized by the generation of platelet-rich thrombi in the microcirculation of many organs of the body.[1] The annual incidence of all TTP syndromes in the general population is about 11 cases per million. It is a fatal condition with a high mortality rate. The diagnosis of TTP relies on clinical features such as thrombocytopenia, hemolytic anemia & routine laboratory findings. i.e. elevated serum LDH. Early recognition and prompt treatment allows the patient not only to recover but survive without long term sequelae.[2]

In disseminated intravascular coagulation (DIC), the mechanisms of coagulation and fibrinolysis become abnormally triggered within the vasculature leading to ongoing coagulation and fibrinolysis.[3] It can present as an acute, life-threatening emergency or may follow a chronic, subclinical course.[4] It is encountered in about 1 percent of patients admitted to tertiary care hospitals. Findings consistent with acute DIC include recent history of sepsis, trauma, malignancy or obstetric complications, bleeding (especially from sites of trauma, drains or catheters), low platelet count, prolonged prothrombin time (PT), thrombin time & activated partial thromboplastin time (aPTT), low levels of plasma fibrinogen, raised plasma D-dimer levels and microangiopathic changes on peripheral blood smear.[5]

Von Willibrand factor (vWF) is an adhesive glycoprotein found on endothelial cells, platelets and plasma.[6] Raised levels of very high molecular weight ultra-large multimers of vWF were found in the plasma of a patient suffering from recurrent TTP. The ultra-large multimers of vWF are found in the endothelium but not in the plasma under physiological conditions. When there is abnormal activation of endothelial cells, this ultra-large vWF promotes aggregation of intravascular platelets and subsequent thrombosis.[7]

A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13 (ADAMTS13) is a zinc metalloprotease composed of 1427 amino acid residues. The stellate cells of the liver are the main source of production of plasma ADAMTS13.[8,9] It is also generated by other cells such as tubular cells of the kidney, vascular endothelium and platelets, though in low levels.[10,11] It cleaves ultra-large von Willebrand factor (vWF) multimers on the endothelial surface. The usual concentration of ADAMTS13 found in plasma is approximately 1 μg/ml and its half-life is 1-2 days. Its concentration remains stable at room temperature for many hours.[12]

Thrombotic thrombocytopenic purpura is thrombotic microangiopathy which is caused by severely

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decreased activity of ADAMTS13.[13] The etiology of acquired TTP is autoimmune inhibition of ADAMTS13 protease thereby causing deficiency of ADAMTS13.[14,15] Although the diagnosis of TTP is primarily clinical, however, nowadays laboratory assays are used for the evaluation of ADAMTS13 in the plasma.[16,17]

A severe secondary deficiency of ADAMTS13 is also seen in the patients of DIC. Kim et al. observed in their study that one of the factors related to poor outcome in sepsis-induced by DIC is the deficiency of ADAMTS13.[18]

This study aimed to compare the levels of ADAMTS13 in patients of acquired TTP and DIC. There is a potential utility of measuring ADAMTS13 level in the diagnosis, management & prognosis of TTP and DIC. The patients with severe ADAMTS13 deficiency have a good clinical response to therapeutic plasma exchange.

METHODOLOGY

It was a cross-sectional comparative study conducted in the Haematology Department of Shaikh Zayed Hospital, Lahore over a period of one year after the approval of study protocol by the ethical review board. A total of 64 diagnosed cases of acquired TTP and DIC of both genders and age ≥15 years were included in the study by consecutive sampling technique. Patients were divided into two groups. Group A comprised of 32 diagnosed patients of TTP and group B included 32 diagnosed patients of DIC. The objectives of the study were explained to the participants and written informed consent was taken. The study proforma gathered their complete information regarding age, gender and type of disease. Patients with acquired/secondary TTP who have undergone plasmapheresis, DIC patients who have received plasma and patients with hepatitis B and C associated liver disease were excluded. Using aseptic measures, 5 ml of venous blood was drawn from each study participant and put in EDTA vial. The sample was mixed and then centrifuged at 2000-3000 rpm for approximately 20 minutes. The obtained plasma was used for the estimation of ADAMTS13 levels by enzyme-linked immunosorbent assay (ELISA) kit by Shanghai Korain Biotech Company. Reference range of ADAMTS13 level was 0.05 ng/ml-15.0 ng/ml.

STATISTICAL ANALYSIS

The data was entered and analyzed by using the Statistical Package for the Social Sciences (SPSS) version 23.0. The data for age and ADAMTS13 levels were expressed by using mean and standard deviation (SD). The ADAMTS13 levels between the two groups were compared by using the independent t-test. A p-value of ≤0.05 was considered significant.

RESULTS

In group A, there were 5(15.6%) males and 27(84.4%) females. In group B, 14(43.8%) patients were males and 18(56.2%) were females. The mean age of patients in group A was 43.1±13.9 years and in group B was 49.9±14.1 years.

In group A, there were 15(46.9%) patients of age 20-40 years while 11(34.4%) and 6(18.7%) patients were of age 41-60 years and >60 years, respectively. In group B, there were 11(34.4%) patients of age 20-40 years, while 11(34.4%) patients were of age 4-60 years and 10(31.2%) patients were >60 years old. The levels of ADAMTS13 were low in 17(53.12%) patients in group A and 6(18.75%) patients in group B. This difference was statistically significant (p<0.004) (Figure 1).

The mean ADAMTS13 levels in patients of group A and B were 0.83±0.8 ng/ml and 1.2±0.5 ng/ml, respectively. A statistically significant difference (p-value=0.016) was found when means levels of ADAMTS13 in both groups were compared. When stratified the ADAMTS13 levels in both groups with respect to age, it was reported that there was a significant difference of ADAMTS13 levels in group A and group B among patients with age >60 years (p<0.008) (Table 1).

The ADAMTS13 levels in both groups were compared with respect to gender, it was reported that there was a significant difference of ADAMTS13 levels in group A and group B among females (p<0.031) (Table 1).

DISCUSSION

Diagnosing and distinguishing the thrombotic microangiopathy from other closely related diseases and their variants become a challenge due to significant variability among certain clinical and biological criteria as well as the methods of von Willebrand disease assay analysis particularly in the early stage of the disease. The understanding of the pathogenicity of TTP has been increased due to recent advances in the description of ADAMTS13. Severe ADAMTS13

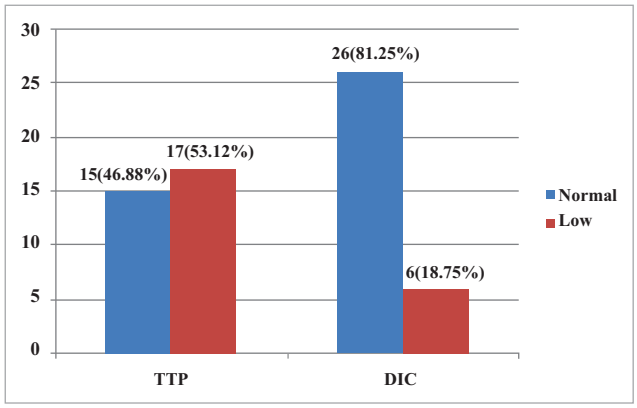

Figure 1: ADAMTS13 Levels in Patients of TTP and DIC

Table 1: Comparison of ADAMTS13 Levels in Both Groups with Respect to Demographic Variables

Demographic Variables		Groups	Frequency	Mean±SD	p-value
Age	20-40 years	TTP	15	0.77±0.86	0.106
		DIC	11	1.24±0.40	
	41-60 years	TTP	11	1.01±1.01	0.941
		DIC	11	1.04±0.62	
	>60 years	TTP	6	0.64±0.66	0.008*
		DIC	10	1.60±0.57	
Gender	Male	TTP	5	0.98±0.94	0.491
		DIC	14	1.32±0.67	
	Female	TTP	27	0.80±0.87	0.031*
		DIC	18	1.26±0.50	

***p-value significant ≤0.05**

deficiency is now accepted as an abnormality specified for TTP.[19-21] The level of ADAMTS13 was found to be deficient, surprisingly, not in all thrombotic microangiopathy but exclusively in TTP.[20,21]

In our study, most of the patients with TTP were females. Five (15.6%) patients were males and 27(84.4%) patients were females. Similarly, the female-to-male ratio was more than 4:1 in a study by Zheng et al.[22] Vesely et al. reported that 69% of the patients were females.[23] In another study by Veyradier et al., out of 111 TTP patients, 64(57.7%) were females and 47(42.3%) were males.[24]

In our study, the mean age of the TTP patients was 43.1±13.9 years. Zheng et al. reported that the median age of patients was 47 years.[22] The mean age of the patients was 40 years in a study by Veyradier et al.[24]

We found a deficiency of von Willebrand factor-cleaving protease in plasma samples of 17(53.12%) patients of TTP. This is in concordance with other studies. Matsumoto et al. and Peyvandi et al. found that 56(52%) of 108 patients and 48(48%) of 100 patients of TTP had low ADAMTS13 levels, respectively.[14,25] Veyradier et al. conducted a prospective study on 66 patients of TTP and found that 47(71%) patients were deficient in ADAMTS13 levels.[24] Zheng et al. and Kremer-Hovinga et al. also reported that 60% and 80% of patients had low ADAMTS13 levels, respectively.[22,26] In contrast, Vesely et al. found low levels of ADAMTS13 in 33% TTP patients.[23]

In our study, out of 32 DIC patients, 14(43.8%) were males & 18(56.3%) were females and the mean age of the patients was 49.9±14.1 years. This is in contrast to the study by Ono et al. According to them, the majority of the DIC patients 65(59.6%) were males and the mean age of DIC patients was 56.9±21.3 years.[27]

Our study showed that only 6(18.75%) patients with DIC had low ADAMTS13 levels suggesting that the deficiency did not result from consumption coagulopathy. This is in concordance with the study conducted by Ono et al. in which decreased levels of ADAMTS13 were found only in few patients with sepsis-induced DIC. They included 109 patients with sepsis-induced DIC and deficiency in ADAMTS13 levels was found in 17(15.6%) patients. Clinical and laboratory data showed that the DIC patients with low ADAMTS13 levels had decreased serum albumin levels, suggesting that decreased ADAMTS13 activity in these patients was partially caused by the reduced synthetic activity of liver reflected by reduced synthesis of albumin in the liver.[27] Further studies should be done to detect the level of ADAMTS13 autoantibodies in patients of TTP & DIC to solidify the diagnosis.

CONCLUSION

The ADAMTS13 levels were low in 53.12% patients of TTP and 18.75% patients of DIC. There exists a significant difference in the ADAMTS13 levels in patients of acquired TTP and DIC.

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Original Article

Levels of Placental Growth Factor (PLGF) in Preeclamptic Gravid Women Taking Methyldopa

Qurat-ul-Ain, Nazish Jamil, Amna Irum, Ghazal Mansoor, Hassan Jamil, Noor-ul-Ain

ABSTRACT

Objective: To estimate the serum levels of pro-angiogenic placental growth factor (PLGF) in preeclamptic gravid women taking methyldopa at different gestational ages in Sharif Medical City Hospital (SMCH), Lahore.

Methodology: This cross-sectional comparative study was performed over a period of six months after approval by the ethical committee of the institution. A total of 62 gravid women with gestational age between 18 weeks to term (40 weeks) presenting to the Obstetrics & Gynaecology Department of Sharif Medical City Hospital (SMCH) were enrolled in the study. They were categorized into 41 women with preeclampsia and 21 gravid normotensive subjects (as a control group) by stratified sampling technique. After obtaining informed written consent, a 3 ml blood sample was taken using aseptic measures and serum PLGF levels were estimated using human soluble PLGF enzyme-linked immunosorbent assay (ELISA) kit. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.

Results: The average serum concentration of PLGF was higher (536±77 ng/L) in preeclamptic gravid women than normotensive gravid women (206±29 ng/L). A statistically significant difference was observed in both groups (p=0.004). The levels of PLGF were increased in gravid women with preeclampsia during 18-28 and 29-35 weeks of pregnancy whereas the PLGF level was decreased during 36-40 weeks in preeclamptic women.

Conclusion: It has been concluded that the serum levels of pro-angiogenic factor PLGF were higher in gravid women with preeclampsia taking methyldopa than normotensive pregnant women. This is credited to the use of alpha methyldopa in these patients. This study suggests that alpha methyldopa has a precise positive effect in increasing PLGF levels and improving placental & endothelial cell function in preeclamptic gravid women.

Keywords: *Pro-angiogenic factor. Placental growth factor. PLGF. Preeclampsia. Alpha methyldopa.*

INTRODUCTION

Preeclampsia (PE) is characterized by hypertension and proteinuria in gravid mother and can lead to growth restraint of fetus developing at late pregnancy. Hypertensive diseases of pregnancy such as persistent hypertension, pregnancy-induced hypertension (PIH) and preeclampsia remain principal reasons for maternal and neonatal morbidity and mortality. Preeclampsia occurs in 5-8% pregnancies all over the world.[1]

According to the International Society for the Study of Hypertension in Pregnancy (ISSHP) classification, preeclampsia is defined as newly onset hypertension of more than 140/90 mmHg after 20 weeks gestation, proteinuria greater than 300 mg/day or a spot urine protein/creatinine ratio ≥30 mg protein/mmol creatinine.[2]

In preeclampsia, the reendothelialization of cytotrophoblasts is diminished and the route of spiral arteries into myometrium is insufficient, resulting in small-caliber resistance vessels. Placental ischemia and hypoxia occur due to depressed placentation. The initiating event in preeclampsia has been assumed to be reduced uteroplacental perfusion as a consequence of atypical cytotrophoblast invasion of spiral arterioles.[3,4]

The molecular mechanisms of preeclampsia depend upon angiogenic factors which also contribute to its chief phenotypes such as hypertension and proteinuria. Nowadays novel anti-angiogenic proteins including soluble endoglin (sEng), soluble FMS-like tyrosine kinase-1 (sFlt-1) and one pro-angiogenic protein i.e. placental growth factor (PLGF) are expressed in varying levels at different gestational ages. An imbalance of pro and antiangiogenic serum biomarkers formed by the placenta play a role in endothelial dysfunction.[4,5]

Pro-angiogenic placental growth factor is the main participant of the vascular endothelial growth factor (VEGF) family and it is formed principally by the placenta. It has a key role in angiogenesis and trophoblastic invasion of the maternal spiral arteries. Maternal serum levels of PLGF are decreased in 11 to 13 weeks of gestation leading to impaired placentation and preeclampsia.[5]

The etiology of PE is associated with dysregulation in angiogenic factors.[6] It has been reported by different studies that the plasma/serum PLGF concentration in women with preeclampsia is lower than in controls with normal blood pressure. The reduction in serum PLGF concentration is observed at the commencement of early second trimester (after 12th week of gestation) of

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hypertensive gravid subjects.[7,8]

Alpha methyldopa is one of the drugs used for the treatment of hypertension and the management of PE in gravid women. Methyldopa stimulates central alpha-adrenergic receptors by alpha-methyl norepinephrine resulting in a decreased sympathetic outflow of norepinephrine to the heart, kidney and peripheral vasculature.[9]

Alpha methyldopa may have a precise effect on endothelial cell function and/or placenta in PE mothers by changing angiogenic proteins. It is not yet clear whether angiogenic factors are concerned with the pathophysiology of PE directly or are merely indicators of the course of illness however antihypertensive treatment with alpha methyldopa has been correlated with a noteworthy reduction in mothers serum levels of sFlt-1 (anti-angiogenic) and upsurge in maternal serum of PLGF (pro-angiogenic) which plays a supportive role in the switch of progression of illness.[9]

Imbalance of pro and anti-angiogenic factors produced by the placenta may perform the main part in facilitating dysfunction of endothelium which leads to the progression of hypertensive disease of pregnancy. This study was conducted to determine the levels of pro-angiogenic factor PLGF in women with preeclampsia taking methyldopa and compare them with control subjects. It will help to understand the pathogenesis of preeclampsia and its management with alpha methyldopa. It will also have an impact on decreasing morbidity and mortality in these patients.

METHODOLOGY

This cross-sectional comparative study was performed over a period of six months after approval by the ethical committee of the institution. A total of 62 gravid women with gestational age between 18 weeks to term (40 weeks) presenting to the Obstetrics & Gynaecology Department of Sharif Medical City Hospital (SMCH) were enrolled in the study. They were categorized into 41 women with preeclampsia and 21 gravid normotensive subjects (as a control group) by stratified sampling technique. The inclusion criteria for the PE group was age 18-30 years, singleton non-molar pregnancy, nonsmoker, no history of hypertension before pregnancy and taking alpha methyldopa. The exclusion criteria for both groups were patients with a history of persistent hypertension, kidney disease, liver disease, cardiovascular illness and diabetes mellitus that may risk the mother or fetus and any metabolic disorder before or after pregnancy. The diagnosed hypertensive gravid women with BP ≥ 130/90 mmHg and proteinuria greater than 300 mg/24 hrs or 1+ and more on the dipstick in the 20th week of gestation with midstream urine sample were labeled as having preeclampsia. The relevant history was filled in questionnaires including age, presenting complaints, obstetric history, smoking habits and medication intake. The duration of gestation was calculated by the last menstrual period (LMP), further confirmed by first or early second trimester ultrasound. After obtaining informed written consent, a 3 ml blood sample was taken using aseptic measures and serum PLGF levels were estimated using human soluble PLGF enzyme-linked immunosorbent assay (ELISA) kit. The estimation of PLGF was done in different periods of pregnancy i.e. 18-28, 29-35, and 36-40 weeks of gestation.

STATISTICAL ANALYSIS

The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24. In the comparison of various groups, the mean and standard error of the mean was calculated. An independent t-test was applied to compare the levels of pro-angiogenic PLGF serum marker in preeclamptic and normotensive subjects. The significance of difference was taken as p-value ≤0.05.

RESULTS

This study included 41 women with preeclampsia and 21 normotensive pregnant control subjects. The subjects with PE had an increased body mass index (BMI). The mean level of PLGF in preeclamptic pregnant women was 536±77 ng/L whereas the average concentration of PLGF in normotensive control subjects was 206±29 ng/L. The PLGF levels were significantly higher in preeclamptic gravid women than normotensive control subjects (p-value =0.004). These results are shown in table 1 & figure 1.

During 18-28 weeks of pregnancy, there were increased levels of PLGF factor in preeclamptic gravid women (524±149.6 ng/L) as compared to normotensive gravid women (198±49.41ng/L). The difference was significant statistically (p=0.05) (Table 1 & Figure 2).

During 29-35 weeks of pregnancy, the mean level of PLGF in gravid women with preeclampsia was 615±116.55 ng/L whereas the mean level of PLGF in normotensive control subjects was 193±46.24 ng/L. The results were not statistically significant in this gestational period (p=0.056). These results are shown in table 1 and figure 3.

In 36-40 weeks of pregnancy, the mean levels of PLGF in preeclamptic and normotensive gravid women were 189±39 ng/L and 311±82 ng/L, respectively. There was no statistically significant difference with a p-value=0.174 (Table 1 & Figure 4).

DISCUSSION

Preeclampsia is a hypertensive disorder of pregnancy beginning from the 20th week of gestation and is characterized by proteinuria, hypertension and multiorgan dysfunction. Hypertension in pregnancy is

Table 1: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) at Different Gestational Age

Gestational Age (Weeks)	Groups	Number of subjects (n)	Mean±SEM	p-value
18-40	Preeclamptic Gravid Women (Taking Methyldopa)	41	536±77	0.004
	Normotensive Gravid Women (Control Group)	21	206±29	
18-28	Preeclamptic Gravid Women (Taking Methyldopa)	14	524±149.6	0.056
	Normotensive Gravid Women (Control Group)	7	198±49.41	
29-35	Preeclamptic Gravid Women (Taking Methyldopa)	17	615±116.55	0.155
	Normotensive Gravid Women (Control Group)	9	193±46.24	
36-40	Preeclamptic Gravid Women (Taking Methyldopa)	10	189±39	0.174
	Normotensive Gravid Women (Control Group)	5	311±82	

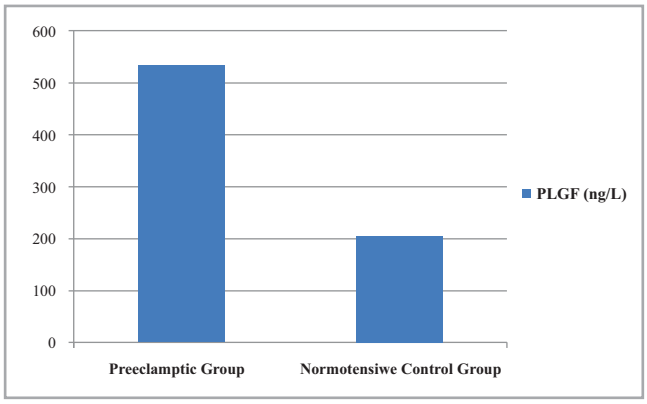

Figure 1: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 18-40 Weeks

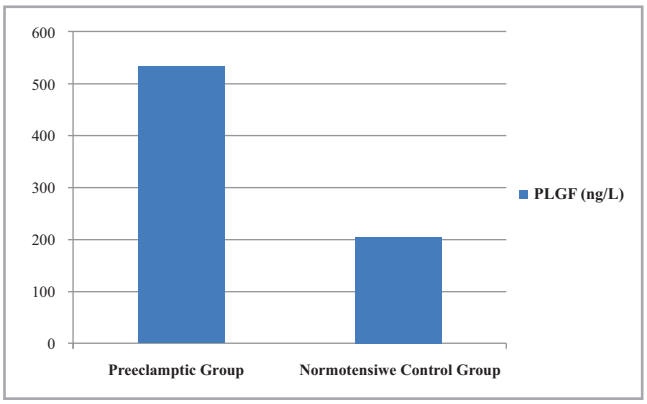

Figure 2: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 18-28 Weeks

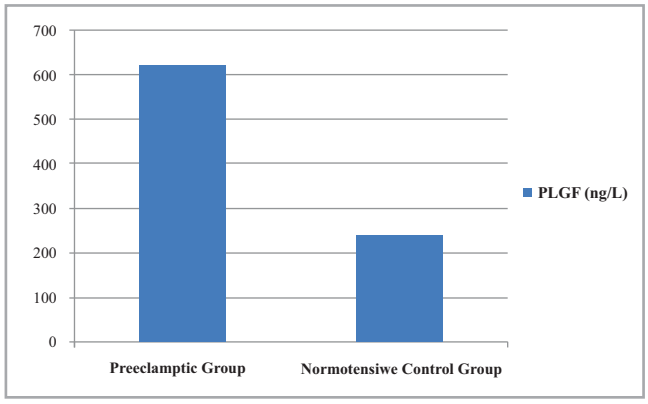

Figure 3: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 29-35 Weeks

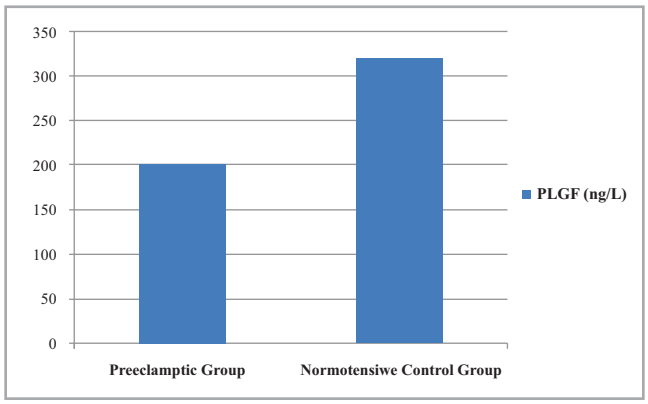

Figure 4: Comparison of PLGF Levels in Preeclamptic and Normotensive Gravid Women (Control Group) during 36-40 Weeks

associated with high morbidity and mortality.[10] In Pakistan, the incidence of pregnancy-induced hypertension is comparatively high.[11] However, biomarker screening and follow up studies are extremely insufficient.

Pregnancy accompanies the appearance of numerous hormones and humoral factors as a part of the adaptations of developing a fetus in the maternal environment. The process of angiogenesis is crucial for the survival of a fetus in the fetal-maternal association. Hypertension in pregnancy has been found to be correlated with changes in the circulatory level of angiogenic and anti-angiogenic factor and vascular complaints.[12,13]

In normotensive pregnancies, pro-angiogenic factor PLGF levels differ during different gestational ages. According to different studies, there is marked variation in PLGF levels within the same gestational

age. It may be due to the difference in sample handling, processing and laboratory techniques. There is no definite cutoff value to predict PE and other hypertensive disorders of pregnancies.[14] Some studies provided the association between the alteration of angiogenic proteins (sFlt-1 & PLGF) and subsequent development of preeclampsia. Hirashima et al. reported that the concentration of PLGF is decreased before the onset of hypertension in pregnancy particularly in PE.[15] Maynard et al. and Levine et al. both observed a decline in PLGF levels in women with preeclampsia taking no antihypertensive medicines.[16,17] But in our study, PLGF levels were higher in preeclamptic pregnant women than control subjects with normal blood pressure and the difference was statistically significant (p=0.004). The PE gravid women were on methyldopa which may have a definite role in improving placental & endothelial cell function and increasing the levels of pro-angiogenic proteins. The analysis of this factor (PLGF) concentrations in different periods of pregnancy was performed and the periods were18-28, 29-35, and 36-40 weeks of gestation. The serum levels of PLGF were high (524±14 ng/L) in 18-28 weeks with statistically significant difference (p=0.056), highest (615±11 ng/L, p=0.15) in 29-35 weeks but declined (189±39 ng/L, p=0.174) near the term in 36-40 weeks, indicating that there is no definite cutoff value of PLGF levels in PE gravid women. But the pattern is surely revealing a relationship between the PLGF factor and PE in different phases of pregnancy.

There are a few studies reporting the influence of methyldopa on the angiogenic factors. According to a study by Khalil et al., the antihypertensive drugs like methyldopa have no protective effect on PE gravid women by changing the proangiogenic (PLGF) proteins in pregnancy.[9] This is in contrast to the present study which indicates a significant alteration in PLGF angiogenic protein levels in PE gravid women of 18-40 weeks (p=0.004).

The present study observed that there were amplified maternal serum levels of PLGF in PE gravid women at 18-28 weeks and 29-35 weeks of pregnancy as compared to the normotensive control group. In 36-40 weeks of pregnancy, serum levels of PLGF were decreased in preeclamptic gravid women than normotensive subjects.

The increasing level of serum PLGF may reveal either additional placental production of PLGF or reduced binding to local circulating and membrane-bound receptors, but the particular mechanism for this needs further investigation. The results of the present study strongly recommend further research on the effect of alpha methyldopa on the levels of serum proangiogenic factor PLGF in preeclamptic gravid women on a large scale.

CONCLUSION

It has been concluded that the serum levels of pro-angiogenic factor PLGF were higher in gravid women with preeclampsia taking methyldopa than normotensive pregnant women. This is credited to the use of alpha methyldopa in these patients. This study suggests that alpha methyldopa has a precise positive effect in increasing PLGF levels and improving placental & endothelial cell function in preeclamptic gravid women.

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Original Article

Breast Phyllodes Tumor: A 6-year Retrospective Series Analysis at Mayo Hospital/King Edward Medical University, Lahore, Pakistan

Madiha Arshad, Shahida Niazi

ABSTRACT

Objective: This study was conducted to determine the frequency and salient pathological features of the different subtypes of phyllodes tumors (PT) at Mayo Hospital/King Edward Medical University, Lahore, Pakistan.

Methodology: It was a cross-sectional study conducted at the Histopathology Section of the Pathology Department of Mayo Hospital/King Edward Medical University, Lahore, Pakistan. The study was approved by the ethical committee of the institution. The histopathological records of 30 patients with a diagnosis of PT reported in 6 years from 1st January 2013 to 31st December 2018 were reviewed. The tumors were categorized into benign, borderline and malignant subtypes based on the World Health Organization (WHO) criteria. Other parameters analyzed were the age at the time of presentation, tumor size, tumor laterality, the type of surgical specimens received, the status of surgical margins and recurrence of the tumor. The Statistical Package for the Social Sciences (SPSS) version 24 was used for analyzing the data.

Results: Out of 30 cases, there were 15(50%) cases of benign PT, 6(20%) cases were classified as borderline and 9(30%) cases were placed in the malignant category. The age of the patients ranged from 25 to 80 years with a mean of 41.07±11.13 years. The size of the tumors ranged from 4-22 cm with a mean of 10.53±4.13 cm. The surgical specimens comprised of 2(6.67%) wedge biopsy samples, 20(66.67%) lumpectomy specimens, 4(13.33%) simple mastectomy and 4(13.33%) cases of modified radical mastectomy specimens. These specimens included 4(13.33%) recurrent cases of phyllodes tumor.

Conclusion: Our study concluded that benign phyllodes tumor was the most common type (50%) of phyllodes tumor followed by malignant category (30%). Recurrence of the tumor was reported in 4(13.33%) cases. All 3 subtypes of phyllodes tumors are prone to recurrences so wide surgical excision is the preferred therapy.

Keywords: *Phyllodes tumor. Breast tumor. Lumpectomy. Mastectomy. Wide surgical excision.*

INTRODUCTION

Phyllodes tumor (PT) is an uncommon breast tumor that accounts for less than 1% of all primary tumors of the breast and 2.5% of all tumors in the fibroepithelial group.[1] The tumor originates from the neoplastic proliferation of the mesenchymal stromal cells.[2] These tumors can occur at any age but are more common in women over the fourth decade of life with early onset in Asian countries.[1] The histological hallmark is a leaf-like pattern produced due to a predominantly intracanalicular ductal architecture and elongated cleft-like spaces lined by epithelial & myoepithelial cells within a hypercellular spindle cell stroma which is more accentuated around the lactiferous ducts.[3] Johannes Muller was the first to describe this tumor in 1838 and he called it Cystosarcoma Phyllodes (the word *phyllodes* is the Greek term for "leaf-like").[4] In the year 1981, the World Health Organization (WHO) introduced the new terminology of phyllodes tumor for this pathological breast entity and later in 2003, WHO International Histological Classification group proposed its subdivision into 3 categories: benign, borderline and malignant on the basis of 4 microscopic features. These features included stromal atypia & hypercellularity, stromal overgrowth over the epithelial component, number of mitotic figures and infiltration into the tumor margins.[5] Heterologous malignant elements with areas resembling a liposarcoma, osteogenic sarcoma and chondrosarcoma categorize the tumor into the malignant category irrespective of other histological characteristics.[3,6] Stromal over growth is histologically defined as the "presence of stroma without the epithelium in at least one low power field as observed with a 4X microscope objective". Stromal mitosis is seen in the mitotically active areas of the tumor and counted per 10 HPF.[6]

Grossly, PTs are large with a firm tan white cut surface with cleft-like spaces resembling a leaf. Hemorrhage and necrosis may also be seen. Patients with PTs usually present with painless, palpable and fast-growing tumor nodules.[3] Phyllodes tumor is locally aggressive & infiltrative and usually does not metastasize to the regional axillary lymph nodes. Axillary dissection is therefore not recommended.[2] The route of tumor metastases is usually bloodstream. It may metastasize to the lungs, bones & pleura.[7,8] The overall metastatic rate reported by WHO is 0% for benign PT, 4% for borderline and 22% for malignant PT.[5] Phyllodes tumor exhibits a very high recurrence rate for which a wide margin of surgical excision is

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crucial to control its recurrence and relapse.[9] A 1 cm margin of excision is therefore recommended for all the three subtypes of phyllodes tumors.[10] The differential diagnosis of malignant PT is any high-grade sarcoma and metaplastic carcinoma.[6,8]

This study was planned to determine the salient pathological features of phyllodes tumor and categorize it into benign, borderline & malignant subtypes. Meticulous histopathological examination of surgical specimens is considered the gold standard for this categorization.[1] Like all morphological grading systems, this grading system is also subject to interobserver variability especially at the interphase between the 3 grades. Many PTs show focal areas with benign, borderline and malignant characteristics intermixed within the same tumor which necessitates careful gross examination and histological assessment as a crucial component for accurate categorization.[6]

METHODOLOGY

It was a cross-sectional study conducted at the Histopathology Section of the Pathology Department of Mayo Hospital/King Edward Medical University, Lahore, Pakistan. The study was approved by the ethical committee of the institution. The histo-pathological records of 30 patients with a diagnosis of PT reported in 6 years from 1st January 2013 to 31st December 2018 were reviewed. The histopathology reports and blocks were retrieved. Fresh slides were prepared & reviewed to reconfirm the previous diagnosis and classify the tumors into benign, borderline and malignant categories based on the 4 histological parameters proposed by the WHO (Table 1).[5] Other parameters noted were the age at the time of presentation, types of surgical samples received, tumor size, tumor laterality, the status of the surgical resection margins and axillary lymph nodes in cases of the modified radical mastectomy specimens. All information was retrieved from the computer records of these patients. Retrospective records were also studied to see whether the patient had presented with a recurrent tumor or with an initial tumor mass.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 24 was used for analyzing the data. The results were compiled and tabulated as frequencies & percentages. Quantitative variables like age & size of the tumors were expressed as mean values±SD.

RESULTS

Our study comprised of 30 female patients with histologically diagnosed phyllodes tumor of the breast. The age of the patients ranged from 25 to 80 years with a mean of 41.07±11.13 years. All females in the benign & borderline category were in the age range of 25-55 years. In the malignant group which comprised of 9 cases, 2 patients were 25 years old & one was 80 years old. The remaining 6 cases were in the age range of 40-55 years. Phyllodes tumors were categorized according to the WHO criteria into 3 groups as benign, borderline and malignant. Out of 30 cases, there were 15(50%) cases of benign PT, 6(20%) cases were classified as borderline and 9(30%) cases were placed in the malignant category (Table 2). The histopathological photographs of benign, borderline and malignant tumors are shown in Figure 1-6.

Table 1: "Three-Tiered Grading System for Phyllodes Tumors Based on 2012 WHO Classification of Tumors of the Breast"[5]

Criteria	Benign	Borderline	Malignant
Stromal Cellularity and Atypia	Minimal	Moderate	Marked
Stromal Overgrowth	Minimal	Moderate	Marked
Mitosis/10 High Power Fields	0-4	5-9	≥10
Tumor Margins	Well circumscribed with pushing tumor margins	Zone of microscopic invasion around tumor margins	Infiltrative tumor margins

Table 2: Frequency Distribution of Phyllodes Tumor into Benign, Borderline and Malignant Category

Tumor Type	Frequency (n=30)	Percentage
Benign	15	50%
Borderline	6	20%
Malignant	9	30%
Total	**30**	**100%**

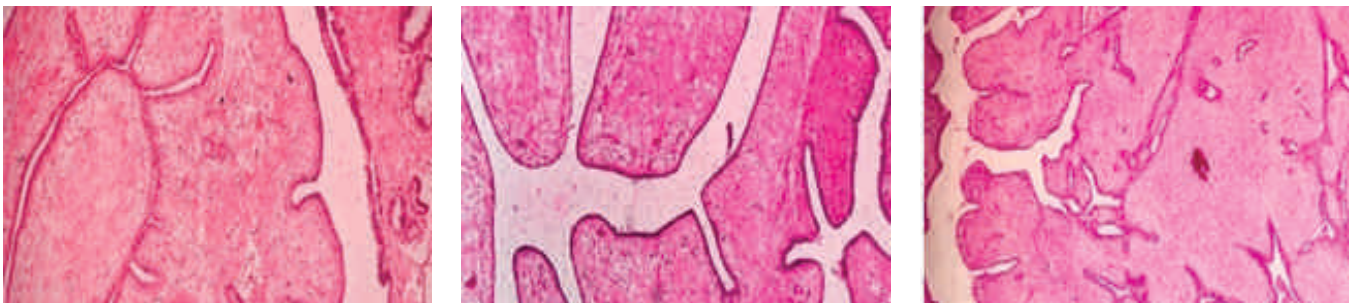

Figure 1: Benign Phyllodes Tumor: Low Power Microscopic Images Demonstrating the Typical Leafy Stromal Fronds Capped by Epithelium, Increased Stromal Cellularity & Overgrowth

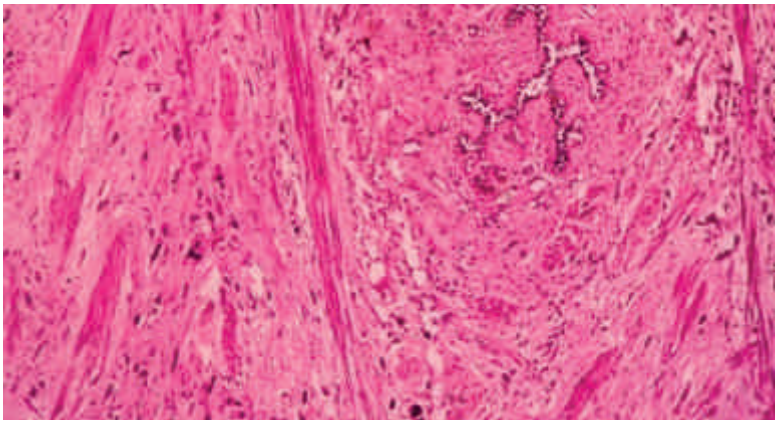

Figure 2: Borderline PT: Low Power View Showing Increased Stromal Cellularity & Overgrowth and the Benign Appearing Ductal Component on the Upper Right Hand Side of the Field. Moderate Degree of Pleomorphism can be Appreciated in the Stromal Component

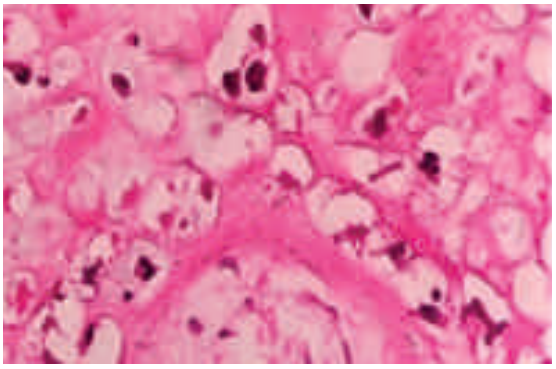

Figure 3: High Power View Microscopic Image Showing Lipomatous Heterologous Sarcomatous Component in a Case of Malignant PT

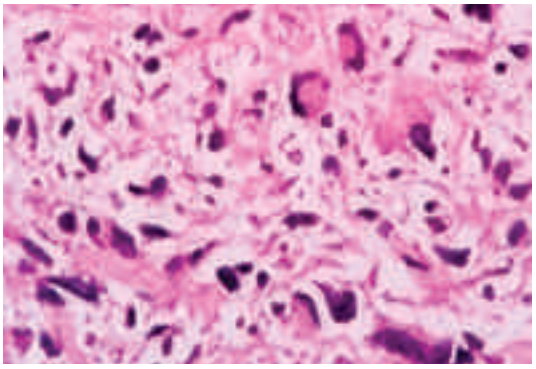

Figure 4: High Power View of Malignant PT Showing Marked Atypia, Pleomorphism & Rhabdoid Cells in the Stromal Component

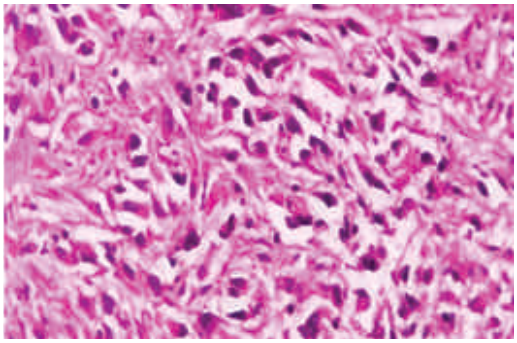

Figure 5: Malignant PT Showing a High-Grade Sarcoma Like Pattern in the Stromal Component

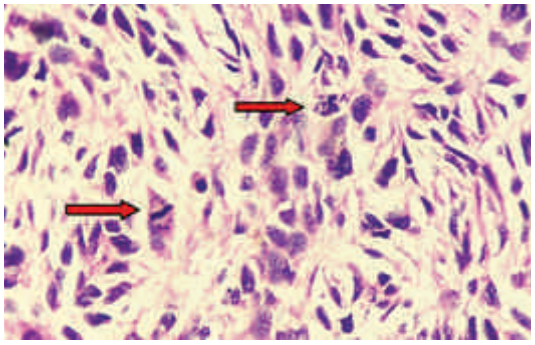

Figure 6: High Power View of Malignant PT Showing Marked Nuclear Atypia, Bizarre Appearing Cells & Mitotic Figures (Arrows) in the Stromal Component Resembling a High-Grade Sarcoma

Table 3: Clinical and Pathological Features of Phyllodes Tumor (n=30)

Clinical and Pathological Features		Frequency	Percentage
Surgical Specimens	Wedge biopsy	2	6.67%
	Lumpectomy	20	66.67%
	Simple Mastectomy	4	13.33%
	Modified Radical Mastectomy	4	13.33%
Laterality	Right Sided	13	43.33%
	Left Sided	17	56.67%
No. of Mitosis/10 HPF	0-4 (Benign)	15	50%
	5-9 (Borderline)	6	20%
	10 or ≥10 (Malignant)	9	30%
Status of Resection Surgical Margins	Involved	8	26.67%
	Not involved	17	56.67%
	Not Assessed/Indeterminate Status	5	16.67%
Recurrent Cases		4	13.33%
Lymph Node Status in 4 Modified Radical Mastectomy Specimens Cases		None Involved	0%
Size of the Tumor		**Range**	**Mean±SD**
	Benign Phyllodes Tumor	4-12 cm	8.87±3.34 cm
	Borderline Phyllodes Tumor	8-12 cm	10.67±3.25 cm
	Malignant Phyllodes Tumor	8-22 cm	13.22±4.39 cm
Age of the Patients	Benign Phyllodes Tumor	25-55 years	38.93±8.04 years
	Borderline Phyllodes Tumor	25-55 years	41.83±8.90 years
	Malignant Phyllodes Tumor	25-80 years	44.11±15.34 years

The size of the tumors ranged from 4-22 cm with a mean of 10.53±4.13 cm. The tumor size varied according to the categorization as benign, borderline & malignant with the largest sized tumor masses observed in the malignant category as compared to the benign and borderline categories of PT. The surgical specimens comprised of 2(6.67%) wedge biopsy samples, 20(66.67%) lumpectomy specimens, 4(13.33%) simple mastectomy and 4(13.33%) cases of modified radical mastectomy specimens. These specimens included 4(13.33%) recurrent cases of phyllodes tumor. Out of a total of 8 mastectomies, 6 mastectomies were performed for malignant PTs and 2 were performed for borderline PTs. No axillary lymph node involvement by tumor deposits was reported in any of the 4 radical mastectomy specimens. The clinical and pathological features of phyllodes tumor are shown in table 3.

DISCUSSION

Phyllodes tumors show a variety of histological changes.[5] Benign PTs show a slight increase in stromal cellularity, a mild degree of cellular atypia and 0-4 mitosis/10 HPF with pushing borders. Borderline PTs show a moderate increase in stromal cellularity, atypia & stromal overgrowth over the epithelial components with mitosis ranging from 5-9 mitosis/10 HPF and areas of microscopic invasion into the tumor margins. Malignant PTs show marked stromal cellularity, an extreme degree of cellular atypia & pleomorphism and stromal overgrowth with >10 mitosis/10 HPF with grossly visible infiltrative tumor margins.[3,5,11]

In our study, the mean age of the patients was 41.07±11.13 years with the age range of 25 to 80 years. In the malignant group which comprised of 9 cases, 2 patients were 25 years old & one was 80 years old. The remaining 6 cases were in the age range of 40-55 years. All females in the benign & borderline category were in the age range of 25-55 years. The left-sided breast was involved in 17 patients and the right-sided breast in 13 patients. In the present study, no significant correlation between laterality and phyllodes tumor was appreciated. In a study carried out at Jinnah

Postgraduate Medical Centre, Karachi, the age of patients ranged between 19 to 66 years with the mean age of 40 years. Similarly, they reported 12 cases involving the left breast and 16 cases involving the right breast.[12] A study carried out on 26 patients of PTs in Farhat Hached Hospital, Tunisia reported the mean age of 40 years.[8]

In the present study, there were 15(50%) cases of benign PT, 6(20%) cases of borderline PT and 9(30%) cases were classified as malignant. A retrospective study carried out in Fudan University, Shanghai Cancer Centre classified 168(41.6%) cases into benign PT, 184(45.5%) cases as having borderline PT and 52(12.9%) cases were placed in the malignant group.[11] In another study carried out over a period of 10 years at the Cancer Hospital Chinese Academy of Medical Sciences, there were 125(55%) cases of benign phyllodes, 55(24%) cases of borderline phyllodes and 47(21%) cases of malignant PT.[13] In a study conducted by Karim et al., 65 PT were analyzed. Out of these, 34(52.3%) cases were classified as benign, 23(35.3%) cases as borderline and 8(12.3%) cases were placed in the malignant category.[14] Two malignant phyllodes tumors in the present study showed osteogenic sarcoma, chondroid & liposarcoma-like stromal areas along with benign glandular elements.

A major diagnostic dilemma is the distinction between a cellular fibroadenoma and benign PT. Both entities show many overlapping features and areas resembling a fibroadenoma are frequently observed histologically in some PTs although the incidence of such occurrence has not been documented.[3] In the present study, such areas were observed as a focal finding in 3 cases which included 2 cases of benign PTs and 1 case of borderline PT. It is important to differentiate the two entities since both have different surgical implications.[6] According to a study published in the American Journal of Clinical Pathology, it was established that presence of any of the following three histological findings like stromal fragmentation, stromal hypercellularity & overgrowth, infiltration into the surrounding fat, stromal heterogeneity, subepithelial stromal condensation and nuclear atypia favour the diagnosis of a benign PT rather than a cellular fibroadenoma.[15]

Repeated local recurrence is the most important prognostic feature of this tumor having an estimated recurrence rate of 40% collectively for the 3 subtypes of PTs.[9,10] Most recurrences of PTs occur within 2 years after diagnosis. These recurrences are more common in the malignant and borderline PTs.[1,13] Even benign PTs can show repeated recurrences and are prone to rapid growth.[9] In the present study, 4 patients presented with tumor recurrences. Two patients had malignant PT & both presented with recurrences in the first 2 years of clinical diagnosis. The 3rd case was a borderline PT & presented within 6 months with recurrence which was reported as malignant. The 4th case with 5 times recurrences had no previous record available but the present diagnosis was malignant PT.

Wide local excision of the tumor is the standard recommended surgical treatment of any type of PT and mastectomy is recommended for the recurrent & overtly malignant tumors.[11] Therefore, all patients of PT, whether benign, borderline or malignant must be kept on close follow-up for tumor regrowth & recurrence.[9,10] An excision margin of 1 cm is therefore considered essential to prevent tumor recurrence.[10,16]

The tumor size in the present study varied depending on the types of PTs. All malignant tumors were huge, protuberant nodular masses measuring in the range of 8 cm to 22 cm (mean=13.22 cm). In another study, the mean tumor size was 8.3 cm (Range 1.5-25 cm).[17] Abdelkrim et al. reported the tumor size between 1.5-40 cm (mean=7.8 cm).[8] The larger the size, the greater the chances of the tumor being malignant.[13] Tumors more than 10 cm qualify as giant phyllodes tumor.[18] Most patients with malignant PTs present with a rapid increase in tumor size.[7]

In the present study, a modified radical mastectomy with axillary dissection was performed in 4 malignant PTs, none of which exhibited axillary involvement which is according to the well known biological feature of this tumor. Borderline and malignant phyllodes tumors show a rare potential for (although very rare) distant metastatic spread.[8] It is only the stromal component that metastasizes. To prevent recurrence and distant metastasis of malignant phyllodes tumor, negative surgical margins should be obtained.[11]

The role of radiotherapy and chemotherapy in the treatment & management of PT has not been clearly defined due to the rarity of the tumor.[19] Molecular and genetic studies reveal the expression of β-catenin and insulin-like growth factors I and II in the stromal component of PT. Several immunohistochemical stains p53, Ki67, CD117, epidermal growth factor receptor (EGFR), p16 and vascular endothelial growth factor (VEGF) are positive in the stromal cells.[6,18] Alterations in chromosome 3p and 1q are common in recurrent

PTs.[20] Recently MED12 mutations have also been discovered in these tumors associated with aberrantly activated estrogen signaling.[3] Due to a lack of resources and high cost, molecular studies and immuno-histochemical stains were not performed in the present study.

CONCLUSION

Our study concluded that benign phyllodes tumor was the most common type (50%) of phyllodes tumor followed by malignant category (30%). Recurrence of the tumor was reported in 4(13.33%) cases. Phyllodes tumor of the breast requires correct histopathological categorization based on tumor margin, stromal atypia and overgrowth, stromal hypercellularity and mitosis. Proper diagnosis, categorization and management are crucial because of its ability to recur and causing considerable morbidity & distress to the patient. Complete surgical removal of the tumor with a 1 cm grossly uninvolved margin is essential to prevent local recurrences & relapse. Mastectomy is recommended for the recurrent & overtly malignant PTs but axillary lymph node dissection is not required. The accurate histological categorization of PTs into benign, borderline and malignant categories remains a challenging task even with the most experienced breast pathologists.

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Original Article

Correlation of Femoral Length and Forearm Plus Little Finger Length for Measurement of Intramedullary Nail Length for Femoral Fracture Fixation

Atif Shahzad, Farooq Azam Khan, Nisar Ahmed, Jawad Asghar Chishti, Zahid Bashir, Zakria Tariq

ABSTRACT

Objective: To determine the correlation between the femoral length and the forearm plus little finger length for the measurement of intramedullary femoral nail length for the femoral fracture fixation.

Methodology: This cross-sectional study was conducted in the Orthopaedic Department of the Services Hospital, Lahore. The study was approved by the ethical committee of the institution. The study included 120 adults of either gender and age 18-50 years with femoral shaft fractures by nonprobability consecutive sampling technique. After taking informed written consent from the patients, their demographic profile was noted on proforma. The femoral length was recorded with the help of a measuring tape from the superior pole of the patella to the greater trochanter of the intact femur and recorded in centimeters. The forearm plus little finger length was measured with the help of a measuring tape from the olecranon process of the ulna to the tip of the little finger and recorded in centimeters. The length of the nail used was recorded as well. Data analysis was done by the Statistical Package for the Social Sciences (SPSS) version 23.

Results: In this study, the mean age of the patients was 33.12±10.65 years. There were 77(64.2%) male and 43(35.8%) female patients. According to body mass index, 29(24.2%) patients were obese and 91(75.8%) patients were non-obese. A total of 62(51.7%) patients had left-sided fracture and 58(48.3%) patients had the right side involved. In this study, the mean femoral length was 41.47±1.64 cm. The mean length of the forearm plus little finger was 41.47±1.71 cm. Correlation of femoral length and length of forearm plus little finger was positively significant (r=0.885, p-value <0.00001).

Conclusion: Measurement of the forearm plus little finger length is a convenient method to determine the femoral length for intramedullary nailing. In our study, a significant correlation is found between the forearm plus little finger length and the femoral length. It can also be used in patients with bilateral fractures of the femur. Hence, it can be implemented in clinical practice to avoid other expensive and possibly harmful means.

Keywords: *Femoral fracture. Forearm plus little finger length. Femoral length. Intramedullary nail.*

INTRODUCTION

Femoral shaft fractures account for the majority of the fracture of the lower limb. The incidence of diaphyseal femur fractures in adults is approximately 10 per 100,000 people annually.[1] The fractures of femoral shaft usually occur in males of age 15-24 years with increased incidence in elderly males and females.[2] The femoral shaft fractures are usually diagnosed clinically and the patient presents with an inability to bear weight, deformed shape of thigh and severe pain.[3] Most of the fractures occur due to high-speed trauma with associated injuries.[4]

The fractures of the shaft of the femur were treated initially with the use of wooden splints, fabrics encased with wax, clay or gum stiffened bandages and plaster of paris.[3,5] Presently, these fractures are not managed conservatively except in underdeveloped countries or war surgery.[6]

The most common operative treatment modality for these fractures is intramedullary nailing.[7,8] It is mandatory to evaluate the personality of fracture including its type, extent of the fracture, the status of soft tissue and comminution for the definitive procedure.[9,10]

Intramedullary nailing provides a stable environment for osteosynthesis. It does not require external fixation and any special postoperative care. It allows early movement of the joint and weight-bearing.[10] Its healing time is rapid with shorter hospitalization. Malunion and shortening of the limb are less common after intramedullary nailing.[9,10] Preoperative femoral nail length can be measured by putting the nail over the other normal thigh and doing a radiograph. This method is mostly cost-ineffective and causes the necessary exposure to radiation. Patients can also be assessed by measuring the length from greater trochanter to the superior pole of patella but this method is inconvenient and painful.[11,13] A new method of femoral length determination has been introduced which involves the measurement of the forearm length extending from olecranon tip to the end of the little finger.[9,11-13]

The rationale of this study was to establish the correlation between the femoral length and the forearm plus little finger length for the measurement of intramedullary femoral nail length. This study has not been done in our population and there is no established

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local data on this correlation. This study can facilitate us to generate evidence about this correlation for the local population and we can use this correlation for the measurement of intramedullary femoral nails in patients with femoral shaft fractures especially in bilateral fractures and in places where the preoperative radiographic facility is not available.

METHODOLOGY

It was a cross-sectional study conducted in the Orthopaedic Department of Services Hospital, Lahore. The study was approved by the ethical committee of the institution. The study included 120 adults of either gender and age 18-50 years with femoral shaft fractures by nonprobability consecutive sampling technique. The diagnosis of femoral shaft fractures was confirmed on the radiograph. The exclusion criteria of the study was patients with open fracture of femur, ipsilateral fracture of neck of femur, pertrochanteric fracture & supracondylar fracture on radiographs, bilateral fractures of radius & ulna, carpal bones, 5th metatarsal & phalanges of little finger, congenital or traumatic deformity of forearm & hand and previous healed fracture of shaft of femur revealed by history and radiographs.

After taking informed written consent from the patients, their demographic profile was noted on proforma. All patients were examined by the orthopedic surgeon. The femoral length was recorded with the help of a measuring tape from the superior pole of the patella to the greater trochanter of the intact femur and recorded in centimeters. The measurement of the length of the forearm plus little finger was done using a measuring tape from the olecranon process of the ulna to the tip of the little finger and recorded in centimeters. The length of the nail used was recorded as well.

STATISTICAL ANALYSIS

The Statistical Package for the Social Sciences (SPSS) version 23 was used for data analysis. Frequency and percentage were calculated for gender and body mass index (BMI). Mean and standard deviation (SD) were calculated for age, femoral length and forearm plus little finger length. The correlation of femoral length with forearm plus little finger length was calculated using the Pearson correlation coefficient (r) with a p-value of ≤0.05 taken as significant.

RESULTS

The study participants had a mean age of 33.12±10.65 years. The age of the patients ranged from 18-50 years. Fifty two (43.3%) patients were <30 years old and 68(56.7%) patients were ≥30 years of age. There were 77(64.2%) male and 43(35.8%) female patients. According to BMI, 29(24.2%) patients were obese and 91(75.8%) patients were non-obese. A total of 62(51.7%) patients had a left-sided fracture and 58(48.3%) patients had the right side involved.

In this study, the mean femoral length was 41.47±1.64 cm with a range of 36-45 cm. The mean length of the forearm plus little finger was 41.47±1.71 cm with a range of 35-45 cm. Correlation of femoral length and length of forearm plus little finger was positively significant i.e. r=0.885, p-value <0.00001 (Figure 1).

On stratifying data we found that the correlation of femoral length and length of forearm plus little finger was positively significant in patients aged <30 years (r=0.914, p-value <0.00001) as well as in patients aged ≥30 years (r=0.842, p-value <0.00001). The correlation of femoral length and length of forearm plus little finger was found significant in both male (r=0.686, p-value <0.00001) & female (r=0.933, p-value <0.00001) patients. Correlation of femoral length and length of forearm plus little finger was positively significant in obese patients (r=0.893, p-value <0.00001). Correlation was also found significant (r=0.903, p-value <0.00001) in non-obese patients (Table 1).

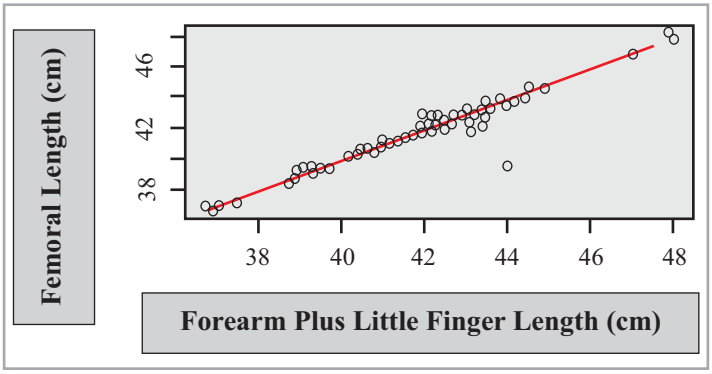

Figure 1: Scatter Graph Showing Linear Correlation of Femoral Length with the Forearm Plus Little Finger Length

Table 1: Correlation of the Femoral Length and Forearm Plus Little Finger Length with Respect to Demographic Variables

Demographic Variables		Pearson Correlation Coefficient (r)	p-value
Age	<30 years	0.914	<0.00001
	≥30 years	0.842	<0.00001
Gender	Male	0.686	<0.00001
	Female	0.933	<0.00001
BMI	Obese	0.893	<0.00001
	Non-obese	0.903	<0.00001

DISCUSSION

The femur is the main weight-bearing bone of the lower limb. It is the strongest tubular bone of the body.[6,8] Femoral shaft fractures are fixed mostly with intramedullary nailing in adults.[1,3] The method being used nowadays to determine femoral nail length is the measurement of the distance from the superior pole of the patella to the superior aspect of the neck of the femur.[1] The other common methods to determine femoral nail length are radiography, radio-opaque ruler, nail template or Kuntscher oximeter. All of these mentioned methods require an intact opposite femur for correct estimation. In case of bilateral femur fracture, this estimation becomes difficult, inaccurate and is done on less comminuted side.[8]

Estimation of the forearm plus little finger length from the olecranon process tip to the tip of the little finger is a new technique to determine the intramedullary nail length. This method is convenient, cost-effective and does not involve the exposure to the radiation.[8,12]

In the current study, the patients had a mean age of 33.12±10.65 years with more male patients (64.2%) as compared to females (35.8%). Similar results were found in a study by Naik et al. in which the mean age of the patients was 35.8±9.2 years. Sixty eight patients were males and 32 were females.[9]

In this study, the mean femoral length was 41.47±1.64 cm with a range of 36-45 cm and the mean length of the forearm plus little finger was 41.47±1.71 cm with a range of 35-45 cm. Correlation of femoral length and length of forearm plus little finger was positively significant (p-value <0.001). Similar results were reported by other studies.[9,13-15]

We found that the correlation of femoral length and length of forearm plus little finger was positively significant in patients of age <30 years as well as in patients of age more than 30 years. Similarly, this correlation was also significant in both male and female & obese and non-obese patients. So, there results indicated that there is no effect of age, gender and BMI of the patients on the correlation. Similar results were found in other studies with no significant association of demographic variables with the femoral length and length of forearm plus little finger.

In a study by Naik et al., the mean femoral length and forearm plus little finger length were 39.85±2.44 cm and 39.87±2.73 cm, respectively and the correlation of these values was significant (p<0.001). The patient's age, gender and BMI did not affect this correlation.[9] A study conducted in Liverpool, the United Kingdom in 2018 also concluded that the length of the forearm plus little finger represents an ideal length for the nail of the femur. In this study, the mean length of the forearm plus little finger was 38.86±2.83 cm and the mean length of the intramedullary nail was 38.56±2.77 cm. Correlation between these two variables showed significant results with r=1.[13] Another study conducted in India showed that the correlation between the femoral length and the forearm plus little finger length was significant (p<0.001) with no effect of age, gender and BMI.[14]

A study was conducted in Nepal in which preoperative assessment of K-nail length was done in 500 patients by three different methods and compared. The first method measured the length of K-nail from the tip of the greater trochanter to the medial joint line of the knee. In the second method, length from olecranon process tip to the end of the little finger was noted and 3rd measurement was taken from the greater trochanter tip to the superior pole of the patella. The mean length of all three measurements was analyzed and the length from the tip of the olecranon process to the end of the little finger was found more convenient and best method for assessment of intramedullary nail length.[15]

CONCLUSION

Estimation of the length of the forearm plus little finger is a convenient method to determine the femoral length for intramedullary nailing. In our study, a significant correlation is found between the forearm plus little finger length and the femoral length. It can also be used in patients with bilateral fractures of the femur. Hence, it can be implemented in clinical practice to avoid other expensive and possibly harmful means.

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Original Article

Serum Vitamin D, Superoxide Dismutase and Catalase Levels in Females with Uterine Fibroid

Nazish Jamil, Qurat-ul-Ain, Ejaz Ahmad, Sana Javaid, Umer Jamil, Shafeen Zulfiqar

ABSTRACT

Objective: To determine the serum vitamin D, superoxide dismutase and catalase levels in females with uterine fibroids.

Methodology: This study was performed in the Obstetrics & Gynaecology Department of Sharif Medical City Hospital, Lahore after taking approval from the ethical committee of the institution. It was a case-control study that included 25 females of reproductive age (25-45 years) having uterine fibroid diagnosed on ultrasonography & 25 females were enrolled as age and gender matched controls. Informed written consent was obtained from all patients before taking the blood sample. Serum vitamin D, SOD and CAT levels were measured by ELISA kit.

Results: This study analyzed serum vitamin D and antioxidant enzymes SOD and CAT levels in uterine fibroid women which were compared with healthy women (non-fibroid females) of the same age group. The mean levels of vitamin D and antioxidant enzymes SOD & CAT in patients with uterine fibroid were 11.29±1.08 pmol/L, 0.13±0.0019 U/ml and 3.30±0.0264 U/L respectively. In the control group the levels of vitamin D, SOD & CAT were 17.88±2.16 pmol/L, 0.49±0.0034 U/ml and 5.26±0.376 U/L respectively. The levels of vitamin D and antioxidant enzymes SOD and CAT were found significantly decreased in females with uterine fibroid as compared to the controls.

Conclusion: The levels of vitamin D and antioxidant enzymes SOD and CAT are decreased in uterine fibroid females in comparison to the healthy females. It indicates a positive relationship between reduced levels of vitamin D, SOD and CAT to the risk of development of uterine fibroid. So, vitamin D and antioxidants supplementation might reduce the risk factor of uterine fibroid and can be helpful in the non-surgical treatment of uterine fibroid.

Keywords: *Uterine fibroid. Serum vitamin D levels. Superoxide dismutase (SOD). Catalase (CAT). ELISA.*

INTRODUCTION

Uterine fibroids also called leiomyomas are benign monoclonal tumors of smooth muscle cells originating from myometrium.[1] Uterine fibroid is the most common benign gynecological tumors appearing in 60-80% of women in the reproductive age group.[2] At 50 years of age the incidence rate of uterine fibroid may reach approximately 70-80% in the western population.[3] According to the literature majority of the patients present with uterine fibroid usually between ages of 28-52 years.[4] Majority cases of fibroids are asymptomatic and remain undiagnosed while other present with multiple symptoms such as heavy menstrual bleeding, dysmenorrhea, chronic abdomino-pelvic pain. Some women feel only pressure effects such as bloatness and bowel disturbances.[5] Most of the fibroids located beneath the endometrium affect the endometrial blood supply to the embryo which further ceases the growth and early development of embryo causing subfertility abortions and late pregnancy complications.[5,6] Normal vitamin D levels play an important role to decline the growth of the fibroid. Normal levels of vitamin D in both children and adults is >30 ng/ml (67.41 pmol/L) and vitamin D levels <20 ng/ml (44.94 pmol/L) are considered as vitamin D deficiency.[7] Decreased levels of serum vitamin D is considered as a risk factor for the development of uterine fibroid. Vitamin D is known to act as an antifibrotic agent causing fibrosis of human uterine fibroid by acting on transforming growth factor beta-3 (TGF β3). Vitamin D inhibits the growth of uterine fibroid by different mechanisms. It declines the growth of cells and induces apoptosis through the suppression of catechol-O-methyltransferase (COMT) and downregulation of cyclin-dependent kinase (CDK1).[8] Vitamin D is also a membrane antioxidant causing a reduction in the oxidative stress which leads to shrinkage of uterine fibroids.[9] Another mechanism of action of vitamin D is that it acts as a profibrotic factor and inhibits the growth of the tumor by acting on vitamin D receptor (VDR). Vitamin D receptor is a nuclear transcription factor that induces growth arrest and enhances the process of apoptosis. Vitamin D also inhibits the TGF β3 dependent key profibrotic factors which cause a paradoxical increase in matrix metalloproteinases (MMPs) in uterine fibroids. So, serum vitamin D inhibits the expression of MMPs which cause shrinkage of fibroid.[10] Vitamin D shows antiproliferative action and regulates different regulatory genes and controls the activity of cyclin-dependent kinases (CDKs), which further arrest the G0-G1 phase by interrupting the S phase and decreasing the number of cells in the uterine fibroid. Another important factor involved in the development of uterine fibroid is oxidative stress which leads to the

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deposition of newly formed extracellular matrix (ECM) in the uterine fibroid.[11] The main cause of oxidative stress is generation of reactive oxygen species (ROS) such as NO, H_2O_2 and hydroxyl radical all of which are involved in an imbalance between connective tissue production and degradation resulting in deposition of extracellular matrix in uterus and also an increase in the production of cytokines and growth factors, angiogenesis, proliferation and inhibition of apoptosis. All these processes simultaneously promote the development of uterine fibroid.[12] Several antioxidant enzymes play a major protective role against oxidative stress such as SOD and CAT. Superoxide dismutase catalyzes superoxide ion to hydrogen peroxide and CAT further catalyzes H_2O_2 into O_2 and H_2O which protect the cell and maintain cellular redox balance.[13]

The objective of this study was to find out the levels of serum vitamin D and serum antioxidant enzymes SOD and CAT in females of reproductive age group with uterine fibroid in comparison to the healthy controls (non-fibroid females). The study focused on the probable therapeutic role of vitamin D supplementation and antioxidants in the uterine fibroid.

METHODOLOGY

This study was conducted at the Obstetrics & Gynaecology Department of Sharif Medical City Hospital, Lahore. In this study, 50 participants were enrolled who underwent a gynaecological ultrasound scan at our institution. Out of 50, 25 women were diagnosed with the uterine leiomyomas on ultrasound and 25 served as control subjects (non-fibroid females). The patients with history of total thyroidectomy, total hysterectomy, osteoarthritis and parathyroid disorder were excluded from the study. All patients variables including age, height, weight, age of menarche, menopausal status, alcohol drinking, smoking status, any past medical history, any past surgical history, present medication and also the purpose of the study were recorded in the form of a questionnaire. Using aseptic techniques, 5 ml of the venous blood sample was collected from the median cubital vein after taking informed written consent. Serum was separated from the blood after centrifugation and serum vitamin D, SOD and CAT levels were measured using a standard ELISA Kit.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23 and expressed as the mean±standard deviation. The results of the two groups were compared by applying independent t-test. A p-value ≤0.05 was taken as statistically significant.

RESULTS

The study showed a significant decline in serum vitamin D levels in uterine fibroid women (11.29±1.08 pmol/L) as compared to the healthy females (17.88±2.16 pmol/L) (Figure 1). Table 1 showed that the results were statistically significant with a p-value of 0.027.

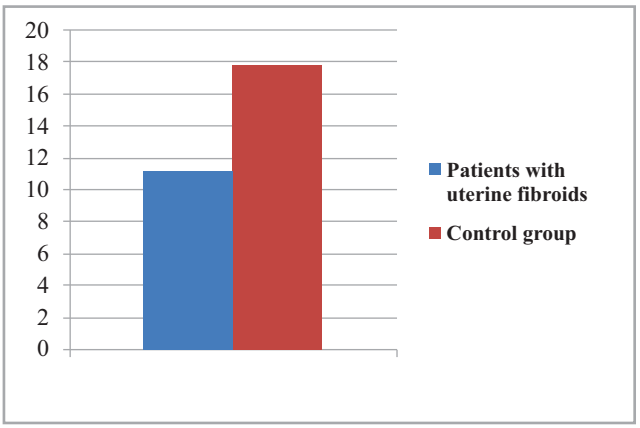

Figure 1: Levels of Vitamin D (pmol/L) in Study Subjects

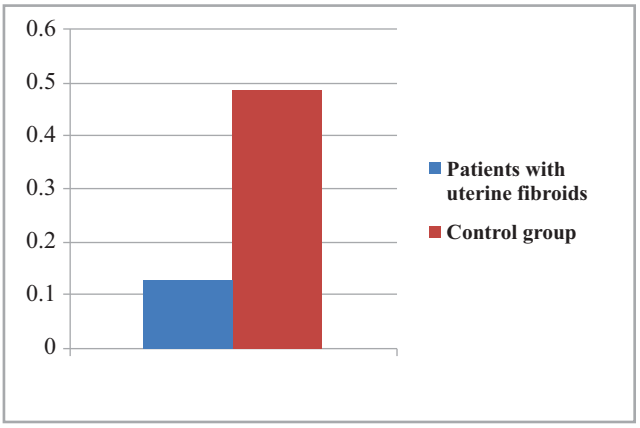

Figure 2: Levels of SOD (U/ml) in Study Subjects

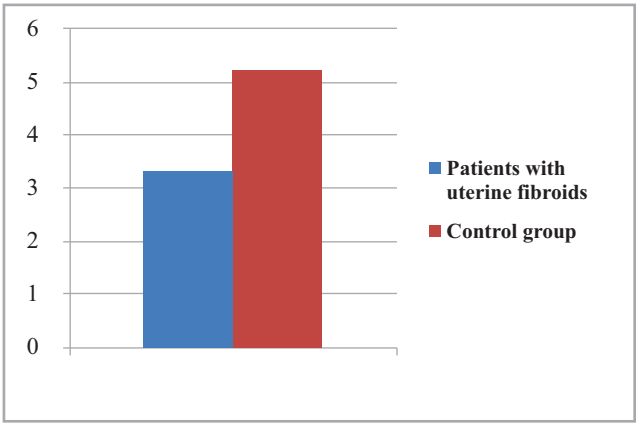

Figure 3: Serum CAT (U/L) Levels in Study Subjects

Table 1: Comparison of Vitamin D, SOD & CAT Levels in Patients with Uterine Fibroid and Control Group

Study Variables	Patients with Uterine Fibroids	Control Group	p-value
Vitamin D	11.29±1.08 pmol/L	17.88±2.16 pmol/L	0.027
SOD	0.13±0.0019 U/ml	0.49±0.0034 U/ml	0.011
CAT	3.30±0.0264 U/L	5.26±0.376 U/L	0.016

The levels of antioxidant enzyme SOD and CAT in uterine fibroid women were 0.13±0.0019 U/ml and 3.30±0.0264 U/L respectively. In the healthy females, the SOD & CAT levels were 0.49±0.0034 U/ml and 5.26±0.376 U/L respectively. The levels of SOD and CAT obtained were statistically significant in uterine fibroid females with p-values of 0.011 and 0.016 respectively (Figure 2, 3 & Table 1).

DISCUSSION

Oxidative stress plays a major role in different profibrotic gynecological disorders such as fibroids, endometriosis and postoperative adhesions. Several powerful antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect cells against the oxidative damage and maintain the cellular redox balance.[14] Vitamin D is a strong antioxidant and the main regulator of calcium homeostasis. It has the property to decrease the whole process of lipid peroxidation that is why vitamin D is comparable to other anticancer drugs like tamoxifen and its 4 hydroxy metabolites.[15] The current study shows a significant decrease in the levels of vitamin D, SOD and CAT in a patient with fibroid uterus compared to healthy controls (non-fibroid females). The results obtained were statistically significant and we found that vitamin D, SOD and CAT are inversely related with the occurrence of uterine fibroid.

In another study by Sabry et al., the serum vitamin D levels were measured in females with uterine leiomyomas. A statistically significant relationship of low serum vitamin D and the incidence of uterine leiomyoma was found (p-value= 0.01) coinciding with the results of the present study. According to them, normal levels of vitamin D act as a protective factor in uterine fibroid as it has an ability to cause apoptosis and inhibit the proliferation of leiomyoma cells which leads to shrinkage of uterine fibroid.[16] In another study by Baird et al., it was indicated that sufficient vitamin D is associated with reduced risk of uterine fibroid. He randomly selected 35-49 year old females and their fibroid status was determined by ultrasound screening. Vitamin D status was assessed in stored plasma by radioimmunoassay. After exposure to sun rays, it was seen that each 10 ng/ml increase in 25(OH) D was associated with a 20% lower risk of fibroid, strengthening the importance of normal vitamin D levels indicated in the present study.[17] Another study by Abdelraheem et al., identified an inverse relation between serum vitamin D level and uterine fibroid volume and also pointed out an inverse relationship between serum vitamin D level and mean number of uterine fibroid. The results were similar to the present study.[18] Brakta et al. also demonstrated that decreased serum vitamin D levels are associated with an increased risk of uterine fibroid.[19] Halder et al. determined whether vitamin D_3 inhibits TGF β3 induced fibrosis related gene expression in fibroid cells. He concluded that vitamin D_3 acts as an antifibrotic factor that reduces TGF β3 which automatically inhibits phosphorylation of SMAD2 and limits the fibrosis of uterine fibroid, so vitamin D_3 is potentially useful therapeutically in the uterine fibroid.[20] According to Halder et al., vitamin D is a potent anti-estrogenic receptor and can reduce estrogenic action in uterine leiomyomas that's why vitamin D might be used as a treatment option in the uterine fibroid.[21] Another research conducted by Halder et al., pointed out that vitamin D_3 acts as an antitumor agent and treatment with vitamin D shrinks uterine fibroid cells in an Eker rat model.[22] These results potentiate the therapeutic significance of vitamin D pointed out in the present study. According to Blauer et al., vitamin D affects the cell growth of the uterine fibroid. He found that normal vitamin D levels inhibited growth in uterine leiomyomas 12% in comparison with the controls. This study strengthens the results of the current study according to which low vitamin D levels were seen in uterine fibroid females thereby further potentiating the need for vitamin D supplementation in females with fibroids.[23]

According to Ciavattini et al. and Paffoni et al., decreased serum levels of vitamin D are strongly associated with the prevalence of uterine leiomyomas similar to our results.[24,25] According to Mitro et al., there is no indicated relationship between decreased serum vitamin D levels and the prevalence of uterine fibroid. In his analysis of 3,590 from the National Health and Nutrition Examination Survey (NHANES) 2001-2006 no significant relationship was observed between uterine leiomyoma and serum vitamin D contrary to the results of the current study.[26]

Superoxide dismutase is a main key antioxidant enzyme that plays a protective role in lipid peroxidation and is responsible for the elimination of superoxide radicals. In our study SOD levels were decreased in patients with uterine leiomyomas as compared to the

healthy females. Our results are consistent with another study by Pejic et al. According to them, serum levels of SOD in the blood are decreased 20% in patients with uterine myomas in comparison to the controls. Activated oxygen metabolites are responsible for cell damage and reduced antioxidant SOD might be responsible for uterine malignancy.[27] This is in accordance with the current study. In some other studies, contrary to the result of our study, SOD levels in different tissue with neoplastic changes appeared to be increased and remained unchanged in comparison to the healthy females.[28] Pejic et al. demonstrated that serum levels of SOD in leiomyoma and the different gynecological patient is decreased comparatively to the healthy subjects but serum levels of CAT remain unchanged in myoma patients,[29] which is contrary to the present study. Manoharan et al. concluded that there is an increased serum CAT level in cervicitis patients and decreased levels of CAT in myoma patients,[30] similar to the current study.

In our study, it has been observed that there is a decreased serum level of vitamin D and antioxidants SOD and CAT in uterine fibroid female as compared to the non-fibroid uterus females (controls). Our study results support that decreased levels of serum vitamin D and antioxidants are strongly associated with the occurrence of uterine leiomyomas.

CONCLUSION

The levels of serum vitamin D, SOD and CAT are decreased in uterine fibroid females in comparison to the non-fibroid uterus females. Vitamin D and antioxidant supplementation on a regular basis in daily routine might reduce the risk of occurrence of uterine fibroid. Hence, vitamin D and antioxidants can be useful as a non-surgical tool in treating uterine fibroids.

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Original Article

Effectiveness of Repeating Valsalva Maneuver in Paroxysmal Supraventricular Tachycardia Patients Nonresponsive to Initial Valsalva Maneuver and a Single Dose of Verapamil

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Muhammad Amad Abbasi, Raafe Iqbal

ABSTRACT

Objective: To determine the effectiveness of repeating valsalva maneuver in paroxysmal supraventricular tachycardia (PSVT) patients nonresponsive to initial valsalva maneuver and a single dose of verapamil.

Methodology: It was a descriptive case series conducted in the Emergency Department of the Punjab Institute of Cardiology, Lahore. The study was approved by the ethical committee of the institution. A total of 100 patients were enrolled by nonprobability consecutive sampling technique by taking informed written consent. The patients of any age or gender presenting with an acute episode of PSVT confirmed on ECG and nonresponsive to initial valsalva maneuver & a single dose of verapamil were included. Patients were given a trial of the valsalva maneuver. The heart rate and rhythm of the patients were continuously observed with a cardiac monitor. Repeat ECG was done 1 minute after performing the valsalva maneuver. The data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 24.

Results: The mean age of the patients was 56.48±15.57 years. Seventy four (74%) patients were males and 26(26%) patients were females. Out of 100 patients nonresponsive to initial valsalva maneuver and a single verapamil dose, reversion of supraventricular tachycardia was noted in 27(27%) patients. There was a statistically significant association between gender, BMI, smoking and SVT reversion.

Conclusion: The reversion rate of paroxysmal SVT with repeat valsalva maneuver after the failure of an initial trial of valvalsa maneuver and a single dose of verapamil is satisfactory. The reversion of supraventricular tachycardia was noted in 27% of cases in our study.

Keywords: *Valsvalva maneuver. Paroxysmal supraventricular tachycardia. Verapamil.*

INTRODUCTION

Paroxysmal supraventricular tachycardia (PSVT) is a tachyarrhythmia which arises from the atrial and/or atrioventricular nodal tissue and has a sudden onset & termination. It is a narrow-complex tachycardia with a regular, rapid rhythm except for multifocal atrial tachycardia and atrial fibrillation. Supraventricular tachycardia (SVT) is a frequent clinical disease occurring in all ages and its treatment is a major challenge.[1,2]

The prevalence of SVT is 2.25 per 1000 population per year in the United States and is responsible for 50,000 visits to the emergency department per year.[3] Supraventricular tachycardia is classified into atrioventricular reentrant tachycardia (AVRT), atrioventricular nodal reentrant tachycardia (AVNRT), atrial tachycardia, atrial flutter and atrial fibrillation.[4] The patients with SVT can be asymptomatic or symptomatic. The clinical features include palpitations, dizziness, chest pain, difficulty in breathing and anxiety. The risk factors of SVT are caffeine, alcohol, nicotine, psychological stress and Wolff-Parkinson-White (WPW) syndrome.[5] It is diagnosed by an electrocardiogram (ECG) showing a rapid heart rhythm of 150-240 beats per minute and narrow QRS complexes[6] (Figure 1). The complications of paroxysmal SVT include heart failure, pulmonary edema, myocardial infarction in patients with poor left ventricular function. Electrophysiological studies have determined that the etiology of SVT is the impulse generation and conduction abnormalities.[1,2]

Acute management of PSVT is to control the heart rate and prevent hemodynamic instability. Cardioversion with sedation is recommended if the patient has low blood pressure. The first-line treatment in hemodynamically stable patients is the vagal

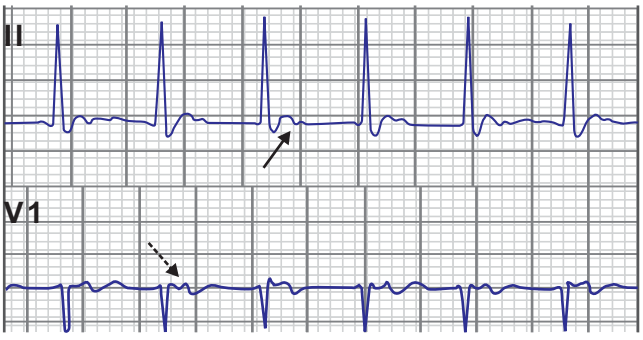

Figure 1: "Electrocardiographic Clues to the Diagnosis of Atrioventricular Re-Entrant Tachycardia and Atrioventricular Nodal Re-Entrant Tachycardia Include a P Wave Following the QRS Complex, as can be Seen in Lead II (Solid Arrow) and Lead VI (Dotted Arrow), and a Short RP Interval"[3]

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maneuvers. Vagal maneuvers slow the AV nodal conduction and interrupt the reentry pathway. They comprise of valsalva maneuvers, carotid sinus massage, bearing down and face immersion in ice water.[7]

The valsalva maneuver is a safe and the most effective internationally recommended vagal maneuver for the first-line emergency treatment for SVT.[8,9] The vagal maneuvers increase the parasympathetic tone by raising the intrathoracic pressure and stimulate the baroreceptors in the carotid bodies & aortic arch.[10] The success rate of vagal maneuvers varies from 6% to 54%.[11]

In the standard valsalva maneuver, the patient is in a semirecumbent or supine position and he is asked to exhale forcefully against a closed glottis after inspiration. The duration of strain is 10-15 seconds. Blowing hard into a 10 ml syringe that moves its plunger can also be used for this purpose.[12]

In the modified valsalva maneuver, the patient performs a standard strain in the semirecumbent position. Then lies in the supine position with leg raise for 15 seconds at 45º. This boosts the venous return and vagal stimulation. The efficacy of modified valsalva maneuver for SVT reversion is greater as compared to the standard method.[12]

If vagal maneuvers fail, pharmacological therapy is given such as calcium channel blockers or adenosine.[6] Adenosine causes many adverse effects such as transient asystole and a sense of impending doom in which patient feels that they are about to die.[13] Verapamil is a calcium channel blocker that inhibits the conduction of action potential through the AV node.[1] The adverse effects of verapamil are bradycardia & hypotension and are not recommended as first-line treatment for SVT. However, studies have reported that a single verapamil dose is safe without any side effects.[7] This study was conducted to determine the efficacy of repeating the valsalva maneuver in the reversion of paroxysmal supraventricular tachycardia in patients nonresponsive to an initial trial of the valsalva maneuver and a single dose of verapamil. Most of the acute episodes of SVT are terminated by verapamil but there is a significant risk of hypotension and bradycardia with more than one intravenous dose. Literature has reported a satisfactory reversion rate of SVT with valsalva maneuver and verapamil but the performance of repeating the valsalva maneuver after the initial trial of valsalva maneuver and a single dose of verapamil has not been evaluated in any previous study. This will help in implementing this practice in an emergency setting in the future to manage acute episodes of paroxysmal SVT and prevent the adverse effects of multiple doses of injection verapamil.

METHODOLOGY

It was a descriptive case series conducted in the Emergency Department of the Punjab Institute of Cardiology, Lahore after approval by the institutional ethical committee. A total of 100 patients were enrolled by the nonprobability consecutive sampling technique after taking their informed consent. The sample size was calculated using a 95% confidence interval, 7.5% margin of error and 17% expected percentage of SVT reversion with valsalva maneuver.[9] The diagnosis of paroxysmal supraventricular tachycardia was confirmed on ECG showing regular narrow complex rhythm and a heart rate of >150 beats/min. The patients of any age or gender presenting with paroxysmal SVT were managed initially with valsalva maneuver. The patients in which SVT was not reverted with the valsalva maneuver, a single dose of verapamil was injected. The SVT patients nonresponsive to both initial valsalva maneuver and a single dose of verapamil were included in the study. The exclusion criteria included patients with sinus tachycardia, atrial flutter, atrial fibrillation, or broad complex or aberrant conduction tachycardia on ECG. The demographic profile of the patients was noted on the proforma and repeat valsalva maneuver was performed. The patients were placed in the semirecumbent or supine position and abdominal pressure was applied for 15 seconds. The heart rate and rhythm of the patients were continuously monitored. The ECG was repeated after 1 minute. The reversion of tachycardia was labeled if the ECG showed a normal sinus rhythm of <100 beats/min. Nonresponsive cases were those in which there was the persistence of paroxysmal SVT on ECG assessed after 15 minutes of a single dose of verapamil.

STATISTICAL ANALYSIS

Data analysis was done by the Statistical Package for the Social Sciences (SPSS) version 24. Quantitative variables like age, body mass index (BMI) were expressed as mean and standard deviation. Qualitative variables like gender, smoking, diabetes mellitus and reversion of tachycardia were expressed as frequency and percentage. A chi-square test was used to compare the demographic variables with the frequency of SVT reversion. A p-value of ≤ 0.05 was considered significant.

RESULTS

In our study, patients had a mean age of 56.48±15.57 years with the age range of 30-80 years. Fifty six (56%) patients were up to 60 years old and 44(44%) were above 60 years of age. Seventy four (74%) patients were males and 26(26%) patients were females. The

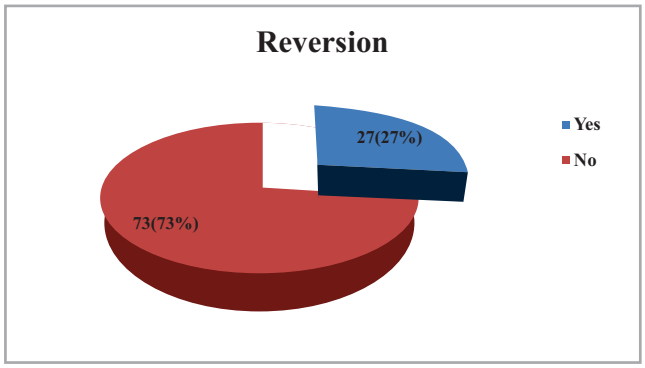

Figure 2: Frequency of SVT Reversion

mean BMI of the study participants was 25.13±2.90 kg/m^2. The minimum BMI was 20.2 kg/m^2 and the maximum BMI was 30.3 kg/m^2. Forty eight (48%) patients had normal BMI while 52(52%) patients were overweight and obese. Out of 100 patients, 23(23%) were smokers and 55(55%) patients had diabetes mellitus. Out of a total of 100 patients nonresponsive to the initial valsalva maneuver and a single verapamil dose, SVT reversion was noted in 27(27%) patients (Figure 2).

Eighteen (66.7%) patients with the reversion of SVT were up to 60 years of age and 9(33.3%) patients were above 60 years old. The correlation of SVT reversion with the age of patients was found statistically insignificant (p-value = 0.1913).

Out of 27 patients who reverted from SVT, 24(88.9%) were males and 3(11.1%) were females. There was a statistically significant association between gender and SVT reversion (p-value = 0.038).

The reversion of SVT was noted in 19(70.37%) patients with normal BMI and 8(29.63%) overweight/obese patients. The correlation of SVT reversion with the BMI of patients was found statistically significant i.e. p-value = 0.0065.

Fifteen (55.6%) patients with SVT reversion were smokers and 12(44.4%) were non-smokers. The difference was statistically significant between the smoking and SVT reversion i.e. p-value=0.001.

Out of 55 diabetic patients, SVT reversion was observed in 14(51.9%) patients and out of 45 nondiabetic patients, SVT reversion occurred in 13(48.1%) cases. The difference was statistically insignificant between diabetes mellitus and SVT reversion i.e. p-value=0.7003. The association of demographic variables with SVT reversion is shown in table 1.

DISCUSSION

Supraventricular tachycardia is a frequent heart disease presenting as a rapid heart rate.[14] The clinicians must diagnose SVT when they detect regular narrow complex tachycardia and also know its different treatment modalities.[15] The treatment of SVT involves vagal maneuver, medication and cardioversion.[14]

In our study, the reversion rate of SVT with repeating valsalva maneuver after an initial valsalva maneuver and a single dose of verapamil was noted in 27(27%) patients. There was a statistically significant association between gender, BMI, smoking and SVT reversion. Many studies have evaluated the reversion of SVT with valsalva maneuver or verapamil but this is the first study that determined the effectiveness of the valsalva maneuver in patients who did not respond to the initial valsalva maneuver and the first dose of verapamil.

The efficacy of the valsalva maneuver as a first-line treatment for SVT varies among different studies. A systematic review reported that the reversion rate of valsalva maneuver in SVT was 19.4%.[10] Walker et al. conducted a retrospective study on 19 SVT patients in Australia with the reversion of 1(5.3%) patient with standard valsalva maneuver and 6(31.7%) patients with modified valsalva maneuver.[16] Another systematic review by Pandya et al. included 3 studies with a total of 316 study subjects. In two studies by Mehta et al. and

Table 1: Comparison of Demographic Variables with SVT Reversion

Demographic Variables		SVT Reversion		Total	Chi-square Statistic	p-value
		Yes	No			
Age Range	Up to 60 years	18(32.14%)	38(67.86%)	56(56%)	1.708	0.1913
	Above 60 years	9(20.45%)	35(79.55%)	44(44%)		
Gender	Male	24(32.43%)	50(67.57%)	74(74%)	4.2615	0.038986*
	Female	3(11.54%)	23(88.46%)	26(26%)		
BMI	Normal	19(39.58%)	29(60.42%)	48(48%)	7.416	0.0065*
	Overweight/Obese	8(15.38%)	44(84.62%)	52(52%)		
Smoking	Smokers	15(65.22%)	8(34.78%)	23(23%)	22.1346	0.001*
	Nonsmoking	12(15.58%)	65(84.42%)	77(77%)		
Diabetes Mellitus	Diabetic	14(25.45%)	41(74.55%)	55(55%)	0.1481	0 .700351
	Nondiabetic	13(28.89%)	32(71.11%)	45(45%)		

***p-value significant ≤0.05**

Wen et al., SVT was induced in patients while the third study by Lim et al. recruited patients presenting to the emergency with SVT. The reversion rate of SVT with valsalva maneuver was 20% while standing & 54% in the supine position by Mehta et al., 53% by Wen et al. and 17.9% by Lim et al. The higher reversion rate in studies by Mehta et al. and Wen et al. might be due to short duration of induced SVT and the chances that patients might not have responded to vagal maneuvers before presenting in the emergency in the study by Lim et al.[17]

A prospective cohort study was conducted in Iraq in which 93 SVT patients underwent a modified valsalva maneuver. The reversion of SVT was seen in 47.3% of patients. Thirty nine (41.9%) patients reverted to sinus rhythm after the first attempt and 5(5.4%) patients recovered after the second attempt of maneuver.[18]

In a randomized controlled trial in England, patients with SVT were randomly allocated into two groups with 214 participants in each group. Patients in one group underwent a standard valsalva maneuver while the patients in the other group underwent a modified valsalva maneuver. Supraventricular reversion was noted in 37(17%) patients with standard valsalva maneuver and 93(43%) patients with modified valsalva maneuver.[9]

A study determined the effectiveness and side effects of verapamil and adenosine in the treatment of SVT. The efficacy of both drugs was the same with more adverse effects with adenosine. The SVT reversion rate was 90.8% with adenosine and 89.9% with verapamil. The adverse effects were greater with adenosine (16.7-76%) in contrast to verapamil (0-9.9%). However, verapamil was associated with a higher rate of hypotension (3.7%) than adenosine (0.6%).[19] Another study done in Pakistan reported a 71.6% reversion rate of paroxysmal SVT with verapamil.[20]

CONCLUSION

The reversion rate of paroxysmal SVT with repeat valsalva maneuver after the failure of an initial trial of valvalsa maneuver and a single dose of verapamil is satisfactory. The reversion of supraventricular tachycardia was noted in 27% of cases in our study.

RECOMMENDATIONS

- Valsalva maneuver can be repeated in patients of SVT nonresponsive to initial valsalva maneuver and a single dose of verapamil. This will prevent the adverse effects of additional doses of verapamil.
- The effectiveness of the modified valsalva maneuver was not evaluated in the study. Further research is required to evaluate the efficacy of both the standard & modified valsalva maneuvers in SVT patients nonresponsive to initial valsalva maneuver & a single dose of verapamil and the results should be compared.
- A multicenter study should be carried out on a large number of study participants.

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Original Article

Evaluation of Tooth Size Discrepancy in Orthodontic Patients with Various Skeletal Patterns

Faiza Malik, Iffat Batool

ABSTRACT

Objective: To determine the frequency of various skeletal patterns in orthodontic patients with malocclusion and evaluate mean tooth size discrepancy ratios in various skeletal patterns.

Methodology: It was a cross-sectional study with consecutive sampling conducted at 28 Military Dental Centre, Lahore. After approval from the ethical committee and written informed consent from patients, the ANB angle of 150 orthodontic patients was derived from corresponding lateral cephalograms to determine the skeletal pattern (Class I, II, III). Their impressions were taken with alginate and poured with hard plaster to form pre-treatment study casts. The mesiodistal widths of all maxillary and mandibular teeth from right first molar to left first molar were measured on the casts using a digital caliper, to calculate the anterior and overall ratios via Bolton analysis.

Results: The frequency of skeletal patterns was such that there were 34.7% patients with Class I skeletal pattern, 51.3% of patients with Class II skeletal pattern and 14% of patients with Class III skeletal pattern. No significant difference in the overall and anterior Bolton ratios in different skeletal patterns ($p>0.05$) was observed upon the application of ANOVA and Tukey's test. However, the anterior ratios for all skeletal patterns showed higher values than the norm, owing to the diversity of skeletal patterns selected. The frequency of individuals with significant tooth size discrepancy i.e. outside ±2SD of Bolton's norms was 12.6% for overall ratio and 36.0% for anterior ratio. No sexual dimorphism was observed.

Conclusion: Class II skeletal pattern showed the highest frequency in this study. No significant difference was found in the tooth size discrepancy ratios in different skeletal patterns and both genders. However, a common trend was noted towards excess tooth material in the mandibular anterior region of all skeletal patterns.

Keywords: *Tooth size discrepancy. Bolton's norms. Bolton analysis. Overall ratio. Anterior ratio.*

INTRODUCTION

Tooth size discrepancy (TSD) is the term used for a relative excess of tooth material in one arch compared to the other arch, or, the disproportion in the size of individual teeth.[1] For occlusal harmony, certain norms must be there for the dimensions of maxillary and mandibular teeth. An appropriate relationship will favor orthodontic treatment results.[2,3] In 1958, Bolton devised two ratios for calculating tooth size discrepancy.[4] He measured the total mesiodistal widths of mandibular anterior teeth and found its ratio to the maxillary anterior teeth (anterior ratio) to be 77.2. Similarly, he calculated the overall ratio to be 91.3 by dividing the total width of all mandibular teeth by maxillary teeth, including first molars and all teeth anterior to them. Any variation from these norms signifies the presence of tooth size discrepancy in either jaw of a particular individual.[5]

Although the size of most natural dentitions is well proportioned, almost 5% of people have a discrepancy among the size of individual teeth.[6] The most common cause is anomalous maxillary lateral incisor(s), followed by premolars and other teeth.[7] One of the first investigators to become interested in the subject of tooth size was G.V. Black. In 1902, he researched the size of innumerable human teeth and established tables of mean values, including the mesiodistal widths of teeth, which are used as important references till date.[3]

Tooth size analysis, developed by Bolton, constitutes an integral segment of diagnosis and treatment planning in orthodontics, which otherwise comprises of history and examination, photographic, radiographic and cast analysis.[6] It determines certain space management issues during the course of treatment.[7] The ratios calculated for each patient are compared with Bolton's norms (overall ratio 91.3, anterior ratio 77.2) to determine whether or not there is a tooth size discrepancy.[3] If the computed ratios are greater than the norms, it signifies a relative excess of tooth material in the mandible. Similarly, a relative excess of tooth material will occur in the maxilla if the computed ratios are less than the norms. A variation greater than 2 standard deviation of the normal ratios and TSD greater than 2 mm is considered clinically significant.[5]

The prevalence of significant TSD varies in different populations since it is affected by variables such as skeletal pattern, gender, race and ethnicity.[7] Several studies have been dedicated worldwide to shed light on it but few relate it to skeletal pattern, which is the relative anteroposterior position of the jaws with reference to the cranial base.[6] This study will broaden our knowledge by providing a database about tooth size discrepancy in a segment of the Pakistani population.

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METHODOLOGY

It was a cross-sectional study with consecutive sampling conducted at 28 Military Dental Centre, Lahore. After approval from the ethical committee, written informed consent was obtained from 150 orthodontic patients of Pakistani ethnicity aged 13 years and above, presenting with malocclusion. Patients included in the study had fully erupted permanent dentition from the first molar to first molar, good quality casts, and absence of caries, extensive restorations or wear of teeth. Patients who were excluded from the study had retained deciduous teeth, morphological dental anomaly, congenitally missing, impacted or extracted teeth and history of previous orthodontic treatment.

Lateral cephalograms of the orthodontic patients were traced and ANB angle was derived to determine the skeletal pattern, such that 0^{o} to 4^{o} was Class I, more than 4^{o} was Class II and less than 0^{o} was Class III. Their intraoral impressions were taken with alginate and pre-treatment study casts were made with hard plaster. The mesiodistal widths of all maxillary and mandibular teeth from the right first molar to the left first molar were measured on the casts using a digital caliper to the nearest 0.01 mm. Bolton analysis was done to calculate the anterior and overall ratios.[4] All measurements were recorded on a specially designed proforma.

STATISTICAL ANALYSIS

All acquired data was entered into the Statistical Package for the Social Sciences (SPSS) version 24 for statistical analysis. Frequency and percentages were calculated for gender and different skeletal patterns. Mean and standard deviation were calculated for overall and anterior ratios in all subjects and also for the three skeletal patterns individually. ANOVA was applied to compare the TSD among three skeletal patterns, followed by Tukey's Test for post HOC analysis. A p-value of ≤ 0.05 was considered to be significant. The frequency of clinically significant tooth size discrepancy was also observed which occurs if Bolton's ratios are computed to be beyond ±2SD of the norms.

RESULTS

The sample selected had a mean age of 14.8±2.77 years. The gender distribution was such that there were 67 males (44.7%) and 83 females (55.3%) among a total of 150 patients. The frequency of skeletal patterns was such that there were 52 class I patients, 77 class II patients and 21 class III patients, as shown in table 1. Class II patients constituted 51.3% of the total sample size, reflecting the fact that class II skeletal pattern is the most prevalent type of malocclusion in this part of the world for which patients seek orthodontic treatment.

The results for the overall ratio (91.35) computed in the present study showed no difference from Bolton's mean of 91.3 (Table 1). The mean anterior ratio was 78.61 which also lies within ±2SD of the normal anterior ratio of 77.2.

The overall ratio was also calculated for individual skeletal patterns and found no meaningful differences from the mean value for the entire sample. The anterior ratios computed for the individual skeletal patterns showed higher values than the norm (Table 1), with class I displaying the highest mean anterior ratio of 79.1, secondly class II with 78.6 and thirdly class III with 77.4. Higher values signify a trend towards excessive tooth material in the mandibular anterior region. No significant difference in the overall and anterior Bolton ratios in different skeletal patterns ($p>0.05$) was observed upon the application of ANOVA and Tukey's test (Table 2).

The current study found no meaningful gender differences in TSD. Both the gender groups had mean overall and anterior ratios close to the means for the entire sample. The mean values for overall and anterior ratios in males were 91.58±2.37 and 78.44±3.02 respectively, while the same for females were found to be 91.16±2.86 and 78.75±3.02 respectively. Both genders displayed a greater range of values for anterior ratio as compared to the overall ratio.

In the current research, 12.6% of the sample displayed a significant overall ratio while 36% of the sample had a significant anterior ratio (Table 3). Among the 12.6% patients with significant overall TSD, 8.6% of individuals showed a trend towards overall maxillary excess while the remainder 4% of individuals had an overall mandibular excess. Among the 36% of patients with significant anterior TSD, 3.3% of individuals displayed anterior maxillary excess, while a very high percentage of 32.6% individuals displayed anterior mandibular excess.

Table 1: Mean Overall and Anterior Ratios

Skeletal Pattern	Frequency (n)	Percentage	Mean Overall Ratio	Mean Anterior Ratio
Class I	52	34.7%	91.43±2.48	79.10±4.01
Class II	77	51.3%	91.33±2.85	78.61±3.63
Class III	21	14.0%	91.22±2.40	77.40±2.98
Total	150	100.0%	91.35±2.65	78.61±3.70

Table 2: Results for ANOVA and Tukey's Test

Skeletal Pattern	Overall Ratio	p-value	Anterior Ratio	p-value
Class I	Class II	0.974	Class II	0.739
	Class III	0.951	Class III	0.179
Class II	Class I	0.974	Class I	0.739
	Class III	0.986	Class III	0.379
Class III	Class I	0.951	Class I	0.179
	Class II	0.986	Class II	0.379

p-value ≤0.05 is significant

Table 3: Frequency of Significant Overall and Anterior TSD

Standard Deviation	Overall Ratio (91.3)		Anterior Ratio (77.2)		Interpretation
	Frequency (n)	Percentage	Frequency (n)	Percentage	
< - 2SD	13	8.6%	5	3.3%	Maxillary excess
> + 2SD	6	4.0%	49	32.6%	Mandibular excess
Total	19	12.6%	54	36.0%	

DISCUSSION

Tooth size discrepancy prevails in 5% of the general population. Regional and ethnic variations are grossly reported in the available literature.[6,8] A significant TSD may present intraorally in the form of rotations, spacing, crowding and improper interdigitation.[2] If diagnosed during the initial phase of treatment planning, difficulties during the finishing stage can be avoided. Also, the extraction pattern, anchorage requirements, treatment mechanics and need for later restoration or prosthesis can be planned ahead.[9]

In the present research, the average overall ratio for 150 subjects was 91.35±2.65. However, the ranges and standard deviations recorded were higher in contrast to Bolton's original study. This can be attributed to the fact that a variety of skeletal patterns were selected in this study, whereas Bolton's original study only included subjects with excellent occlusion. These results are supported by other studies. Al-Omari et al. found the overall ratio to be 92.2 among Jordanian children.[10] Another study conducted by Jaiswal and Paudel in Nepal found the mean overall ratio to be 92.42±1.80 which is slightly greater than the mean calculated in this study for the Pakistani population.[11]

Quraishi et al. carried out a study in Karachi, Pakistan, in which the mean overall and anterior ratios were 91.54 and 78.85 respectively, regardless of the type of occlusion.[12] These values are very close to those of the present study, probably due to similar race and ethnicity of the subjects selected. The anterior ratio calculated in the present research was found to be 78.61±3.70, which lies within ±2SD of Bolton's norm of 77.2.

The minimum and maximum anterior ratios recorded in the current research were 71.90 and 88.70, both in class I skeletal patterns. Another local research by Batool et al. reported a minimum anterior ratio of 66.0 in a class I patient and a maximum anterior ratio of 97.3 in a class III patient.[13] The range is quite large compared to the present study, however, the average anterior ratio of the entire sample is quite comparable at 79.34±5.19.

Bolton's TSD ratios were also calculated for individual skeletal patterns, of which class II was the most prevalent. No significant difference was seen between the overall ratio computed for individual skeletal patterns and the mean overall ratio for the entire sample, as concluded by ANOVA and Tukey's test. These findings are supported by Basaran et al.,[9] O'Mahony et al.,[14] Uysal et al.[15] and Endo et al.[16]

The anterior ratios were also computed for individual skeletal patterns, whereby the mean for each group was higher than the norm. Higher values signify a trend towards excessive tooth material in the anterior mandibular segment. A similar quantitative study conducted by Batool et al. in Rawalpindi, Pakistan, resulted in very high anterior ratios in class II malocclusion, signifying the presence of excess tooth material in the anterior mandibular segment.[13] Such findings suggest that for a clinical orthodontic evaluation, there is a need to set population-specific standards and norms.

In another local research by Asad et al., the difference in Bolton's ratios was statistically insignificant in three skeletal classes.[17] Contrary to the present research, the incidence of significant Bolton's ratios was more in skeletal class III. A Brazilian study by Cancado et al. also showed no significant differences in the overall and anterior ratios of tooth size discrepancies among different Angle malocclusion groups.[18]

To determine the frequency of significant TSD in the current research, values of ratios beyond 2 standard

deviations of Bolton's norms were considered significant.[5] The results displayed 12.6% individuals with an overall ratio beyond ±2SD of 91.3 and 36% individuals with anterior ratio beyond ±2SD of 77.2. These results very closely relate with Freeman's study in which the percentage of significantly different overall and anterior ratios was found to be 13.5% and 30.6% respectively. Freeman's study also concluded that the overall discrepancy could equally be either an excess in the mandible or maxilla. However, the chances for excess tooth material in the anterior mandible (19.7%) were double than that in anterior maxilla (10.8%).[19]

Another important aspect related to tooth size discrepancy is sexual dimorphism. The current study found no meaningful differences in TSD between males and females. The mean overall ratio for males was 91.58±2.37 and for females was 91.16±2.86. Both values are very close to the overall mean of 91.35 and Bolton's norm of 91.3. However, it can be concluded that males showed a trend towards overall mandibular excess while females followed a trend towards overall maxillary excess. As far as the anterior ratio is concerned, males reported a mean value of 78.4±3.02, while females displayed a mean value of 78.75±3.02, both signifying a trend towards excess tooth material in the mandibular anterior region.

A Jordanian study by Al-Omari et al. showed no significant differences in the TSD between males and females.[10] In another study on the Turkish population by Basaran et al., no sexual dimorphism was found among various malocclusions.[9] Endo et al. found no statistically significant sexual difference in anterior and overall ratios in Japanese population.[16] Paredes et al. investigated TSD in a Spanish population and concluded no significant gender differences in tooth-width ratios.[20] Among the local literature available, a notable study by Quraishi et al. in Karachi, Pakistan, reported no sexual differences in tooth size discrepancy.[12] On the contrary, a Nepalese study by Jaiswal and Paudel displayed a significant difference in mean overall ratios between male and female subjects.[11] In another study by Uysal et al. in Turkey, a significantly different overall ratio was found among males and females with normal occlusions.[15]

The clinical implication of Bolton's ratios has been demonstrated time and time again. TSD is a principal factor in space analysis. The ultimate goal of orthodontic treatment is to achieve the best esthetics, function and stability. Management will of course depend on the particular situation of Bolton's discrepancy.[21] This study has explored a segment of Pakistani population presenting for orthodontic treatment in an area of Lahore. During the review of the literature, very few local studies were found on this important subject. The relevant studies were notably from Rawalpindi, Karachi and Lahore. Although these are the three largest metropolitan cities of the country, representing two of Pakistan's most densely populated provinces, more pertinent data is required from the northern and western territories in order to depict a complete picture of the prevalence of TSD among the entire nation.

CONCLUSION

Class II skeletal pattern showed the highest frequency in this study. No significant difference was found in the tooth size discrepancy ratios in different skeletal patterns and both genders. However, a common trend was noted towards excess tooth material in the mandibular anterior region of all skeletal patterns. Further investigations are required to ascertain the racial, ethnic and sexual differences in tooth size discrepancy, and determine its relationship with various types of dental and skeletal malocclusions.

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