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# EDITORIAL

## Drug Standardization: Its Regulation and Methods for Drug Testing

Akbar Waheed Syed, Afifa Siddique

Standardization of drugs is an evolving process, aiming to ensure safety, efficacy and quality of the drugs. The amount of drug substance and its release profile in every drug product should be uniform, within specified limits so that the required dose is delivered to the human body. This uniformity must align with the standards laid down in the pharmacopoeias, drug indexes, formularies and other books defining standards and specifications. All drug products manufactured by all the drug manufacturing facilities must be uniform and the product should be free of impurities for patient's safety and to avoid toxicity.<sup>1</sup>

Many countries have developed their own pharmacopoeias, like United States pharmacopeia (USP), British pharmacopeia (BP), International pharmacopeia (Ph. Int) and Japanese pharmacopeia (JP) and these are the most widely used pharmacopoeias all over the world as well. Pakistan has not developed its own pharmacopeia, so the drug testing is relied upon USP and BP standards. There are approx. 11,000 prescription drugs actively marketed in Pakistan and sold through licensed pharmacies. Over the counter (OCT) products such as pain, cold, flu medicines contribute to significant market in our country as well. Collaborative efforts between the government, academia and industry stakeholders are essential to keep pace with global standards.<sup>2</sup>

A brief overview of bindingness of regulations according to state of law, state of technology and state of science is shown in Table I. In Pakistan, it is regulated by both Ministry of **National Health Services Regulations and Coordination (NHSR&C)** and **Drug Regulatory Authority of Pakistan (DRAP)**. They ensure that drugs manufactured and

distributed in Pakistan meet international standards.<sup>3, 4</sup> **National Essential Medicine List (NEML)**, previously known as **National Formulary of Pakistan (NFP)** is an official publication that lists essential medicines approved for use within Pakistan. It has crucial role in promoting rational drug use and ensuring consistent health care standards.<sup>5</sup> **The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)** is distinctive in bringing together the pharmaceutical industry and the regulatory bodies to collaborate scientific and technical aspects of drugs and thus leading to the development of its guidelines. **Pakistan National Pharmacovigilance Center (PNPC)** was established in collaboration with US in year 2017, and became member of Uppsala Monitoring Center (UMC) by 2018.<sup>6</sup> **The National Pharmacovigilance Centre (NPC)** at DRAP highlights both legal requirement and

**Table I: Binding and Non-Binding Regulations**

		USA	International	Pakistan	British
Must be like this (State of law)	Law	Federal Food, Drug, and Cosmetic Act	-	The Drugs Act 1976 and DRAP* Act 2012	Medicine Act 1968, Misuse of Drugs Act 1971, Misuse of drugs Regulation 2001
	Rules and Regulation	Code of Federal Regulations	-	The Drug Rule 1976, 1978, 1986	MHRA*
Should be like this (state of technology)	Standard	USP*	ICH*, ISO*, WHO*, PIC/S*	NFP*, USP/BP*	BP*
	Guidance	FDA* Guidelines	PDA/ISPE*	PNPC* (GMP* + ICH* + WHO guidelines)	British Guidelines, EMEA* Notes for guidelines
Can be like this (State of science)	Publication	Articles, reviews, systemic analysis and meta-analysis of peer-reviewed, high impact factor journals			

**BP:** British Pharmacopeia, **DRAP:** Drug Regulatory Authority of Pakistan, **EMA:** European Medicines Agency, **FDA:** Food and Drug Administration, **ICH:** International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, **ISO:** International Organization for Standardization, **MHRA:** Medicines and Healthcare products Regulation Agency, **NFP:** National Formulary of Pakistan, **PIC/S:** Pharmaceutical Inspection Co-operation Scheme, **USP:** United States Pharmacopeia, **WHO:** World Health Organization

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social responsibility. Effective pharmacovigilance not only ensures product quality and patient safety but also strengthens a company's reputation, credibility, and competitiveness in the global market. Non-compliance of binding regulations can lead to legal consequences, such as fines, penalties, or the suspension of licenses.<sup>7</sup>

The drug manufacturer companies label their products to indicate which standard was used to access the quality of finished drug product, this is commonly known as specifications. For example, if the manufacturer has claimed on the label of his product, that it complies with USP specifications, then it means that this product is tested as per testing method of US pharmacopeia. If the drug fails any quality test mentioned in its monograph, then it will be considered substandard drug product i.e. unsafe and ineffective. The drug testing methods can be chemical, biological or immunological, depending on the nature of drug. However, these also face distinct challenges that must be addressed to maintain the integrity of drug development and therapeutic applications.

**Chemical assays** have long been foundation of drug standardization, providing precise quantification of active ingredients. They involve the use of chemical methods like titrations, chromatographic and spectroscopic techniques. Their reliability and accuracy make them indispensable, particularly in the production of generics. The exact chemical nature of the drug must be known, and it should be available in pure form. Nevertheless, the complex chemical composition of some drugs and the need for increasingly sophisticated technologies highlight the ongoing challenge of maintaining the consistency across different batches and manufacturers.<sup>8</sup>

**Bioassays**, on the other hand, offer a more holistic approach by measuring the biological activity of a drug. These assays are crucial for assessing some antibiotics like cholistimethate, neomycin etc. A chemical assay is performed to identify the quantity of cholistimethate, then biological assay is done to measure its activity in IU. Biologicals (like insulin, erythropoietin, heparin, adalimumab) are checked for biosimilarity too, which is as crucial as their assays. Biosimilar means that it has been grown in same strain, with same protein sequence and

folding. Moreover, the genetic material used in creating recombinant cell/organism should be 100% same. For example, if any pharmaceutical company wants to have multiple sources of insulin bulk, then insulin coming from all sources must be biosimilar. The inherent variability in the biological systems can lead to inconsistencies, necessitating rigorous validation and standardization of these assays to ensure reproducibility.<sup>9</sup> So, there is always ongoing research to shift analysis from bioassays to chemical method. For example, for insulin it has been changed to high-performance liquid chromatography (HPLC) method.<sup>10</sup> Among these bioassays, the 3-point and 4-point assays are particularly noteworthy. The 3-point assay, utilizing two submaximal doses of the standard and the one dose of unknown drug, allows for a reliable estimation of potency through response plotting. The 4-point assay, which involves two doses of both the standard and unknown drug, enhances the accuracy by calculating the log ratio of doses.<sup>11</sup>

**Immunological assays** are very specific and are used mainly for vaccines. A specific dose of vaccine is given to animals to access the immunogenicity of vaccine, then their blood is checked for antibodies. A specific dose of vaccine is required to make specific amount of antibodies. When desired level of antibodies is produced then this vaccine is considered "up to the standard". Thus, this allows detection and quantification of therapeutic antibodies. While these assays are highly sensitive and specific, they are also susceptible to interference and cross-reactivity, posing challenges in ensuring their accuracy and reliability in clinical setting. They have also gained prominence with the advent of personalized medicine.<sup>12</sup>

In short, the integration of drug testing in drug standardization presents a multi-faceted approach. When harmonized, it can lead to more robust and reliable therapeutic products. However, the challenges associated with each method underscore the need for continuous innovation. As we look to the future, it is imperative that the researchers, regulatory bodies, and industry stakeholders should collaborate to enhance these methods, address their limitations, and develop new strategies for standardization.

Ultimately, the goal remains the same: to ensure that every patient receives a safe and effective medicine,

regardless of where or how it is manufactured. It will continue to evolve as the drug development becomes more complex, and it is our responsibility to guide this evolution towards standards of quality and safety.

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## ORIGINAL ARTICLE

## Seroprevalence of Varicella-Zoster Virus in Females of Childbearing age in Rawalpindi

Muhammad Ali Rathore<sup>1</sup>, Amna Kanwal<sup>2</sup>, Ejaz Ghani<sup>3</sup>, Hina Saeed<sup>4</sup>, Khushbakht Alam<sup>5</sup>, Faraz Ahmed<sup>6</sup>

## ABSTRACT

**Objective:** To determine the seroprevalence of Varicella-zoster virus (VZV) in females of childbearing age.**Study Design:** Cross-sectional study.**Place and Duration of Study:** Virology Department, tertiary care hospital, Rawalpindi, from August 2022 to December 2022.**Materials and Methods:** A total of 200 females, aged 15-49 years, were included in the study. Data of the participants regarding age, socioeconomic status, marital status, and previous history of VZV infection was collected by a predesigned questionnaire. Blood samples were tested for the detection of VZV IgG by Enzyme Linked Immunosorbent Assay (ELISA) of Ratio Diagnostic, Germany. SPSS version 25 was used to analyze the data.**Results:** Out of total 200 samples, 163 (81.5%) were positive for VZV IgG and 37 (18.5%) were negative. The positivity of VZV IgG was highest in age group 2 (20-29 years) with 87 (43.5%) positive cases, followed by 54 (27%) in age group 3 (30-39 years) and 11 (5.5%) in both age group 1 (< 20 years) and age group 4 (40-49 years). Out of 200 participants, 158 (79.0%) were married and 42 (21.0%) were unmarried. Among total, 107 (53.5%) had history of previous VZV infection while 93 (46.5%) had no significant history.**Conclusions:** This study showed significant exposure of VZV in females of childbearing age and subsequent development of immunity. However, a substantial proportion of females were susceptible to VZV infection. This susceptibility in females could potentially pose a future risk of infection during pregnancy, resulting in severe complications and congenital abnormalities.**Key Words:** Childbearing Age, Seroprevalence, Varicella Zoster Virus IgG, Varicella-zoster virus Infection

## Introduction

Varicella-zoster virus (VZV) is HHV-3 and belongs to Herpesviridae family. It is a highly contagious pathogen and is distributed worldwide. Primary VZV infection causes vesicular rash of chicken pox (varicella), while reactivation of latent VZV typically results in localized skin lesions known as shingles (zoster).<sup>1</sup> VZV spreads via inhalation of respiratory droplets, however transmission can also occur through direct contact with infectious vesicular fluid.<sup>2</sup> In temperate regions, VZV primarily affects children, whereas in tropical areas, adults are more commonly affected.<sup>3</sup> While the infection is often benign in children, it may have severe consequences when contracted during pregnancy, posing a

significant risk to both the mother and fetus.<sup>4</sup>

Globally, over 4.2 million cases of VZV occur each year, resulting in 4200 deaths annually. The incidence of VZV infection during pregnancy is 1 to 5 cases in 10,000 in United States.<sup>6</sup> This low incidence is attributed to high VZV seroprevalence (up to 90%) in developed countries.<sup>7</sup> However seroprevalence rates are lower in developing countries, at about 40%. Fewer than 2 % of women who acquire infection in pregnancy give birth to neonates with congenital varicella syndrome.<sup>8</sup>

VZV infection during pregnancy poses significant risks for the expectant mother, especially an increased likelihood of developing pneumonia. Additionally, there is a risk of transmitting the virus to both the developing fetus and the newborns. The risk of development of congenital varicella syndrome is higher if mother gets infection during first 8 to 20 weeks of pregnancy.<sup>9</sup> Congenital varicella syndrome is characterized by scarring of skin, neurological abnormalities, structural eye damage, hypoplasia of limbs, gastrointestinal abnormalities or low birth weight.<sup>10</sup> If VZV infection develops during five days

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before and two days after delivery, the baby might develop potentially life-threatening infection called neonatal varicella.<sup>11</sup> Early onset of zoster in children is also linked to maternal VZV infection during pregnancy.<sup>12</sup>

Infection with VZV leads to the development of antibody response. VZV IgM appears 2 to 5 days after rash appears and remains detectable for 10 weeks.<sup>13</sup> VZV IgG antibody appears early in infection and levels progressively decrease in a few years.<sup>14</sup> Definitive diagnosis of varicella infection is carried out by PCR testing of skin lesion specimens.<sup>15</sup>

There is an overall change in epidemiology of VZV infection after the introduction of varicella vaccine since 1995.<sup>16</sup> Pakistan, like many developing nations, has experienced advances in healthcare infrastructure and vaccine availability in recent years. These factors, coupled with variations in cultural practices and disease exposure, make this study essential to understand the trends in seroprevalence of VZV. Our study will contribute by providing sufficient base line local data for further studies to be carried out in Pakistan. It will also help to implement infection control measures and take proactive steps towards the well-being of expectant mothers and their offspring. So a research was planned to determine the seroprevalence of Varicella-zoster virus (VZV) in females of childbearing age.

## Materials and Methods

After taking approval from institutional review board (BS AHS/VIR-4/READ-IRB/21/924), a cross-sectional study was conducted at department of Virology, tertiary care hospital, from August 2022 to December 2022. By using WHO Calculator taking population proportion 41%, confidence interval 95% and margin of error 5%, the sample size of 200 was calculated.<sup>16</sup> The females between the age 15-49 years (reproductive age specified by WHO) were included in the study. The women with underlying comorbidities or immunocompromised state were excluded from the study. About 3mL of serum sample was collected from each participant by non-probability consecutive sampling. Informed consent was taken from all participants and a questionnaire was used to collect demographic data. The variables included in the data were age, marital status, socio-economic status, history of VZV vaccination and

comorbidities. The indirect ELISA was performed on serum samples for detection of VZV IgG by using Ratio Diagnostic Kit. The tests were performed according to the manufacturer's instructions. The antibody index was calculated for each sample and the values > 1.1 were considered positive. Data was analyzed using Statistical Package of Social Sciences (SPSS) version 25.0. Descriptive statistics like frequencies and percentages were calculated for qualitative variables (marital status, socio-economic status, and history of chickenpox infection, while quantitative variables (age) were expressed as mean and standard deviation.

## Results

A total of 200 females were included in this study for the detection of VZV IgG by ELISA method. Out of 200 participants, 163 (81.5%) were positive for VZV IgG and 37(18.5%) were negative. The participants were divided into four age groups. The positivity for VZV IgG was highest in age group 2 (20-29 years) with 87 (43.5%) positive samples, followed by 54 (27%) in age group 3 (30-39 years) and 11(5.5%) in both age group 1 (< 20 years) and group 4 (40-49 years), as shown in figure-I.

Out of 200 participants, 107 (53.5%) had a previous history of VZV infection while 93

(46.5%) did not give significant history. About 97% of the participants with previous history of VZV infection were positive for VZV IgG, however 63% had positive VZV IgG with no history of previous infection, as shown in figure-II. Among total, 158 (79.0%) were married and 42 (21.0%) were unmarried. Seropositivity was high (49.5%) among females living in joint family system and low (32%) in females not living in joint family system.

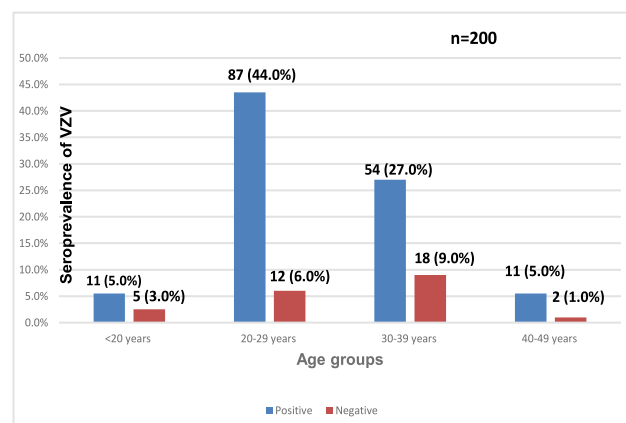
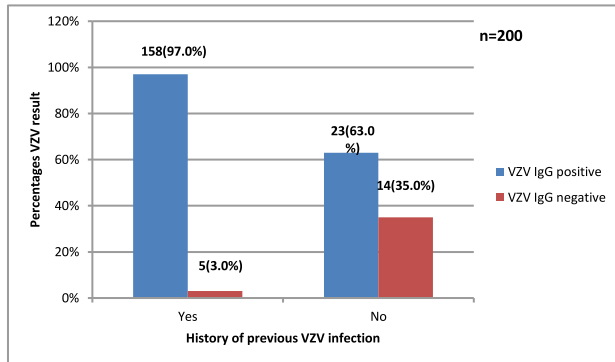


Fig 1: VZV IgG Seroprevalence in Different Age Groups





**Fig 2: History of Previous VZV Infection**

## Discussion

Varicella-zoster virus infection poses a considerable worldwide health challenge, carrying significant consequences, especially in terms of reproductive health and its potentially severe impact on fetus. Gaining insights into the prevalence of VZV is of paramount importance for formulating impactful healthcare strategies in a country like Pakistan. The prevalence of VZV has exhibited an upward trend in recent years, a phenomenon attributed to the introduction of the varicella vaccine.

In this study, seroprevalence of VZV IgG in women of childbearing age was determined. Of total 200 samples, VZV IgG was positive in 163 (81.5%) samples; however, 37 (18.5%) samples were negative. Notably, the highest prevalence was observed in age group 2 (20-29 years) with 87 (43.5%) cases of VZV IgG positivity, followed by 54 cases (27%) in age group 3 (30-39 years) and 11 cases (5.5%) in both age group 1 (<20 years) and age group 4 (40-49 years). Among total, 107 (53.5%) had previous history of VZV infection while 93 (46.5%) did not give significant history.

A study conducted in Pakistan by Naseem *et al.*<sup>9</sup> in 2022, showed that the seroprevalence of VZV IgG was higher (45.2%) in females as compared to males (39.6%). Moreover, it was observed that seroprevalence increased with age of the individuals; with highest seroprevalence of 53.6% in age group 21-30 years. The results of this study were comparable to our study.

A cross-sectional study was carried out in 2016 in Iran by Majidy *et al.*<sup>17</sup> This study reported the seroprevalence of 71.2% for VZV IgG which showed that majority of women had been exposed to the VZV at some point before the study, either through contracting infection or through varicella

vaccination. The outcomes of our study were consistent with this study.

Another study was conducted in 2019 on seroprevalence of VZV IgG among pregnant females in Egypt. They found seropositivity of 88.3% in pregnant females. This study also observed a trend that VZV seroprevalence increased with increasing age of the women. This higher seroprevalence could be due to increased varicella vaccination or previous exposure.<sup>6</sup>

A study conducted by Daulagala and his colleagues in Sri Lanka, found that the seroprevalence of VZV IgG was 77.9% in females. Notably, the highest prevalence (85%) was observed within the age range of 31 to 35 years, consistent with our study. Additionally, a direct relationship was noted between woman's age and the prevalence of VZV IgG. This study also had similar findings as in our study.<sup>18,19</sup>

A review article showed the prevalence of VZV IgG among populations of Gulf countries in 2023. The findings from these studies indicated a wide variation in the VZV seroprevalence, ranging from as low as 15% to as high as 92%. This study reflected a diverse range of immunity levels to VZV within these countries, due to differences in vaccination rates, natural exposure, and other contributing factors.<sup>14</sup>

Another study was carried out in India to assess the prevalence of VZV IgG. Among the entire cohort, 49.9% exhibited seropositivity, and approximately 29% reported a prior history of VZV infection.<sup>5</sup>

The present study demonstrated a rise in the prevalence of VZV IgG within the female population as compared to a previous study conducted in 1999. Since that no similar investigation has been carried out in Pakistan, thereby emphasizing the value of this study in addressing a gap in research. Furthermore, our findings were in alignment with outcomes from other tropical countries, establishing a level of comparability between our results and those of similar studies conducted in those regions.

## Limitations of the study

This study included a relatively small sample size and population of northern Pakistan which may limit the generalizability of the findings to other regions. The assessment of previous VZV infection was based on self-reported history, which might be subject to recall bias. Additionally, the unequal distribution of

participants across age groups may impact the robustness of conclusions.

## Conclusion

This study found significant seroprevalence of VZV in our female population of childbearing age. However, a notable percentage of females remained susceptible to VZV. These susceptible females were at risk of VZV infection during pregnancy in future, potentially leading to fetal abnormalities and severe complications in newborns.

**Conflict of interest:** None

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**CONFLICT OF INTEREST**

Authors declared no conflicts of Interest.

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**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

# A Comparative Study on Benign and Premalignant Histological Changes Induced by E. Coli and K. Pneumoniae in Gall Bladder Mucosa Having Pigment Stones

Saif Abbas<sup>1</sup>, Khalida Moeed<sup>2</sup>, Fauzia Siraj<sup>3</sup>, Hammad Waseem<sup>4</sup>

## ABSTRACT

**Objective:** To observe and compare benign and premalignant histological changes induced by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in gall bladder mucosa having Pigment stones.

**Study Design:** Cross sectional comparative study.

**Place and Duration of Study:** This study was conducted at the department of Anatomy in collaboration with the department of Surgery, Al Nafees Medical College and Hospital (ANMC) from 14<sup>th</sup> November 2017 to 18<sup>th</sup> December 2019.

**Materials and Methods:** Out of total cholecystectomy specimens collected during study duration, only thirty gallbladder specimens having Pigment stones and bile culture positive for *E. coli* or *K. pneumoniae* were obtained. They were divided into two groups comprising fifteen specimens each (n=15). Group 1 had bile culture positive for *E. coli*, while group 2 was positive for *K. pneumoniae*. All specimens were processed in automated tissue processor after tissue processing. Paraffin embedded sections were stained with Haematoxylin and Eosin stain. Slides were examined by consultant pathologist under light microscope. Premalignant histological lesions included hyperplasia, metaplasia and dysplasia. Chi square was applied to compare the statistical association of histological lesions between group 1 and 2.

**Results:** Histopathological analysis revealed that hyperplasia, metaplasia and dysplasia in group 1 and group 2 were found to be statistically insignificant with p values of p= 0.715, p= 0.464 and p= 0.1 respectively.

**Conclusion:** The histological patterns of benign and premalignant findings in both group 1 and group 2 showed minimal variation. Although this study did not observe any progression of premalignant lesions to carcinoma, the eradication of bacteria remains crucial to prevent the development of histopathological lesions in the gallbladder.

**Key Words:** *Dysplasia, E. Coli, Hyperplasia, K. Pneumoniae, Metaplasia and Gallbladder.*

## Introduction

Gall bladder is an organ connected to the hepatobiliary system. Simple columnar epithelium lines its perimeter.<sup>1</sup> The lamina propria and muscularis externa are located underneath the epithelium. Submucosa and muscularis mucosae are absent. The histopathological diseases found in

gallbladder range from cholelithiasis on one side of spectrum and carcinoma on another end. Its malignancy is always fatal.<sup>2</sup> Gall bladder cancer is the most common cancer of biliary tract and fifth most common cancer among gastrointestinal tract related cancers.<sup>3</sup> It is ranked 21<sup>st</sup> most common cancer worldwide in terms of mortality. On gender basis, females are three time more affected by gallbladder cancer than male. Both benign and malignant diseases of gallbladder may have bacterial infections as an underlying cause.<sup>4</sup> Chronic bacterial infections, polyps, and other conditions are among well-established risk factors for gallbladder cancer.<sup>5</sup> The frequent bouts of gallbladder epithelial injury and healing encourage a backdrop of chronic inflammation that leads to increasing morphological abnormalities through a sequential cascade of metastasis-dysplasia till carcinogenesis.<sup>6</sup> *Helicobacter* and *Salmonella* species are established risk factors for gastric cancer and gallbladder

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dysplasia.<sup>5</sup> Certain virulent strains *E. coli* and *Klebsiella* are currently being investigated for their role in colorectal cancer. *E. coli* and *Klebsiella* species along with *Enterobacter* and *Enterococcus* are frequently found in bile cultured from gallbladder specimens having cholelithiasis and cancer but their association with histopathological lesions is yet not clear.<sup>7</sup> *E. coli* and *Klebsiella* have a tendency to alter the normal histology of the gallbladder by producing beta glucuronidase and phospholipase. They encourage the formation of gallstones and their presence in bile may result in premalignant changes in the gallbladder.<sup>8</sup> The objective of this study was to compare benign and premalignant histological changes induced by *E. coli* and *K. pneumoniae* species in gall bladder mucosa, having Pigment stones. Gallbladder cancer is complex disease with poor prognosis and worst outcomes. If bacterial toxins produced by chronic bacterial infection are responsible for carcinogenesis, eradication of causative microbes by antibiotic therapy is essential in order to prevent chronic illnesses and patient health care.<sup>9</sup> So, the rationale was to identify the bacterial risk factors linked to gallbladder cancer before creating preventive and therapeutic strategies.

## Materials and Methods

It was cross sectional comparative study conducted at the department of Anatomy in collaboration with the department of Surgery at ANMC and hospital from 14th November 2017 to 18th Dec, 2019. A total of thirty(n=30) gallbladder specimens were selected out of cholecystectomy specimens collected during study duration based on selection criteria of pigment stones and bile culture positive for *E. coli* or *K. pneumoniae*. The sampling technique was convenient sampling. The study protocol was approved by Ethical Review Committee Board (ERCB) vide their letter dated F.2/IUIC-ANMC/EC-94/2015. The inclusion criteria were based upon cholecystectomy specimens having pigment stones and bile culture positive for *E. coli* or *K. pneumoniae*. Patients of male and female genders with mean age of 44±5 were selected for study purpose with informed consent. Patients diagnosed with biliary malignancy, prior biliary surgical procedures and gallbladder specimens with cholesterol stones were excluded from studies. 4-5 ml of bile was aspirated

from each gallbladder sample by sterile syringes. Bile was subjected to gram staining procedure, bile culture and Analytical Profile Index (API 10s) to identify gram negative bacteria i.e. *E. coli* or *K. pneumoniae*. Thirty gallbladder specimens having Pigment stones were divided into two groups. Gallbladder specimens with Pigment stones were collected from patients after cholecystectomy having concomitant biliary infection with *E. coli* were included in Group 1 (n=15). Gallbladder specimens with Pigment stones were collected from patients after cholecystectomy having concomitant biliary infection with *K. pneumoniae* species. Were included in Group 2 (n=15). Longitudinal sections of gall bladder tissue were removed from body and fundus of gallbladder and fixed in 10% formalin for further tissue processing. Haematoxylin and Eosin stain (H & E) were used to study benign and premalignant histological lesions and dysplasia of gallbladder specimens in both group 1 and 2 under light microscope. Benign histological lesions observed were Hyperplasia (Increased number of cell production in normal tissue). Diffuse or local hyperplasia was noted in any gallbladder tissue section. Premalignant histological lesions observed were Metaplasia (conversion of one cell type into another) and Dysplasia (Presence of abnormal cells within a tissue). Pyloric (resembling pyloric glands) or intestinal metaplasia (showing goblet cells) was observed in each gallbladder tissue section. Low grade dysplasia showing stratification of epithelial cells and hyperchromatic nuclei was also noticed. High grade dysplasia showed marked cellular disorganization, pleomorphism, loss of polarity and increased mitosis of epithelium under light microscope. Both Benign and premalignant histological lesions of gallbladder compromise spectrum of chronic cholecystitis and gallbladder cancer.<sup>10</sup> The benign and premalignant histological lesions like hyperplasia, metaplasia and dysplasia in groups 1 and 2 were observed and compared with each other. The chi-square test or Fisher's exact test for categorical variables in group 1 and 2. A *p* value of <0.05 was considered statistically significant. All statistical analyses were performed by using SPSS version 22.

## Results

Hyperplasia whether diffuse or focal was observed in

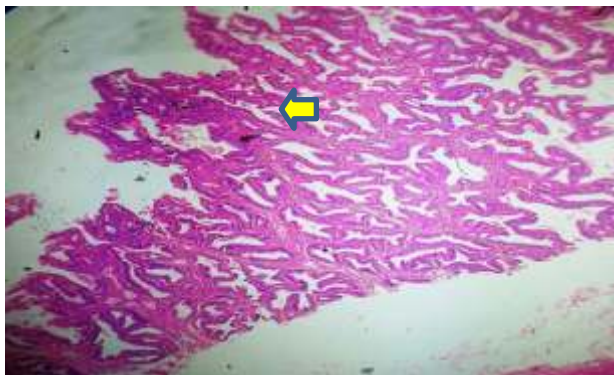
gallbladder tissue under high power lens (10x40). Hyperplasia was present in 8 out of 15 (53%) percent of gallbladder specimens in Group 1. Hyperplasia was present in 7 out of 15(47%) percent of gallbladder specimens in group 2. Metaplasia was observed in gallbladder tissue under high power lens (10x40). Metaplasia was present in 9 out of 15(60%) percent of gallbladder specimens in group 1. Metaplasia was present in 47% (7 out of 15) percent of gallbladder specimens in group 2. Dysplasia was observed in gallbladder tissue under high power lens (10x40). Low grade focal dysplasia was present in 1 out of 15 (7%) of gallbladder specimens in group 1. Low grade focal dysplasia was present in 7% (1 out of 15(7%) of gallbladder specimens in group 2. There was no statistically significant association between benign and premalignant histological lesions of group 1 and 2.

**Table I: Demographic Data**

	<b>E. coli (Group 1)</b>	<b>K. pneumoniae (Group 2)</b>
Number of Cases (n)	15	15
Age (mean in years)	44 $\pm$ 5	44 $\pm$ 5
Gender Male	5	4
Gender Female	10	11

**Table II: Premalignant Histological Lesions Observed in Gall Bladder Specimen of Group 1(E. Coli) and Group 2 (K. Pneumoniae)**

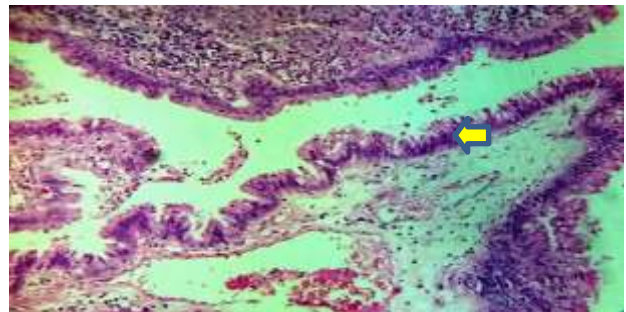
	Group I		Group II		p-value
	Present	Absent	Present	Absent	
<b>Hyperplasia</b>	53% (8)	47% (7)	47% (7)	53% (8)	.715
<b>Metaplasia</b>	60% (9)	40% (6)	47% (7)	53% (8)	.464
<b>Dysplasia</b>	7% (1)	93% (14)	7% (1)	93% (14)	0.1



**Figure 1: Photomicrograph Showing Gallbladder Specimen Having Hyperplasia (H&E stain) (Specimen No.6 of Group 2) (10x)**



**Figure 2: Photomicrographs Showing Gallbladder Specimen Having Metaplasia (H&E Stain). (Specimen No.5 of Group 1 (10x)**



**Figure 3: Photomicrographs Showing Low Grade Dysplasia in Gall Bladder (H&E stain ,10x)**

## Discussion

*E. coli* and *K. pneumoniae* were the most common bacteria in bile cultured from cholecystectomy specimens. Numerous regional and international studies support our findings that *E. coli* and *K. pneumoniae* are the most common bacteria isolated from bile.<sup>11</sup> Biliary bacteria in more predominantly present in gallbladder having pigment stones.<sup>12,13</sup> Hyperplasia was present in higher proportion i.e. 53% in group 1 gallbladders infected with *E. coli* but was statistically insignificant in both groups 1 and 2. Similarly, metaplasia was found in higher percentage i.e.60% in group 1 but was statistically insignificant in both groups. Low grade dysplasia was found only in single specimens of group 1 and 2.

Hyperplasia is a benign lesion frequently found in cholecystectomy specimens. It can be local, segmental, diffuse or annular. Adenomyomatous hyperplasia of the gallbladder is a benign condition characterized by hyperplasia of the gallbladder wall mucosa and muscularis propria with hallmark epithelial invaginations forming cystic pockets known as Rokitansky-Aschoff sinuses. This peculiar type of hyperplasia can mimic gallbladder cancer and present diagnostic challenge for health providers.



Hyperplasia is frequently in chronic inflammations of gallbladders.<sup>14,15</sup>

Metaplasia of both types i.e. pyloric and intestinal is found in histopathological lesions of chronic cholecystitis.<sup>16</sup> Pyloric gland metaplasia is found in association with bacterial infections, old age and gallstones. Intestinal metaplasia is less common of both types but with increased risk association for gallbladder cancer.<sup>17</sup> Bacterial infections with chronic cycle of inflammation and repair tend to increase metaplasia of gallbladder.<sup>18,19</sup>

Dysplasia is the second last step in development of gallbladder cancer following metaplasia.<sup>20</sup> Depending on other factors, whether gallbladder cancer eventually develops or not, dysplasia is incidental finding in bacterial cholecystitis. Low grade dysplasia is usually focal. High grade dysplasia is significantly related to adenocarcinoma of gallbladder.

Bacterial role has been implicated in many histopathological lesions of gastrointestinal tract leading to cancers.<sup>21,22</sup> *H. pylori* is a well-known and well-established risk factor for gastric cancers. It is responsible for the production of toxins, reactive oxygen species and inflammatory gastritis. Each component directly or indirectly contributes to altering normal host cellular pathways.<sup>23</sup> *Salmonella* species was recently declared as risk factor for gallbladder cancer by researchers. In chronic stages of cholecystitis, it releases toxins responsible for damaging DNA of host cells.<sup>24</sup> Similarly, *Chlamydia* infection is considered as risk factor for lung cancer.<sup>24</sup> *Fusobacterium* species is considered carcinogenic bacteria for colorectal cancers.<sup>24</sup> Certain pathogenic of *E. coli* modify host cell immune mechanism by virulence factors and inflammatory pathways in colorectal cancers. They can lead to altered cellular growth.<sup>25</sup> This role of bacterial infections in gastrointestinal diseases prompted our interest to explore the role of *E. coli* and *K. pneumoniae* infected bile and histopathological lesions of gallbladder in their presence.

In our study, the mean age of patients was  $44 \pm 5$  years. Hyperplasia was more prevalent in Group 1 but not statistically significant. Supporting are findings are observation of hyperplasia in *Pylori* infected bile albeit to a lesser extent.<sup>26</sup> As comparison, one more study found hyperplasia to a

lesser percentage in *H. pylori* bile positive samples.<sup>27</sup> One Indian study found hyperplasia to be prevalent in majority of cases.<sup>28</sup> One study demonstrated statistical association between degree of fibrosis and *H. pylori*.<sup>29</sup> This difference of more prevalence of hyperplasia in our study might be due to chronic stage of cholecystitis.

In our study metaplasia was more in group 1 as compared to group 2. Both of them were statistically not significant. In both groups, metaplasia was found in higher percentage overall as compared to one study conducted in Pakistan.<sup>27</sup> The study conducted in Pakistan only showed metaplasia only in 3% of *H. Pylori* positive gallbladder specimens. One study in India showed no metaplasia in *H. Pylori* infected gallbladder.<sup>29</sup> One study showed both pyloric and intestinal metaplasia to larger percentage in *H. pylori* infected gallbladder specimens supporting our findings.<sup>30</sup> Differences of metaplasia proportion in our study and other studies might be due age, sex and chronic phase of cholecystitis.

In our study, low grade focal dysplasia was present in single specimen both groups 1 and 2 but statistically insignificant. Dysplasia being incidental finding was not reported in one study conducted in Pakistan<sup>27</sup> and other in India.<sup>29</sup> One study conducted in Turkey found low grade dysplasia in 2% of gallbladder specimens.<sup>30</sup> In our study, although statistically insignificant but benign and premalignant histopathological lesions are observed in gallbladder having Pigment stones and bile infected with *E. coli* or *K. pneumoniae*. Exact pathological mechanisms and virulence factors of *E. coli* and *K. pneumoniae* contributing to gallbladder lesions is poorly understood due to paucity in research. They have carcinogenic potential for premalignant lesions in gallbladder like salmonella is not known till date. Our limitations of research study are primarily due to fact that it is observational study. A definite link from premalignant lesion to dysplasia cannot be established only with bile having *E. coli* or *Klebsiella* species. Molecular, host cell genetics and virulence factors of pathogenic strains of *E. coli* and *Klebsiella* need to be studied and explored on research basis to assess their risk.

## Conclusion

The histological patterns of benign and premalignant findings in both group 1 and group 2 showed minimal variation. Although this study did not observe any

progression of premalignant lesions to carcinoma, the eradication of bacteria remains crucial to prevent the development of histopathological lesions in the gallbladder.

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#### CONFLICT OF INTEREST

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

## Prevalence of Dysmenorrhea and Predictors of Its Pain Intensity Among Female Medical Students: A Cross Sectional Study

Barira Afzal<sup>1</sup>, Saliha Hussain<sup>2</sup>, Sanabil Gul<sup>3</sup>, Sidra Hamid<sup>4</sup>

## ABSTRACT

**Objective:** The objective of this study is to determine the prevalence of dysmenorrhea and to assess the predictors of pain intensity during dysmenorrhea among female medical students at Rawalpindi Medical University.

**Study Design:** Descriptive Cross-sectional study

**Place and Duration of Study:** The study was conducted from May 2023 to October 2023 at Rawalpindi Medical University.

**Materials and Methods:** A validated, self-structured and self-administered 24 items questionnaire regarding dysmenorrhea was used to assess the prevalence and pain intensity of dysmenorrhea focused on 277 undergraduate medical students, excluding those with any pre-existing pelvic pathology. Participants were selected through convenience sampling and data was analyzed using SPSS version 26.

**Results:** The study involved 277 female medical students, showing an 83.2% dysmenorrhea prevalence. Based on visual analog scale, most of the research participants experienced moderate to severe pain (49.6%), along with symptoms like depressed mood (50.7%) and mood swings (51.4%). Common outcomes were limited daily activities (57.5%) and emotional instability (55%). Heavy bleeding ( $p=0.041$ ,  $OR=3.56$ ) and stress ( $p=0.041$ ,  $OR=2.108$ ) were significant predictors. BMI and caffeine consumption showed no significant improvement, but the pain's duration during periods significantly affected pain scale scores, with the "more than 3 days" group reporting the most severe pain.

**Conclusion:** Dysmenorrhea is widespread among female medical students, causing substantial disruptions in their daily routines. This condition is closely linked to heavy bleeding and stress, highlighting the importance of addressing these factors. Understanding the timing and duration of pain during the menstrual cycle is crucial for developing effective treatment strategies.

**Key Words:** Dysmenorrhea, Education, Medical Students, Prevalence, Predictors.

## Introduction

Primary dysmenorrhea, a condition characterized by painful menstruation in the absence of pelvic disease, is one of the most common gynecological concerns globally.<sup>1</sup> In a comprehensive systematic review and meta-analysis encompassing 38 studies with the participation of 21,537 women, the prevalence of dysmenorrhea was identified to be notably prevalent, affecting 71.1% of young

females.<sup>2</sup> This discomfort can be categorized into two distinct forms: primary dysmenorrhea, characterized by lower abdominal pain during menstruation without any concurrent pelvic pathology, and secondary dysmenorrhea, which associates with various pelvic and extra-uterine pathologies.<sup>3</sup>

Among young females, primary dysmenorrhea's major risk factors include the presence of heavy menstrual flow, a family history of dysmenorrhea, and endeavors at weight loss.<sup>4</sup> Furthermore, a meta-analysis identified additional risk factors such as poor sleep quality, skipping breakfast, elevated stress levels, and a lack of physical exercise.<sup>5</sup> The repercussions of dysmenorrhea extend beyond mere physical discomfort; it exerts a detrimental influence on the overall quality of life for affected individuals, impacting their physical, mental, emotional well-being, and social relationships.<sup>6</sup> Women with dysmenorrhea have increased

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sensitivity to pain, even outside of menstruation, which may lead to a higher risk of chronic pain conditions like fibromyalgia. Dysmenorrhea significantly impacts quality of life, mood, and sleep, but can be effectively managed with non-steroidal anti-inflammatory drugs (NSAIDs) as first-line therapy.<sup>7</sup>

Particularly noteworthy is the high prevalence of dysmenorrhea among university students<sup>8, 9</sup>, significantly impairing their daily activities and occasionally leading to school absenteeism. Notably, medical students report the highest incidence of dysmenorrhea-related distress<sup>10</sup>, making it one of the primary causes of psychological distress among female medical students.<sup>11</sup>

However, the existing body of research on this subject within the context of medical students in Pakistan is notably limited, with no specific studies conducted in the Rawalpindi region. Therefore, there is a pressing need to examine the prevalence of dysmenorrhea and its associated predictors concerning pain intensity among the student population at Rawalpindi Medical University, Rawalpindi, Pakistan. This research endeavor aimed to fill this critical gap in our understanding of the condition's impact and characteristics among this specific demographic.

The rationale of this study was to increase awareness that dysmenorrhea is a medical condition necessitating appropriate medical care, despite common misconceptions and cultural norms. This would enable medical students to address it effectively, leading to enhancements in individuals' psychological, social, mental, and physical well-being, as well as their overall quality of life. Objectives of this study was to determine the prevalence of dysmenorrhea and to assess the predictors of pain intensity and exploration of other factors contributing to dysmenorrhea among female medical students of RMU from first year to final year MBBS.

## Materials and Methods

This research was a descriptive cross-sectional study that utilized a questionnaire-based approach and spanned a period of six months, from May 2023 and to October 2023. The study focused on a cohort comprising 277 undergraduate female medical students studying in Rawalpindi Medical University.

Individuals with any pre-existing pelvic pathology were excluded from the study in accordance with our exclusion criteria as they might experience secondary dysmenorrhea. Prior to embarking on this study, we sought and successfully obtained ethical approval Ref no.424/IREF/RMU/2023 from the esteemed Ethical Review Board at Rawalpindi Medical University, situated in Rawalpindi, Pakistan<sup>12</sup>. Additionally, our research adhered to ethical standards by obtaining informed consent from each of the participating students.

To gauge the prevalence of dysmenorrhea among the female medical students of Rawalpindi Medical University, we employed a self-administered questionnaire containing a comprehensive set of 24 items specifically designed for this purpose. The questionnaire underwent a thorough validity check process including a comprehensive review by an expert in the field, as well as a preliminary assessment involving a sample of 20 students. Hence the pilot study was successfully conducted, and appropriate modifications were incorporated into the questionnaire to enhance its accuracy and relevance, based on the feedback received during this validation phase.

For the purpose of participant selection, we employed a convenience sampling technique, which allowed us to conveniently approach and engage with the target population, given the practical constraints and available resources. The sample size was calculated to be 277, using the EPI info software with 95% confidence interval, margin of error 5%, expected frequency 50% and population size considered to be the total female medical students of RMU. Those students with any diagnosed pelvic pathology were excluded from the study. The questionnaires were administered by our team of investigators directly to the study participants. Written informed consent was taken from all participants. To streamline the process and ensure data accuracy, questionnaires were collected on the same day they were distributed.

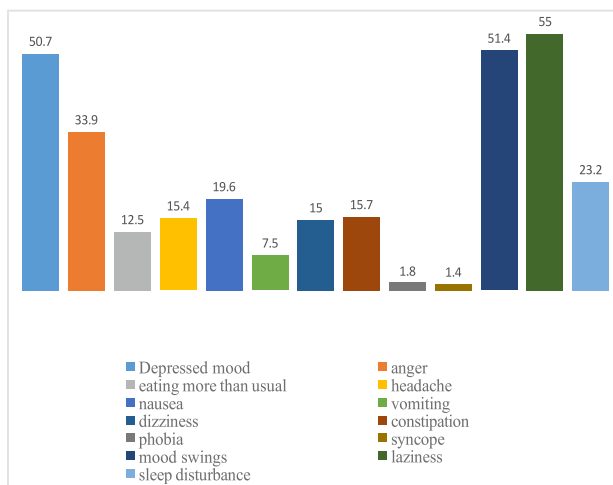
Upon collection, the data from the completed questionnaires were entered into a Microsoft Excel spreadsheet. Subsequently, data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26. The data obtained was described through frequencies and percentages and

further analyzed by applying statistical tests like chi square test, one-way ANOVA and logistic regression. Confidence interval was kept at 95% and p-value was set at 0.05.

## Results

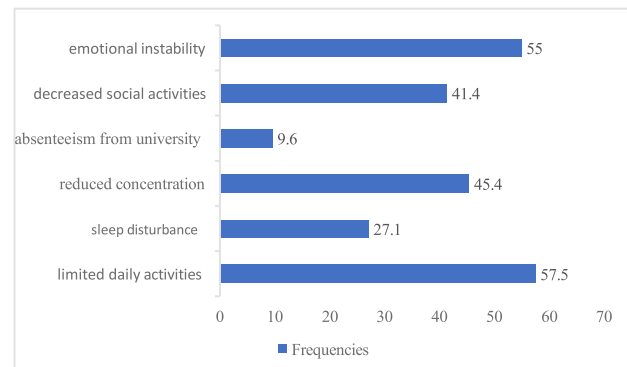
The study included 277 female medical students and prevalence of dysmenorrhea was found to be 83.2% (N=230). All of the participants of the study were aged between 18-26 years with 54.5 percent of the participants aged 21-23 years. The mean age of participants was 20.8± 1.5 years. The participants were from all years of study from first to final year (fifth year) of medical school as per convenience sampling. Among these, the majority of participants, 49.6% (137), reported experiencing "moderate to severe (4- 6)" pain. Following this, 27.5% (76) of respondents reported "mild pain (1-3)," while 12.0% (33) experienced "very severe (7-9)" pain. A smaller proportion, 7.6% (21), reported "No Pain (0)," and only 3.3% (9) described their pain as the "Worst Pain Ever (10)." These findings suggest that, within this population, the prevalence of moderate to severe pain is notably higher than mild or very severe pain. This categorization of pain intensity was done by employing the visual analogue scale in the questionnaire.

The majority of participants reported experiencing "depressed mood" (50.7%, N=140) and "mood swings" (51.4%, N=142) as common symptoms associated with primary dysmenorrhea. Besides these, sleep disturbance was also found in 23.2% (64) of participants. A summary of symptoms is presented in the figure below.



**Fig 1: Frequencies of Symptoms (N=277)**

"limited daily activities" (57.5%, N=159) and "emotional instability" (55%, N=152) were the most frequently reported outcomes related to primary dysmenorrhea among the participants.



**Fig 2: Frequencies of Outcomes (N=277)**

The prevalence of dysmenorrhea was found to be higher (94.1%, N=260) among students who experienced heavy bleeding (more than 3 towels per day). A statistically significant relation was found ( $p=0.041$ ) and females who suffered from heavy bleeding had more than 3 times ( $OR=3.5$ ) risk of having dysmenorrhea as compared to others. Similarly, a significant relation was found between stress and dysmenorrhea ( $p=0.041$ ) and students who had stress were found to have greater prevalence (51.1%, N=141) of dysmenorrhea as compared to others (34.1%, N=94). Other factors such as duration of cycle, days of menstruation, age of menarche, family history, and physical exercise were found to be statistically insignificant ( $p \geq 0.05$ ). However, a higher prevalence (85.7%, N=237) was found among participants who don't do physical exercise regularly. Following table shows a summary of predictors:

According to the results of logistic regression model, heavy periods ( $p$  value=0.041,  $OR=3.5646$ ) and stress ( $p$  value=0.041,  $OR= 2.108$ ) were significant predictors of dysmenorrhea.

The relationship between pain scale and BMI based on ANOVA test showed that the mean pain scale scores for "underweight" and "overweight and obese" groups were similar (2.82), while the "normal" group had a slightly lower mean (2.72). The  $p$  value was found to be 0.677 so there were no significant differences in pain intensity scores among the three BMI groups.

The mean pain scale score for each group showing

**Table I: Association Between Dysmenorrhea and Various Pain Related Factors in Female Medical Students of RMU**

Dysmenorrhea	Yes	%	No	%	P	OR	95% C.I.
<b>Variables</b>							
<b>Amount of bleeding</b>					0.041	3.546	1.0353-11.945
Less than 3 towels	185	81.9	41	18.1			
More than 3 towels	48	94.1	3	5.9			
<b>Stress</b>					0.041	2.018	1.028-3.96
Yes	119	51.10%	114	48.90 %			
No	15	34.10%	29	65.90 %			
<b>Regularity of Cycle</b>					0.075	0.495	0.228-1.075
Yes	200	85.8	33	14.2			
No	33	75	11	25			
<b>Physical Exercise</b>					0.093	1.99	0.894-4.4
Yes	30	75	10	25			
No	203	85.7	34	14.3			
					0.851	0.929	0.430-2.009
<b>Day Scholar</b>	183	84.3	34	15.7			
<b>Hostelite</b>	50	83.3	10	16.7			
<b>Days of Menstruation</b>					0.184	0.626	0.314-1.25
Less than 5 days	57	79.2	15	20.8			
More than 5 days	166	80.9	29	15.3			
<b>Duration of cycle</b>							
Less than 21 days	20	90.9	2	9.1	0.671		
More than 21 days	213	83.5	42	16.5	0.452	2	0.328-12.184
<b>Age of Menarche</b>					0.635	1.173	0.608-2.27
Before 12 or at 12	99	85.3	17	14.7			
After 12	134	83.2	27	16.8			
<b>Family History</b>					0.772	0.901	0.446-1.82
Yes	159	83.7	31	16.3			
No	74	85.1	13	14.9			

**Table II: Logistic Regression Analysis of Dysmenorrhea with Stress and Heavy Bleeding**

Predictor	B	Sig.	AOR (adjusted odds ratio)	95% C.I (confidence interval)	Constant
Heavy Period	1.266	0.041	3.546	1.053-11.94	2.071
Stress	0.702	0.041	2.018	1.028-3.96	1.507

the interval between menstrual cycles was 3.09 for "less than 21 days", 2.73 for "21-35 days" and 2.71 for "more than 35 days". In this case, the *p* value for the ANOVA test was not statistically significant (0.178) so the gap between consecutive menstrual cycles does not appear to have a significant impact on the intensity of pain in dysmenorrhea.

The mean pain scale score for each group with

regards to consumption of cups of caffeine was 2.84 for 1 cup, 2.55 for 2 cups, and 2.75 for more than 2 cups. The ANOVA results provide evidence that there are no significant differences in pain intensity among these three groups.

The mean pain scale score for each group with regards to duration of pain during periods was 1.94 for "A few hours before periods", 2.69 for "Few hours before and few hours after start of periods", 2.79 for "1st day of periods only", 3.15 for "from 1st to 3rd day" and 3.33 for "more than 3 days". It suggested that the severity of pain varies depending on when the pain occurs in relation to the menstrual phase, with the "more than 3 days" group experiencing the most severe pain on average. In this case, the *p* value was significant and showed that the timing of pain during menstrual cycle has a significant impact on pain intensity.

The data revealed that dysmenorrhea led 27.9% of the students to resort to over the counter (OTC) medication for pain relief. 14.7% used hot water bags for pain relief, while 6.3% opted for herbal medication and 51.1% chose to rest only to get through dysmenorrhea.

## Discussion

The prevalence of dysmenorrhea in our sample female medical students was notably high, with 83.2% of participants reporting the experience of menstrual pain. This percentage is in line with the global trends of prevalence of dysmenorrhea in young females with 85.1% in Palestine<sup>13</sup>, 80% among 15-17-year-old females in Australia<sup>14</sup>, 94% in Oman<sup>15</sup>, 72.4% in India<sup>16</sup> and 94% among Egyptian nursing students<sup>17</sup>. This high prevalence suggests that dysmenorrhea is a common issue among this population and emphasizes the need for effective management strategies. This difference in prevalence among different populations may be due to differences in culture, genetics, social and personal factors and different criteria to define dysmenorrhea.<sup>18</sup>

The results of our study indicate that 12% of the participants have very severe pain while 3.3% categorized their dysmenorrhea as the worst pain ever. However, the majority of participants reported to have moderate to severe pain (49.6%). Previous studies investigating severe dysmenorrhea pain show very varied results with percentages ranging

from 0.9% in Korea<sup>15</sup> to 21.7% in Iran<sup>19</sup> and 59.8% in Bangladesh.<sup>15</sup> This highlights that a substantial portion of individuals in our sample and in other populations worldwide experiences pain that may significantly impact their well-being. Our study excluded the cases of pre-existing pelvic pathologies, however, those suffering from undiagnosed pelvic pathologies may make up a portion of the participants suffering from moderate to severe pain or worst pain ever.

The presence of dysmenorrhea led to the outcomes of disruptions in daily activities and emotional instability, reported by 57.5% and 55% of the participants, respectively. This is also reflected in a study in Australia which revealed that 53% Western Australian young girls said that dysmenorrhea limited their activities<sup>14</sup>. Furthermore, 45.4% of the participants of our study also said that they suffered from reduced concentration as a result of dysmenorrhea. This may have serious implications on academic productivity of students while suffering from dysmenorrhea. However, only 9.6% of the participants in our study reported absenteeism from university as a result of dysmenorrhea in contrast to 28.3% in female medical students at a university in Jeddah Saudi Arabia<sup>20</sup>. This rate is also much lower than that reported in nursing students in Spain.<sup>21</sup> This unique finding of our study suggests that female medical students in Pakistan may feel compelled and pressurized to attend classes and daily tasks despite being in disruptive pain.

Our study identified common symptoms associated with dysmenorrhea, such as depressed mood (50.7%), laziness (55%) and mood swings (51.4%). These symptoms underscore the emotional and psychological toll that dysmenorrhea can exact on individuals. Physical fatigue and emotional instability were the most common symptoms shown by a study in North Palestine. Additionally, 23.2% of our participants reported sleep disturbance and 33.9% of the participants said that they experienced increased anger during dysmenorrhea. In a study conducted in Saudi Arabia among medical female students, the most common symptoms that accompanied dysmenorrhea were also depressed mood and anger.<sup>22</sup> We suspect that these effects of dysmenorrhea on mood and daily activities are both due to the dysmenorrhea and also the hormonal

changes in the body accompanied by the menstrual phase. These also emphasize the broad and undeniable impact of menstrual pain on various aspects of life.

The relationship between dysmenorrhea and different predictors of dysmenorrhea was explored. Notably, a statistically significant association was found between heavy bleeding and dysmenorrhea, with 94.1% of students who experienced heavy bleeding reporting dysmenorrhea, and students who suffer from heavy bleeding having more than three times the risk of experiencing dysmenorrhea. These results are similar to a previous study in Saudi Arabia<sup>20</sup> in which heavy period was the first predictor of dysmenorrhea and a study in Athens, Greece<sup>23</sup> which found a good correlation between heavy and painful periods. This finding suggests that heavy bleeding may be a significant risk factor for dysmenorrhea and it is essential for healthcare providers to consider the presence of heavy menstrual bleeding when evaluating and managing dysmenorrhea in their patients. Heavier bleeding accompanied by a higher amount of prostaglandins release may explain the association between heavy menstrual bleeding (HMB) and dysmenorrhea. HMB may also be linked to other common symptoms associated with dysmenorrhea like fatigue and laziness due to being a cause of anemia in women.

Furthermore, our results identified stress as another significant predictor of dysmenorrhea. Among students, those who reported experiencing stress had a higher prevalence of dysmenorrhea (51.1%) to those without stress (34.1%). This is also in line with the results of similar investigations in Saudi Arabia<sup>20</sup>, Iran<sup>19</sup> and Taiwan<sup>24</sup>. The underlying pathophysiology of this association is given by several studies by relating stress and a cascade of neuroendocrine responses<sup>19</sup>. Stress management and emotional well-being should, therefore, be integral components of dysmenorrhea treatment and prevention strategies.

The logistic regression analysis confirmed that heavy periods and stress were robust predictors of dysmenorrhea, further emphasizing the importance of addressing these factors in the assessment and management of menstrual pain. Other factors, including the regularity of the menstrual cycle, physical exercise, days of menstruation, age of

menarche, and family history, were found to be statistically not significant in their association with dysmenorrhea. These results suggest that these factors may not play a significant role in determining the likelihood of experiencing dysmenorrhea among female medical students. However, our findings were in disagreement to results from Dammam and Iran<sup>19</sup> which revealed a statistical association between family history of dysmenorrhea and presence of dysmenorrhea (This inconsistency may be due to variations in study populations and sample sizes).

BMI, cycle interval, and caffeine had no notable impact on pain scores or dysmenorrhea. A Saudi study confirmed no BMI-dysmenorrhea link.<sup>20</sup> Notably, pain duration relative to periods mattered; pain over three days indicated severe discomfort. Understanding pain timing and duration is crucial for dysmenorrhea management.

Although this is among the few such studies conducted in North Pakistan, it has the limitation of having non-probability sampling and being a cross-sectional study which hinders the interpretation of causality. This study only investigates students from Rawalpindi Medical University hence it may not very accurately represent the general female population from all parts of Pakistan. Furthermore, while this study does inquire about the employment of different techniques young women use for the relief of dysmenorrhea such as OTC medication, hot water bags and rest etc., it doesn't delve into the efficacy of such techniques, therefore demanding future research into this aspect. Despite these limitations, this study provides a comprehensive statistical picture of factors surrounding dysmenorrhea and can be used to enhance awareness regarding dysmenorrhea and its predictors and to devise effective strategies to prevent and control dysmenorrhea. These strategies include both non-pharmacologic means like exercise and heat therapy and pharmacologic means such as non-steroidal anti-inflammatory inhibitors and hormonal contraceptives.<sup>26</sup>

## Conclusion

In conclusion, dysmenorrhea is a highly prevalent issue among female medical students leading to a significant proportion of the students to experience moderate to severe pain and associated symptoms



that impact daily life. Heavy bleeding and stress emerged as robust predictors of dysmenorrhea, emphasizing the need for targeted interventions in these areas. Addressing dysmenorrhea is not only important for the well-being of individuals but also for promoting overall health and productivity in the context of medical education and beyond.

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**CONFLICT OF INTEREST**

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**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

## In Vitro Osteogenic Analysis of a Novel Scaffold for Bone Regeneration

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## ABSTRACT

**Objective:** This study aimed to analyse the osteogenic potential of a novel experimental material comprising of nano-hydroxyapatite grafted glass fiber scaffolds using murine osteoblast MC3T3-E1 cell lines through gene expression of biomarkers for osteogenesis.

**Study Design:** In vitro experimental study.

**Place and Duration of Study:** Bone Marrow Transplant Centre and Genetic Resource Centre, Rawalpindi from 10<sup>th</sup> May 2021 to 17<sup>th</sup> June 2022.

**Materials and Methods:** Two types of nano-apatite grafted E-glass fiber scaffolds i.e., 10 wt% E-glass/90wt% Hydroxyapatite (E-10), 20% E-glass/80% Hydroxyapatite (E-20) were used, whereby pure nanohydroxyapatite (E-0) was used as control group. A comparative gene expression of Osteopontin (OP) and Collagen (Col) Type 1 was measured through reverse transcription polymerase chain reaction (RT-PCR).

**Results:** Both experimental groups showed expression of the osteogenic proteins. The level of OP expression was higher in E-10 compared to E-20, whereas the level of Col expression was higher in E-20. Only for E-0, Col expression was almost same as OP whereas for the E-10 and E-20, OP expression was greater.

**Conclusions:** Based on the findings, both experimental scaffolds supported the growth of cells and showed an osteogenic nature. It is anticipated that the experimental scaffolds have potential to be used for bone regeneration.

**Key Words:** Bone Regeneration, E-glass fibres, Gene expression, Hydroxyapatite, Reverse Transcription Polymerase Chain Reaction.

## Introduction

Tissue engineering concepts have promising prospects as they combine the use of scaffolds in combination with techniques at a molecular level to replace missing or diseased tissues.<sup>1</sup> These procedures not only restore the non-functioning tissues, but also utilize specific materials that can be

used to create a fully functional, three dimensional (3D) and structurally sound tissue. These regenerative procedures are now being explored to replace bone defects and loss of bone seen in periodontal diseases. The essence of the treatment aims at eliminating inflammation and promoting periodontal regeneration.<sup>2</sup>

As tissue engineering has evolved with growing research, new materials are now being explored to develop an ideal scaffold possessing an interlinked structure with porosities, reliable mechanical properties and biocompatibility.<sup>3</sup> Hydroxyapatite (HA) has also been widely researched due to its excellent biocompatibility owing to structural similarities to the inorganic or HAP bone phase. It has the remarkable capacity to bond directly to the bone and provide a structural base for cellular attachment and proliferation.<sup>4</sup>

For bone tissue engineering HA scaffolds are primarily opted in non-load bearing areas owing to their inferior physico-mechanical characteristics and slow degradation.<sup>4</sup> To overcome this disadvantage, a new amalgamation of nano Hydroxyapatite (nHA) with E-glass fibres was developed in which the fibres were added at different concentrations to impart

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strength and rigidity to the otherwise brittle nHA. An in vitro trial was conducted to document the osteogenic potential of this novel material.

Our study involves the analysis of the experimental scaffold using murine osteoblast MC3T3-E1 cell lines through gene expression of biomarkers for osteogenesis. Gene expression for two biomarkers namely (Col) Type 1 Collagen and Osteopontin (OP) will be observed through PCR. Expression of the Col Type 1 gene signifies the initial phase of bone formation whereas expression of OP gene comes in the later stages which is consistent with mineralization.<sup>[5]</sup> OP gene expression is a marker for mature osteoblasts signifying an advanced stage in bone formation.<sup>5</sup>

The prospect of studying the behaviour of the cells through gene expression is very promising as the complete information on the cells 'genetic code' can be obtained with polymerase chain reaction (PCR).<sup>6</sup> The study of expression of the genetic code through quantitation of messenger RNA (mRNA) is called gene expression study. The Pfaffl method was used for relative quantification in which change in expression is expressed as a ratio as it can provide useful information about the physiological and pathological processes.<sup>7</sup> In this study real time PCR was employed which is considered a gold standard for quantitative PCR and gene expression studies.<sup>8</sup>

RT-PCR is considered a powerful tool for amplification of small amounts of mRNA due to its high sensitivity, accuracy and quick reading of gene expression.<sup>8</sup> As this technique directly measures the amount of bone proteins being formed by the cells, it leaves no doubt or need for any other analysis as the 'genetic code' of the cell can be read directly and information regarding any function of the cell can be determined. In case of bone tissue engineering, it is an authentic method to determine the progress of tissue formation and identify the stages based on expression of different proteins.

This in vitro study aimed at determining the suitability of the experimental scaffold to be utilized for bone tissue engineering, using murine osteoblasts by measuring the gene expression of biomarkers expressed during different stages of bone formation. The prospects of tissue engineering are promising as the tissue heals naturally with better clinical outcomes.

## Materials and Methods

This In vitro experimental study was conducted in Bone Marrow Transplant Centre and Genetic Resource Centre, Rawalpindi from 10<sup>th</sup> May 2021 to 17<sup>th</sup> June 2022. Permission from the Ethical Review Board of National University of Medical Sciences was obtained on 5<sup>th</sup> April 2021. Murine Osteoblasts MC3T3-E1 (RIKEN Bio Resource Centre, Japan) were used for the osteogenic analysis of the materials. The culture media and related consumables were purchased from GIBCO®. The cell line was cultured in  $\alpha$ -Minimum Essential Medium ( $\alpha$ -MEM), where 10% Fetal Bovine Serum (FBS) and 2 mM Glutamine were added. The cells were stored at 37°C and 5% CO<sub>2</sub>. The osteoblasts were trypsinized using 0.25% trypsin and seeded on to the test materials (E-0, E-10, and E-20 discs) at a concentration of  $3 \times 10^4$  /well. Composition of the materials is given in Table I. The size of the sample discs was kept at 6×2mm. According to the ISO Standard for Direct Contact Test ISO 10993-5, it should cover at least one tenth of the cell layer surface. The experiment was done for Triplicates (n=3), for each sample group (ISO Standard ISO 10993-5). The control plate (E-0) was given a similar treatment, and each well plate was stored at 37°C with 5% CO<sub>2</sub> for 5 days. The experimental cells (cells with discs) and control cells (cells without discs) were detached, for RNA extraction towards the end of the 5-day experiment. The discs were removed from the well plate and 220  $\mu$ L of cell lysis buffer was used. The RNA was extracted from the lysed cells using the RNA extraction machine. A DNAase (1  $\mu$ L) treatment was given to the obtained sample of pure RNA.<sup>9</sup> The confounding variable in this study was the DNA sample in the extracted RNA as that may be responsible for false positive results. Incubation of the sample was done at 37°C for 10 min and DNase-1 was inactivated at 75°C for 10 min.

After decontamination, cDNA synthesis was done by using the enzyme, MMLV Reverse transcriptase (RT) (Thermo Fisher Scientific, USA).<sup>10</sup> Primer synthesis was done by Integrated DNA Technologies (IDT), USA. The primers and their sequences are given in Table II. A real-time PCR (Rotor Gene-Q machine, Qiagen, USA) was run for 35 cycles with initial denaturation at 95°C for 5 min and denaturation at 95°C for 15s followed by annealing/extension at 60°C for 50 s. The control, housekeeping gene was

GAPDH. The cycle threshold (Ct) values of both the target genes, Type 1 Collagen and Osteopontin as well as GAPDH was used for data analysis. Quantification of mRNA expression was done and compared with the relative levels of GAPDH.

The gene expression was measured from an amplification plot generated from the PCR software in which a comparison of cells with and without exposure was done. The Ct values of the target and the reference genes were used for mathematical calculations. The first significant amount of fluorescence in real-time PCR correlated with the amount of template RNA. The target genes in the test (exposed) and the control (unexposed) samples were amplified and their Ct values were noted. A reference gene (GAPDH), unrelated to the target gene, was also amplified to control for the variation in the RNA concentration between the test and the control samples. The difference in the Ct values of the control and the test is called  $\Delta Ct$ . The relative expression ratio (R) of the target gene was calculated based on the  $\Delta Ct$  of the target and the reference gene as follows:

$$\Delta Ct = Ct^{\text{control}} - Ct^{\text{test}}$$

$$\text{Ratio (R)} = 2.0^{\Delta Ct(\text{target})} / 2.0^{\Delta Ct(\text{reference})}$$

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. For comparable variables between groups one-way analysis of variance (ANOVA) was done and for a pair-wise comparison post hoc Tuckey's test was done.

**Table I: Composition of test materials and sample group**

Sample Group	Composition	
E0	No E glass	100 wt% Hydroxyapatite
E10	10 wt% E-glass	90 wt% Hydroxyapatite
E20	20 wt% E-glass	80 wt% Hydroxyapatite

**Table III: Ct and ratio (R) of OP gene expression in murine osteoblasts exposed to E-10, E-20 E-0 and significance of post hoc Tuckey's test.**

Groups	Gene	Cycle threshold test	Cycle threshold control	$\Delta Ct^{\#}$	$2.0^{\Delta Ct}$	Ratio (R) <sup>@</sup>	Expression%	Significance
E-0	Target OP	27.15	16.81	-10.34	0.00077	15.4	100%	-
	Ref GAPDH	31.41	17.23	-14.18	0.00005			
E-10	Target OP	27.74	16.81	-10.93	0.00051	10.2	66.2%	0.009
	Ref GAPDH	31.61	17.23	-14.38	0.00005			
E-20	Target OP	22.90	16.81	-6.09	0.01470	9.30	60.3%	0.001
	Ref GAPDH	26.54	17.23	-9.31	0.00158			

$$\# (Ct \text{ control} - Ct \text{ test}) \quad @ 2.0^{\Delta Ct(\text{target})} / 2.0^{\Delta Ct(\text{reference})}$$

**Table II: F (forward) R (reverse) Primer Sequences for PCR**

Gene	Primer Sequence
Mur-Col-1a1-F	5'-GAGAGGTGAACAAGGTCCCG
Mur-Col-1a1-R	5'-AAACCTCTCTCGCCTCTTGC
Mur GAPDH-F	5'-AAGGTCATCCAGAGCTGAA
Mur GAPDH-R	5'-CTGCTTACCACCTTCTTGA
Mur OP-F	5'-TCTGATGAGACCGTCACTGC
Mur OP-R	5'-AGGTCCTCATCTGTGGCATC

## Results

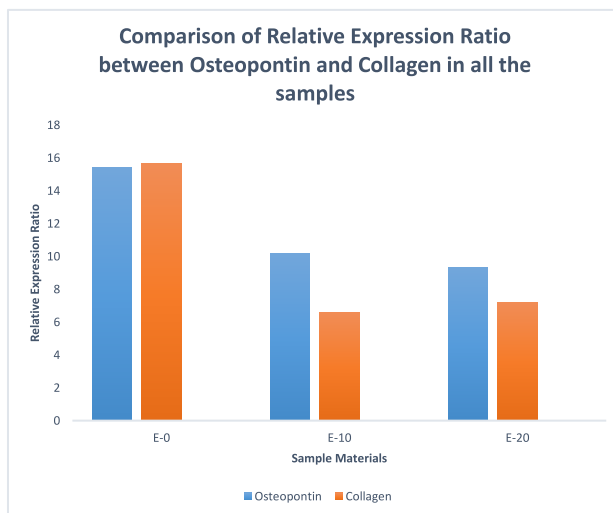
Results of Collagen Type 1 and Osteopontin gene expression include the ratio of relative expression among the test samples (exposed) and control samples (unexposed). The relative expression ratio of OP in the experimental groups was 15.4 (E-0), 10.2 (E-10) and 9.3 (E-20) respectively. The mathematical calculations of the Pfaffl method for OP gene expression are summarised in Table III. The presence of 10% E-glass/90% nHA reduced the level of OP expression from 15.4-fold to 10.2-fold ( $p=0.009$ ). The presence of 20% E-glass/80% nHA reduced the level of OP gene expression from 15.4-fold to 9.30-fold ( $p=0.001$ ).

The relative expression ratio of Col in the experimental groups was 15.69 (E-0), 6.59 (E-10) and 7.22 (E-20) respectively. The mathematical calculations of the Pfaffl method for Col gene expression are summarised in Table 4. The presence of 10% E-glass/90% nHA reduced the level of Col expression from 15.69-fold to 6.59-fold ( $p < 0.001$ ). The presence of 20% E-glass/80% nHA reduced the level of Collagen gene expression from 15.69-fold to 7.22-fold ( $p < 0.001$ ). A Comparison of the relative expression ratio of OP and Col measured and compared in each sample group is shown in Figure 1. Only for E-0, Col expression was almost same as OP whereas for all the other experimental materials, OP expression was greater in comparison to Col.

**Table IV: Ct and ratio (R) of Col gene expression in murine osteoblasts exposed to E-10, E-20 and E-0 and significance of post hoc Tuckeys test**

Groups	Gene	Cycle threshold test	Cycle threshold control	$\Delta Ct^{\#}$	$2.0^{\Delta Ct}$	Ratio (R) <sup>@</sup>	Expression %	Significance
E-0	Target Col	27.16	19.92	-7.24	0.0066	15.69	100%	-
	Ref GAPDH	31.40	20.18	-11.22	0.0004			
E-10	Target Col	28.50	19.92	-8.58	0.0026	6.59	42.03%	p < 0.001
	Ref GAPDH	31.51	20.18	-11.33	0.0003			
E-20	Target Col	27.16	19.92	-7.24	0.0066	7.22	46.02%	p < 0.001
	Ref GAPDH	30.27	20.18	-10.09	0.0009			
	Ref GAPDH	30.22	20.18	-10.04	0.0009			

**# (Ct control – Ct test) @  $2.0^{\Delta Ct(\text{target}) / 2.0^{\Delta Ct(\text{reference})}$**

**Fig 1: Comparison of the Relative Expression Ratio of Osteopontin and Collagen measured and compared in each sample group separately**

## Discussion

Periodontal therapy after loss of periodontal tissue, is aimed at repairing the damaged tissue and encourage regeneration of new bone tissue as treatment therapies based on regeneration have proved to be more effective than limiting infection alone.<sup>11,12</sup> Nano-hydroxyapatite has been used in the past with multiple other components to study its most efficient combinations. In a previous study, n-HA was used in combination with calcium and strontium substitutes at varying proportions and the results showed that not only the material exhibited osteoblastogenicity but was also non-cytotoxic.<sup>13</sup> This experiment studied the osteogenic potential of the novel blend of nHA with E-glass fibres designed

for use in the clinical applications of tissue engineering. Hydroxyapatite is known for its ability to form bone bonds and in addition to allowing cell attachment and proliferation.<sup>14</sup> The current study reaffirmed the ability of nHA to augment bone formation and assist in bone regeneration.

PCR is a highly sophisticated and accurate diagnostic tool which enables monitoring all the activities within the cells.<sup>15</sup> A comparison of gene expression between exposed and unexposed cells was done using relative quantification method. Amount of fluorescence was measured against the cycle number. There was a clear difference between the Ct of OP and GAPDH genes in the test (exposed) samples as compared to the same in the control (unexposed) indicating increased level of OP gene expression in the test as compared to the control. Similar difference was noted for Col as well indicating increased level of Col gene expression in the test as compared to the control.

The control disc containing only nHA had the highest expression ratio for both collagen and osteopontin, 15.69 and 15.4 respectively. The relative expression ratio for OP was found to be inversely proportional to concentration of E-glass fibres. For E-10 the ratio dropped to 10.2 and then a slight decrease in expression to 9.30 folds in E-20. For Col 1 gene expression results in all the materials were similar to OP. The highest ratio was expressed by E-0 (15.69) followed by E-20 (7.22) and then E-10 (6.59). Col Type 1 expression proves that the cells were proliferating on the scaffolds due to the presence of nHA while being able to maintain their phenotype. This finding is similar to previous studies in which

composites of HA have up regulated the Col Type 1 gene expression.<sup>16-18</sup> The difference between the discs could be attributed to their difference in composition as addition of E-glass fibres reduced the osteogenic potential of the materials.

Only for E-0, Col expression was almost same as OP whereas for all the other experimental materials, OP expression was higher in comparison with Col. The discs were able to up regulate the gene expression of both proteins attributing to the natural osteogenic tendency of HA which allows murine osteoblasts to attach, proliferate and differentiate. It has been documented that early expression of OP is consistent with formation of bone matrix and expression later in bone development is associated with remodelling.<sup>19</sup> OP also promotes the osteoblastic attachment to the extracellular matrix so that these cells can play their role during osteogenesis.<sup>20</sup> The five-day cell culture experiment generated optimum results for OP as it's up regulation as early as 3 days has been demonstrated in another study, in which PLGA scaffolds were coated with biomimetic apatites and osteogenic gene expression was measured after seeding the scaffolds with MC3T3 E-1 cells.<sup>21</sup>

Nano Hydroxyapatite is known for its osteoconductive and osteoinductive behaviour and allows the integration of bone tissue to the material surface.<sup>22</sup> HA is an osteoconductive biomaterial.<sup>23</sup> Since natural tissues of the body are on a nanometer scale, the development of nanostructured materials can replicate the extracellular matrix and therefore cells can interact directly with nanometer grain sizes.<sup>24</sup> Therefore these nanoceramics can induce osteointegration, osteogenesis, osteoinduction and osteoconduction owing to a higher surface area-volume ratio.<sup>[24]</sup> Nano hydroxyapatite can regulate the expression of both Col Type1 and OP in this experiment effectively. MC3T3 E-1 cells were expressing these genes even in the absence of the materials and through relative quantification, thus, establishing that the presence of this novel material increased the expression even more.

The major limitation of the study was the absence of a three-dimensional structure of the scaffold but even the 2D structure had still up regulated the expression of both genes owing to the osteogenic nature of nHA. Collagen expression during the initial stages of extracellular matrix deposition has been

extensively documented and has been proved by this experiment as well. The expression of OP as early as five days also signifies that the cells had reached a stage where mineralization of the extracellular matrix was possible. OP gene expression is a marker for mature osteoblasts signifying an advanced stage in bone formation. This information is of paramount importance to a clinician who aims to use this osteogenic biomaterial for bone augmentation. Based on these results the authors recommend the use of this material to conduct animal studies to see the extent of bone formation preferably using 3D scaffolds in the future.

## Conclusion

It was found that all the experimental materials and control materials supported the growth of MC3T3 E-1 cells. The material was also able to demonstrate the induction of mineralization associated gene expression in MC3T3 E-1 cells. The novel material was osteogenic and cells grown on this scaffold were capable of producing an extracellular matrix containing the Col Type 1 and OP bone proteins. This material has the potential to be used as a reinforcing agent and implant material because it will impart strength as well as play a bioactive role. With new research being done on a massive scale, tissue engineering which was just an idea three decades ago, has promising prospects in the future.

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## Conflict of Interest

The authors have no conflict of interest to disclose, and no financial assistance was taken. Data has not been presented previously in any conference.

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**CONFLICT OF INTEREST**

Authors declared no conflicts of Interest.

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**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

# Anterior Inferior Cerebellar Artery (AICA) Loop Variants and Its Effect on Audio-Vestibular Symptoms Using Three-Dimensional Fast Imaging Employing Steady State Acquisition (3-D FIESTA) MRI Sequence

Saerah Iffat Zafar<sup>1</sup>, Syeda Tatheer Fatima<sup>2</sup>

## ABSTRACT

**Objective:** To assess the effect of vascular loop variant anatomy of anterior inferior cerebellar artery (AICA) types in relation to vestibulocochlear and facial nerves within cerebellopontine angle (CPA) on MRI, in patients presenting with otoneurological symptoms.

**Study Design:** Cross-sectional study.

**Place and Duration of Study:** Combined Military Hospital (CMH) Abbottabad for a period of 8 months from July 01, 2022 to February 28, 2023.

**Materials and Methods:** All patients with audio-vestibular symptoms (group I) pertaining to facial or vestibulocochlear nerve referred for MRI brain were included in the study. Specific MRI protocol for the assessment of cerebellopontine angle and its contents- three-dimensional fast imaging employing steady-state acquisition (3-D FIESTA)- was used to delineate the relation of AICA loop to VII and VIII cranial nerves within cerebellopontine angles and internal auditory meatus on both sides. Patients without any symptoms also underwent similar MR protocol to serve as a control group (group II). Vascular loop anatomy of AICA in CPA was recorded and its relation to patient's symptomatology, if any, was documented. Findings were compared with the control group. Social Package for Statistical Studies (SPSS) version 25.0 was used for analysis. Data type was Qualitative (categorical) and Chi square test was applied for drawing results. A  $p$  value of  $\leq 0.05$  was considered statistically significant with 95% confidence interval (CI).

**Results:** Type I was most common variant in both groups followed by Type II and Type III subsequently. No statistically significant difference was found in the AICA loop variant distribution between the symptomatic and control groups for both right ( $p = 0.543$ ) and left ( $p = 0.564$ ) sides with respect to patient's symptoms.

**Conclusion:** AICA loop within cerebellopontine angle demonstrates variant course with respect to internal auditory meatus in proximity to VII and VIII nerves; this variation has no impact on patient's otoneurological symptoms. Other factors need to be addressed and evaluated for such symptoms in the relevant patients.

**Key Words:** Anterior Inferior Cerebellar Artery, Cerebellopontine, Facial, MRI, Vestibulocochlear.

## Introduction

Cerebellopontine angle (CPA) contains many vascular structures and nerves, including anterior inferior cerebellar artery (AICA) which is a branch of basilar artery predominantly arising from its lower third part. Less commonly it may originate from the distal vertebral artery.<sup>1</sup> It supplies lateral pons, inner

ear, middle cerebellar peduncles and antero-inferior part of the inferior cerebellum as well as the 7<sup>th</sup> and 8<sup>th</sup> cranial nerves. Vascular compression through the course of nerves has been proposed to cause neurological symptoms due to direct contact and irritation of the nerves by the adjacent vessels, particularly in closed spaces like internal auditory meatus.<sup>2</sup> Some authors proposed this neurovascular compression to be dynamic, occurring only at times hence this could be missed on routine MRI at the time of examination.<sup>3</sup> AICA can form a vascular loop within the CPA in relation to internal auditory meatus (IAM) after its origin - forming a loop at the base of the meatus, entering part of it or extending more than half of its length, crossing over the vestibulocochlear and facial nerves. These loops have traditionally been graded according to Chavda classification according to which loops within the

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CPA not entering the IAM were classified as type I, those entering the porus acusticus and extending less than 50% of the length as type II, and extension of AICA loop beyond 50% of meatal length were labelled as type III.<sup>4</sup>

Three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) is an ultrafast heavily weighted T2W MRI sequence developed by General Electric (GE) HealthCare for evaluation of thin or small structures against fluid, due to high intrinsic contrast resolution and relative insensitivity to motion.<sup>5</sup> Hence it has been utilised in assessment of abnormalities pertaining to cranial nerves and small vessels within cisterns and inner ear structures. Some centres are using the pre and post contrast T1W images in addition to identifying small retro cochlear lesions and inflammation in investigations of otovestibular symptoms.<sup>6</sup> An equivalent to this MRI sequence is constructive interference in steady state (CISS) by Siemens, working on the same principle.<sup>7</sup>

Many studies have been conducted to identify the relationship between loop variants of AICA in the CPA with the otological symptoms of tinnitus, vertigo, hearing loss or symptoms related to facial nerve such as hemispasm, with the help of these relatively new sequences. This relation was proposed due to close relation of AICA with the VII and VIII cranial nerves in the CPA raising the possibility of neurovascular compression. For this purpose, classification system of Chavda has been utilised as proposed by McDermott *et al.*,<sup>4</sup> in patients with such symptoms. There have been conflicting conclusions regarding the importance of this neurovascular relation. Despite repeated research, no significant effect of AICA anatomical type was identified with the patients' symptomatology.<sup>8,9,10</sup>

The aim of this study was to identify the role of AICA loop variations in causing audio-vestibular and facial nerve symptoms secondary to neurovascular compression of the nerves traversing the internal auditory meatus by comparing the distribution of AICA loop variants between patients with and without vestibulocochlear symptoms. So far, no such study has been conducted in this region; it will help neurosurgeons to properly evaluate and diagnose the root cause of patients with symptoms related to ear by appropriate investigation.

## Materials and Methods

This was a cross-sectional study carried out in Combined Military Hospital (CMH) Abbottabad for a period of 8 months from July 01, 2022 to February 28, 2023. Prior permission was obtained from Ethical Review Board (ERB) of the institution (ETH-63 dated 16 July 2022). A total of 58 patients were included in the study. Random sampling was used for patients and control group selection. Inclusion criteria included patients of all ages and both genders referred to Radiology Department CMH Abbottabad for MRI brain for assessment of otovestibular symptoms like tinnitus, hearing loss, vertigo and facial sensory or motor abnormalities. The patients who were found to have an organic lesion as a cause of these symptoms or those who were unable to undergo MRI examination by virtue of age, technical limitations or claustrophobia were excluded from the study. In addition, all patients undergoing MRI brain without these symptoms formed a number specific control group, matched for age and gender. A total of 58 patients were included in the study distributed into group I (having otovestibular/facial nerve symptoms,  $n = 29$ ) and group II undergoing MR brain for other reasons (control,  $n = 29$ ). Both groups were matched for age and gender (Table I). Data was collected by the author with assistance from the co-author. Non-enhanced MRI brain was done on 1.5 Tesla GE machine including three-dimensional fast imaging employing steady-state acquisition (3D-FIESTA) to determine the location of anterior inferior cerebellar artery with respect to vestibulocochlear and facial nerves in cerebellopontine angle. AICA loop anatomy in the CPA and its relation to facial and vestibulocochlear nerves was ascertained for each patient on either side. Images were interpreted by consultant radiologist. Data was fed into Social Package for Statistical Studies (SPSS) v 25.0 and significance of variant anatomy to the symptoms of patients was determined. Similar MRI sequence was carried out in asymptomatic patients undergoing MRI brain as a control group. Chi square test was applied for assessment of relation between AICA loop variant and symptoms related to VII/VIII nerves. In analyses where at least one case had an expected frequency of less than five, Fisher's exact test was applied. A  $p$  value of  $\leq 0.05$  was considered statistically significant with 95% confidence interval (CI).



## Results

Most of the group I patients ( $n = 22$ , 75.8%) demonstrated symptoms related to the vestibulocochlear nerve (vertigo, dizziness, and tinnitus), with vertigo being the most frequent complaint ( $n = 20$ , 71.4%). Isolated tinnitus was reported in seven patients -out of these, five had bilateral symptoms while two had reported tinnitus in the left ear only; two patients had both tinnitus and dizziness. Facial nerve symptoms (hemispasm and palsy) were reported in two (6.8%) patients. The frequencies of AICA loop types for the right and left ears are illustrated in Table II. There was no statistically significant difference in the distribution of the AICA loop types between the symptomatic and asymptomatic groups for both right ( $p = 0.543$ ) and left ( $p = 0.564$ ) CP angles.

**Table I: Gender and Age for symptomatic and control Groups ( $n = 58$ )**

		Group I	Group II	Total
Gender n %	Male	15 (41.7%)	15 (41.7%)	30
	Female	14 (48.3%)	14 (48.3%)	28
Age (Years)		44.97 $\pm$ 16.46	47.76 $\pm$ 16.90	46.36 $\pm$ 16.60

**Table II: AICA Loop Type Distribution for both Groups ( $n = 58$ )**

AICA Loops		Group I	Group II	Total	p value
Right	Type I	24 (82.8%)	25 (86.2%)	49 (84.5%)	0.543
	Type II	3 (10.3%)	4 (13.8%)	7 (12.1%)	
	Type III	2 (6.9%)	0 (0%)	2 (3.4%)	
Left	Type I	23 (79.3%)	24 (82.8%)	47 (81%)	0.564
	Type II	4 (13.85%)	5 (17.2%)	9 (15.5%)	
	Type III	2 (6.9%)	0 (0%)	2 (3.4%)	

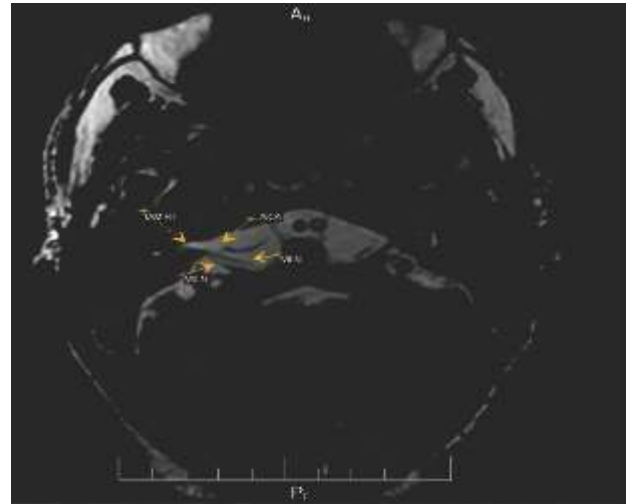
\* The  $p$  value  $\leq 0.05$  was considered statistically significant

## Discussion

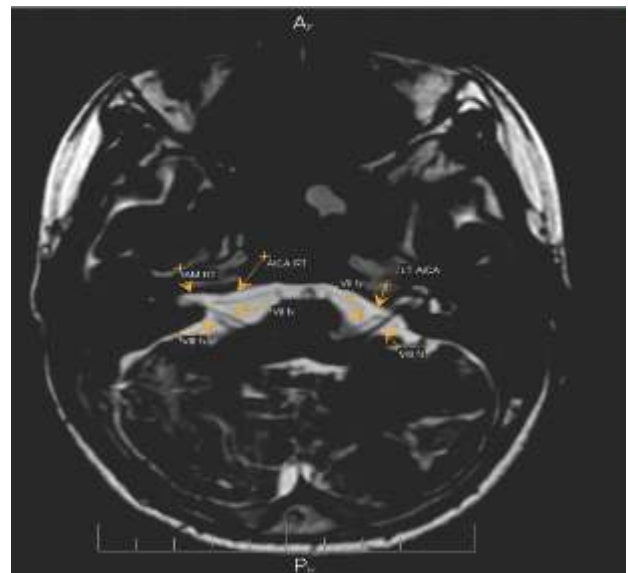
Our study revealed AICA loop distribution to be similar in both symptomatic and non-symptomatic patients (control group). Type I remained the most common variant in both groups at 81.5 % in symptomatic patients, and 84.5% in the control group; no difference in rest of the variants was noted ( $p$  value = 0.5), signifying no role of these vascular loop variants in causation of audio-vestibular symptomatology.

AICA arises as a branch of basilar artery in 98% of normal population. Like majority of vessels, it can have a variant course - traversing the CPA at the origin of IAC close to the meatus (19%– 40%), at the meatus (33%–56%), or entering within the IAC (25%–

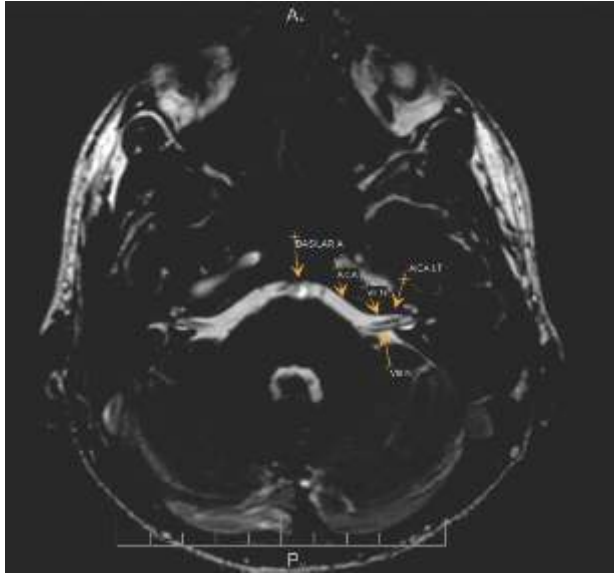
27%)<sup>11</sup> - Figures 1-3. At this juncture it lies in close proximity to the VII and VIII nerves.<sup>12</sup> Superomedially, midbrain borders the CPA, which is the exit point of some important nerves including trigeminal, facial and vestibulocochlear nerves. Eighth nerve travels lateral to the abducens and facial nerve after its exit from the brain; after a short anterolateral course, it accompanies facial and intermediate nerve to enter the auditory canal before dividing into cochlear and vestibular branches.<sup>13</sup>



**Fig 1: MRI 3-D axial FIESTA sequence outlining AICA in the CSF of right cerebellopontine angle. The vessel is seen crossing over the VII/VIII nerve at the level of porus acusticus without entering the internal auditory meatus (type I variant).**



**Fig 2: MRI 3-D FIESTA sequence axial view showing right AICA entering the IAM for less than 25% of its length (type II variant); left sided AICA loop is type I**



**Fig 3: MRI 3D-FIESTA sequence demonstrating type III variant of AICA (on the left) as it passes into the IAM for more than 50% of its length, crossing over VII/VIII nerves**

Neurovascular compression as a cause of audio-vestibular symptoms due to irritation of the nerves has long been debated without a definitive conclusion. With the advent of newer MRI sequences with thin sections, assessment of these nerves and vessels along with points of contact has been made easy. One of the researches carried out in Turkey showed no significant vascular conflict between AICA loop variants and the 8<sup>th</sup> nerve course within the CPA and auditory canal in patients having cochleo-vestibular symptoms.<sup>14</sup> Studies performed in Netherlands and Egypt concurred these findings as they could not establish a diagnostic value to any specific type of AICA causing compression upon the nerve to cause a neurovascular conflict.<sup>15,16</sup> A research carried out to determine the relation between contact of these nerves with AICA loops showed that it was similar in symptomatic and control patients (25% and 21.4%, respectively).<sup>17</sup> No regional study has been conducted in Pakistan so far; in our study, we concluded that no significant relation existed between audio-vestibular symptoms and vascular loop variants within the CPA and IAC, as both the cases and control group demonstrated variation in the AICA anatomy. However, a study with a large number of subjects (n=2622) concluded that loop characteristics of AICA loop in this region as a significant predictor in evaluation of sensorineural symptoms for guidance

regarding its treatment.<sup>18</sup> Ezerarslan *et al.*,<sup>19</sup> showed sudden sensorineural hearing loss was significantly associated with a sub-type of AICA loop variant. Moreover, these patients were less responsive to steroid therapy. These findings led to switching of the management in these patients from oral therapy to microvascular decompression with improved results. Since the treatment required for a neurovascular conflict is microvascular decompression hence it becomes imperative that an authentic diagnosis is made before undertaking this invasive procedure. So far, based on the existing classification, no study has managed to cement the hypothesis of an AICA loop being a cause of audio-vestibular symptoms definitively. Hence routine MRI for identification of AICA variant anatomy, despite being a non-invasive modality, is not recommended by most authors as concluded by a review paper comparing the results of 12 studies.<sup>20</sup>

Despite multiple research world-wide, to our knowledge this is the first study in Pakistan to investigate possible relation between AICA anatomic variants to 7<sup>th</sup> and 8<sup>th</sup> nerve course in CPA for assessment of otovestibular and facial symptoms. The limitations of this study were that symptomatic evaluation was not performed clinically on all patients, and the sample size was small.

### Conclusion

Anterior inferior cerebellar artery within cerebellopontine angle demonstrates variant course with respect to internal auditory meatus in proximity to VII and VIII nerves; this variation has no impact on patient's otoneurological symptoms.

### Conflict of interest

There is no conflict of interest to declare by the authors.

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#### CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

# An Exploratory Study of Reproductive Health Awareness and Information Seeking Behavior Among Saudi Female Students

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## ABSTRACT

**Objective:** To explore sexual reproduction-related awareness and knowledge, information-seeking behavior, and related information resources needed among Saudi female students.

**Study Design:** Descriptive cross-sectional study.

**Place and Duration of Study:** The study was conducted in King Faisal University, Al Ahsa Saudi Arabia from 1<sup>st</sup> September 2023 to 20<sup>th</sup> November 2023.

**Materials and Methods:** A questionnaire-based cross-sectional study was conducted at King Faisal University Saudi Arabia. A sample of 328 preparatory-year female students was evaluated on reproductive health awareness through a randomized sampling method. Data was cleaned in Excel and Analyzed in IBM SPSS 29.

**Results:** The study involved 328 female students and highlights significant findings in reproductive health awareness. Notably, 26.2% (85) experienced irregular menstrual cycles, 99.1% (325) used sanitary pads, and 78.4% (257) and 41.2% (135) discussed their first period, primarily with mothers. TV (17.1%) and news (16.2%) were the primary sources of reproductive health information while 93.9% (307) had contraceptive knowledge, and 84.5% (277) understood reasons for use. Challenges in obtaining information included uncertainty (30.6%) and shame (29.1%). Preferences included information on reproductive/sexual health (21.1%) and sexual/premarital preparation classes (18.3%). The educational status of fathers significantly correlated with sexual and reproductive health awareness and knowledge ( $p=0.040$ ).

**Conclusion:** The study revealed gaps in female preparatory year students' awareness of reproductive health. It underscores the importance of enhancing the accessibility of reproductive health knowledge resources and improving communication channels with parents to attain first-hand appropriate knowledge of reproductive health.

**Key Words:** Awareness, Adolescent Health, Parental Role, Sexual and Reproductive Health.

## Introduction

Sexual and reproductive health is a critical cornerstone for women's empowerment and overall well-being in the field of health. Understanding one's body, making informed judgments about intimacy and relationships, getting access to quality healthcare, and navigating the process of making

reproductive decisions are just a few of the many facets that fall under this broad category.<sup>1</sup> It is in this context that raising females' awareness of sexual and reproductive health issues becomes crucial.<sup>2</sup>

In comparison to other age groups, adolescents and young adults in Western and European countries have disproportionately high rates of avoidable sexual health problems, such as HIV, STIs, and unplanned pregnancies.<sup>3</sup> In this situation, health professionals frequently don't have a thorough grasp of how to provide services that are sensitive to the requirements of teenagers.<sup>4</sup> Studies have shown how important it is for teenage girls to have a greater understanding of sexual and reproductive health since sociocultural norms, healthcare accessibility, educational possibilities, and personal empowerment all have a significant impact on women's health.<sup>5,6</sup>

Adolescent girls frequently have false information or

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misconceptions about reproductive health topics because of the notable lack of sexual reproductive health (SRH) awareness in rural areas of developing countries. This is primarily due to a lack of educational resources and limited access to information facilities.<sup>7,8</sup>

Women's ability to make educated decisions is hampered by the lack of sexual and reproductive health education in the Middle East and North Africa, particularly Saudi Arabia.<sup>9</sup> In Saudi Arabia, SRH is often framed within the context of morality, tradition, and modesty. Consequently, discussions surrounding SRH are regarded as culturally taboo, particularly for unmarried females, who are less knowledgeable and have limited resources to access such information.<sup>10</sup>

The current study has focused on preparatory-year female students of King Faisal University, Al Ahsa, to evaluate their SRH awareness, knowledge, information-seeking attitude, parental communication, and desire to access information resources. The preparatory-year students being at a transitional stage between secondary school and higher education, may indeed be considered a vulnerable group in terms of their awareness of sexual and reproductive health matters. Recognizing the diverse and evolving needs of young females this initiative seeks into the complexities of sexual and reproductive health, aiming to equip students with knowledge, resources, and a supportive community that facilitates informed decision-making and promotes a sense of awareness of their health.

## Materials and Methods

A descriptive cross-sectional study was conducted at the King Faisal University, Al Ahsa Saudi Arabia in the first semester of the academic year from 1<sup>st</sup> September 2023 to 20<sup>th</sup> November 2023. Ethical approval was granted by King Faisal University of Al-Ahsa Research Ethic Deanship (KFU-REC-ETHICS536). The study targeted young female students from the preparatory year to evaluate reproductive health awareness. A sample size of 328 girls was extracted from the total number of enrolled students with a 95% Confidence Interval and a 5% margin of error. The students were enrolled through the systematic randomized sampling as per subject class sessions with their free will to respond to the questionnaire.

The questionnaire was retrieved from published studies and tailored to the Saudi culture, religion, and ethics.<sup>9,10</sup> The questionnaire contained a sociodemographic section of participants and their parents and the awareness of any gynecological illness. The second section contained close-ended knowledge questions with yes, no, and don't know choices about the menstrual cycle, puberty body changes, polycystic ovaries, sexually transmitted diseases, contraception, and sexual reproduction. All "Yes" were correct answers and scored as 1, and "No, and Don't know" scored as 0. Participants with scores above the total median were considered to have a high level of awareness and those below to have a low level of awareness. The knowledge obtained from parents was evaluated on 3 scales Nothing, Some, and a Lot. Their behavior or attitude in discussing SRH issues was assessed with a 4 Likert scale of strongly disagree, disagree, agree, and strongly agree. In addition their sources of sexual and reproductive health information, statements to choose regarding the barriers in the way of SRH awareness, and finally their opinions on the provision of information sources or platforms to improve and enhance knowledge about SRH and pregnancy. Permission from the teaching faculty was retrieved before the teaching session. The researchers distributed the questionnaire to the randomly assigned students. Out of the systematically randomized participants, we received 328 complete voluntary responses.

The data was cleaned in Excel and analyzed in IBM SPSS 29. A thorough statistical analysis was performed on the dataset, incorporating both descriptive and inferential methods. Firstly, a descriptive analysis was conducted to summarize the demographic characteristics of the participants, which include age, gender, and other features. This provided an overview of the study population. Subsequently, inferential analysis such as Fisher's Exact Test was used to see the association between categorical variables. Statistical significance is established at a p-value of < 0.05 and a 95% Confidence Interval. All statistical analyses were performed using IBM's SPSS Software, version 29.0.0.

## Results

Among the 328 female students, most (46%) were 18



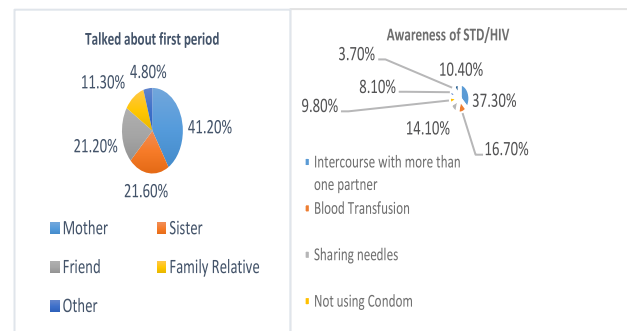
years old. The majority of students resided in the city (71.6%). Maternal and paternal education levels varied, with a higher prevalence of university-educated parents. The majority were single (91.2%), and nearly all had not attended sexual/reproductive health courses. A small percentage reported a family history of gynecological issues shown in Table I

**Table I: Sociodemographic and Other Features of Female Students**

Variables		Frequency (n=328)	Percent
Age	18 Year	151	46.0
	19 Year	119	36.3
	20 Year	38	11.6
	21 Years or Above	20	6.1
Residence	City	235	71.6
	Village	93	28.4
Mother's Education Level	Uneducated	9	2.7
	Primary Education	27	8.2
	Intermediate Stage	55	16.8
	Higher School	109	33.2
	Diploma	16	4.9
	University	112	34.1
Father's Education Level	Uneducated	6	1.8
	Primary Education	32	9.8
	Intermediate Stage	46	14.0
	Higher School	104	31.7
	Diploma	21	6.4
	University	119	36.3
Marital Status	Married	29	8.8
	Single	299	91.2
Have Ever attended Courses in Sexual/Reproductive health as part of compulsory or elective programs	No	318	97.0
	Yes	10	3.0
Family history of any gynecological problem, treatment, or Operation	No	299	91.2
	Yes	29	8.8
Weight (Kg)	Mean (SD)	55.5 (17.8)	
	Range	30-162 Kg	

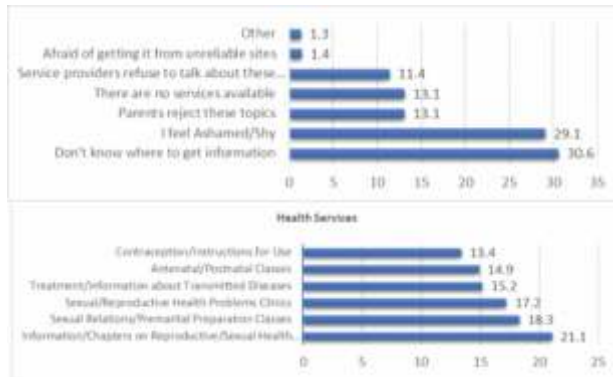
Various aspects of menstrual cycle were asked and a notable 26.2% experienced irregular menstrual cycles, with 99.1% using sanitary pads. Of those with irregular cycles, 75% had consulted a doctor. Only 3% used cloth as an alternative to pads. The majority 78.4% discussed their first period, and 80.8% noticed hair growth on their face and body. Facial acne was found prevalent, affecting 61.9% of participants.

Figure 1 shows the people with whom female students discussed their first period. The majority (135) talked to their mothers, followed by sisters (71) and friends (70). A smaller percentage sought guidance from the relatives (37), while (16) chose other individuals. Regarding the awareness of STDs/HIV, the majority knew multiple partners can be a cause of these infections. Blood transfusion, sharing needles, unprotected intercourse, and the use of public restrooms were acknowledged as potential transmission routes.



**Fig 1: Female Students Talked about their First Period and awareness of STD/HIV**

The primary sources of reproductive health included TV 17.1%, followed by news 16.2%, health professionals 12.6%, and friends 11.4%. The top graph in Figure 2 laid out the hurdles faced by female students in obtaining reproductive health information. The most common challenges included uncertainty about where to get information and feelings of shame or shyness. A smaller percentage encountered obstacles such as parental rejection of these topics, limited availability of services, and service providers refusing to discuss these matters. Fear of unreliable sources and other unspecified barriers contribute to the complexities students face in accessing reproductive health information. The graph at the bottom revealed the preferences of female students regarding reproductive and sexual health services. The majority express a desire for information or chapters on reproductive/sexual health issues. Additionally, there was interest in sexual relations/premarital preparation classes and sexual/reproductive health problems clinics. Services related to the treatment/information about transmitted diseases, antenatal/postnatal classes, and contraception/instructions for use were also considered valuable.



**Fig 2: Barriers to access information, and required health services/resources to provide.**

Table II shows that 41.8% were aware that irregular menstrual cycles affect fertility. While 91.5% recognized premenstrual symptoms, only 60.4% were familiar with polycystic ovary syndrome. Understanding the consequences of polycystic ovary disease was evident in 62.8% of participants. Awareness of sexually transmitted diseases was high at 81.1%, with 75.9% acknowledging preventive measures. Contraceptive knowledge was known well widespread (93.9%), but 84.5% understood the reasons for contraceptive use. Few married participants reported using contraception (3.4%).

**Table II: Knowledge about Reproductive Health Issues/Problems**

		No	Don't Know	Yes
Does an Irregular menstrual cycle affect fertility?	N	29	162	137
	%	8.8	49.4	41.8
Do you know about premenstrual symptoms?	N	13	15	300
	%	4.0	4.6	91.5
Do you know about the term polycystic ovary syndrome?	N	83	47	198
	%	25.3	14.3	60.4
Polycystic ovary disease can lead to irregular menstrual cycles, unwanted body hair growth, weight gain, and difficulty getting pregnant	N	5	117	206
	%	1.5	35.7	62.8
Have you heard about sexually transmitted diseases?	N	40	22	266
	%	12.2	6.7	81.1
Is there anything a person can do to avoid STDs and HIV?	N	10	69	249
	%	3.0	21.0	75.9
Have you heard about contraceptives?	N	8	12	308
	%	2.4	3.7	93.9
Do you know why to use contraceptives?	N	25	26	277
	%	7.6	7.9	84.5
If you are married, have you ever used any type of contraception?	N	34	7	11
	%	10.4	2.1	3.4

Table III shows the learning behavior and information-seeking attitude of female students. A large proportion of 57.3% learned a lot about body changes during puberty from their parents. Similarly, 57.9% also acquired a lot of knowledge about menstruation. However, fewer students 15.2% gained extensive information about the male and female reproductive system, 11.3% contraception, and 6.7% sexual relations. While 43.9% have talked about reproductive health matters, 64.3% found it easy to obtain information. About 35.4% expressed interest in attending sexual and reproductive health courses. Communication comfort varied, with higher agreement on discussing these matters with mothers than with fathers.

**Table III: Assessment of Knowledge and Information Seeking Attitude of Female Students**

Knowledge Obtained from Parents		Frequency (f)	Percent (%)
The body changes during puberty	Nothing	37	11.3
	Some	103	31.4
	Alot	188	57.3
About menstruation	Nothing	31	9.5
	Some	107	32.6
	Alot	190	57.9
About the male and female sexual reproductive system	Nothing	154	47.0
	Some	124	37.8
	Alot	50	15.2
About contraception	Nothing	201	61.3
	Some	90	27.4
	Alot	37	11.3
About sexual relations between males and females	Nothing	230	70.1
	Some	76	23.2
	Alot	22	6.7
Information Seeking Attitude Toward Reproductive Health			
Have you ever talked to anyone about sexual and reproductive health matters?	No	184	56.1
	Yes	144	43.9
Do you think it is easy to get information about sexual and reproductive health?	No	117	35.7
	Yes	211	64.3
Would you like to attend any courses on sexual and reproductive health?	No	212	64.6
	Yes	116	35.4
I feel that I can discuss issues related to sexual reproduction with my mother only.	Strongly Disagree	78	23.8
	Disagree	111	33.8
	Agree	118	36.0
	Strongly Agree	21	6.4
I feel that I can discuss issues related to sexual reproduction with my parents (both mother and father)	Strongly Disagree	208	63.4
	Disagree	98	29.9
	Agree	19	5.8
	Strongly Agree	3	.9

Table IV explored factors associated with reproductive health awareness among female students. Participants who answered correctly having scores above the total median score had a high level of awareness and those below had a low level of awareness. The educational status of fathers significantly correlated with the awareness of reproductive health among female students ( $p$ -value = 0.040). Notably, when fathers were uneducated, the awareness level was 0.0%, contrasting with 78.6% awareness when fathers had a university education. Similarly, the Preparatory year students for the College of Engineering were having the highest awareness (78.3%) with  $p=0.036$ . Other factors like age, marital status, residence, and other socio-demographic factors were found non-significant.

**Table IV: Association of Reproductive Health Knowledge Level with Demographic Variables**

Variables			Knowledge level of Reproductive Health		p-value
			Low Level	High Level	
Educational Status of Father	Uneducated	N	0	6	0.040
		%	0.0%	100.0%	
	Primary Education	N	6	26	
		%	18.8%	81.3%	
	Intermediate Stage	N	19	27	
		%	41.3%	58.7%	
	Higher School	N	38	66	
		%	36.5%	63.5%	
Preparatory Year Students' Bachelor program colleges	Diploma	N	4	17	0.036
		%	19.0%	81.0%	
	University	N	30	89	
		%	25.2%	74.8%	
	College of Business Administration	N	46	141	
		%	24.6%	75.4%	
	College of Clinical Pharmacy	N	4	4	
		%	50.0%	50.0%	
	College of Engineering	N	5	18	0.036
		%	21.7%	78.3%	
	College of Science	N	20	39	
		%	33.9%	66.1%	
	College of Medicine	N	15	14	
		%	51.7%	48.3%	
	College of Computer Science.	N	7	15	0.036
		%	31.8%	68.2%	

## Discussion

Our study provided a comprehensive understanding of reproductive health awareness among 18–to 21-year-old female university students. The exploration

of communication challenges, information-seeking behaviors, and the impact of educational strategies sheds light on the dynamics shaping their reproductive health perspectives.

Yadav et al.<sup>5</sup> stated that the age group 14–17 years is the most vulnerable group when it comes to reproductive health due to the lack of proper resources about SRH makes them seek information from unreliable places and sources, which can be dangerous because of myths and misconceptions contrary to this in the current study the students' age range was 18-21 and most of them showed better understanding in this regard.

There is a pivotal role of mothers in reproductive health discussions, with 41.2% of students confiding in their mothers in the study. This aligns with previous literature emphasizing the influential role of maternal figures in shaping adolescents' perceptions of reproductive health by Richards et al.<sup>11</sup> who stated that young females considered maternal figures as a source of advice, information, and resources. The presence of sisters and friends as significant confidantes highlights the importance of peer and familial support networks.

Our findings revealed varied learning behaviors among participants, with a substantial percentage gaining extensive knowledge about body changes during puberty and menstruation. However, fewer students possessed an in-depth understanding of the male and female reproductive systems and contraception. Published studies<sup>12,13</sup> have similarly identified a deficit in this profound comprehension, which can be attributed to inadequate education, limited knowledge, and insufficient awareness. These findings advocate for the implementation of targeted educational interventions that address specific aspects of reproductive health suggesting a need for targeted educational interventions addressing specific aspects of reproductive health.<sup>7</sup>

The study by Olamijuwon et al.<sup>2</sup> highlighted the substantial influence of social networks on sexual health information in contemporary society. Our research aligns with this finding as participants obtain reproductive health information primarily through television followed by news, advertisements, radio, and a minor percentage through social media. This underscores the importance of utilizing diverse communication

channels within social networks for reproductive health education. Therefore, educational interventions should leverage these varied channels to maximize their effectiveness.

Various hurdles are faced by students in accessing reproductive health information by the participants. The prevalence of uncertainties about information sources and feelings of shame or shyness are echoes of the findings from previous studies highlighting societal barriers to open discussions about reproductive health.<sup>3,4</sup> Hailemariam et al.,<sup>14</sup> also stated various hurdles, which included socio-cultural barriers, health system barriers, perceived legal barriers, inadequate information regarding sexual reproductive health services, and low parent-adolescent communication. Addressing these emotional and social barriers is crucial for effective reproductive health education.

The high educational status of fathers emerged as a notable factor with good awareness and knowledge scores of female students. This aligns with existing literature emphasizing the influence of parental education on adolescent health outcomes by Mikkonen et al.,<sup>15</sup> where parental education was a strong independent predictor of young females' sexual educational attainment. A study in India<sup>16</sup> stated that young college girls felt more convenient with their mothers to attain SRH information irrespective of maternal education which is also similar to the current findings. In Bangladesh<sup>7</sup>, the mother and female relatives were the source of information about SRH, which often contained misconceptions that may harm female health which is not consistent with the current study.

The study reflected that participants were aware of the transmission of sexually transmitted diseases (STDs). The recognition of high-risk behaviors, such as intercourse with multiple partners, aligns with literature emphasizing the importance of understanding transmission modes for effective prevention which parallels the findings of Kalichman et al.<sup>17</sup> However, gaps in knowledge, such as the uncertainty of transmission of STDs/HIV, highlight areas for focused education. Most of them had regular menstruation and used sanitary pads, which is contrary to the above-cited Bangladesh study where the majority used cloth as a vaginal pad, and also with Saudi western region studies<sup>18,19</sup> where the

majority of participants had revealed irregular menstrual cycles. Current study participants were aware of polycystic disease (PCOD) and its effects on menstrual irregularity and had facial hair. The consequence events of PCOD must be highlighted and young girls be informed to avoid its unhealthy effects on the female body and reproductive functions.<sup>20</sup>

## Conclusion

The study revealed gaps in female preparatory year students' awareness of reproductive health. It underscores the importance of enhancing the accessibility of reproductive health knowledge resources and improving communication channels with parents for young females to attain first-hand appropriate knowledge regarding all aspects of reproductive health. This can contribute to heightened awareness of sexual and reproductive health.

## Limitation of Study

The limitation of the study lies in its design and may not represent the broader population or students of other universities. Also, it would have been better if we had included the awareness of preparatory male students which could have served as a compatible comparison with females but due to logistics issues this comprises a second limitation of our study.

## Recommendation

Future research endeavors should encompass secondary school and university girls across all regions of KSA. By conducting comprehensive studies with large, representative population samples, we can identify and address gaps and deficiencies in knowledge and communication related to sexual and reproductive health on a large scale.

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#### CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

## To Determine the Biometric Markers Like Cheiloscopy Patterns in Pakistani Population

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## ABSTRACT

**Objective:** To determine the prevalence of cheiloscopy patterns in Pakistani population.**Study Design:** Cross-sectional study.**Place and Duration of Study:** The study was conducted at Post Graduate Medical Institute (PGMI), Lahore, Capital diagnostic Centre CDC Islamabad, HITEC Taxila for a duration of 12 months from 1<sup>st</sup> September 2021 to 31<sup>st</sup> August 2022.**Materials and Methods:** The lip prints of 320 males and females were taken by applying lip shades and then cellophane tapes (2 inches). They were transferred to an A4 sheet (white A4 ROCO Premium 80g copy papers) and then observed with magnifying hand lenses Deluxe magnifier (70mm diameter). The data was analyzed by IBM SPSS statistics 27.0. Chi square test was applied and a *p* value of  $\leq 0.05$  was considered statistically significant.**Results:** The results showed differences in all individuals in both lip prints. The predominant lip pattern was type III, observed in 159 (99.4%) male participants while it was observed in all the female participants. Type I' observed in 37 (23.1%) female participants and IV are greater in females 160(100%) than males. Type II (was) greater in males 152 (95.0%) than females.**Conclusion:** Lip prints are useful tool that can be used as biometric markers in dentistry and forensic odontology.**Key Words:** Cheiloscopy, Forensic Sciences, Lip Prints, Identification.

## Introduction

Outer surface of lip has many ridges and troughs leading to the formation of a distinctive format termed as lip prints. Evaluation of these prints is termed as cheiloscopy (cheilos refers to lips and skopien means to see).<sup>1</sup>

Establishing human identity is a complex process involving multiple domains. It relies on definitive

strategies and techniques, including analyzing biometric data, precise administration and assessment of dental records, or any other forensic evidence.<sup>2</sup> Forensic dentistry focuses on post-mortem identification of an individual and identification of the culprit from evidences that may be left.<sup>3</sup> The patterns on lips can play a crucial role in determining a person's identity. They can be used to confirm whether an individual was present at a scene of misconduct, making lip prints valuable as biometric tools.<sup>3</sup> Lip print evaluation can be useful in predicting gender, ethnic groups, the kind of criminal violation, in addition to forecasting the number of people associated with the unlawful offence.<sup>4</sup>

Lip prints can be found on exteriors of fabric, crockery, cigarette butts, envelopes, and eating utensils, and is usually seen as visible (patent) or hidden (latent). Hidden lip prints can be developed by administering soluble lipid staining dyes, certain reagents, chromatic dyes, aluminum powder, cobalt oxide, Nile red and magnetic powder. Conventional lipsticks generate a pattern of lip that can effortlessly be observed which is termed as visible lip print.<sup>4</sup> The creases and grooves on the lips begin to develop

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approximately on the 42<sup>nd</sup> day of gestation, and once fully formed, they remain permanent.<sup>4</sup> The patterns of lip have to be recorded within a day of expiration to avoid inaccurate findings that would change these prints from autopsy.<sup>5</sup> Lip prints can also be used in gender determination.<sup>6</sup>

Lip impressions were primarily studied in research where patterns without friction ridges were used to provide evidence. Lips also contain creases and wrinkles which are not present in finger prints and these can be categorized into multiple kinds for human certification.<sup>7</sup>

Any unlawful act requires recognition of both the affected and the accused.<sup>8</sup> Comparative interpretation is required in most cases of the chance imprints which are found on certain surfaces. The chances of these patterns developing from a region of the skin where friction ridges are absent have been observed. Odonatological, dactyloscopic and genetic records are possibly the most frequent strategies exercised in this aspect, enabling quick and safe recognition methods, as these cannot be always available sometimes it becomes inevitable to look for advance and novel methods.<sup>9</sup> The tendency of criminals to use deceptive methods during illegal activities has led to the use of lip prints as an additional tool in forensic dentistry.<sup>10</sup> Since the literature lacks data regarding the occurrence of patterns of lips in Pakistani population. This study aimed to determine the prevalence of lip prints in the Pakistani population to identify any gender-based differences.

## Materials and Methods

This cross-sectional study was conducted in Oral Biology Department of Post Graduate Medical Institute, Lahore and Capital Diagnostic Center, Islamabad, from 1<sup>st</sup> September 2021 to 31<sup>st</sup> August 2022. The Ethical approval was taken from ethical review committee PGMI Lahore Registration no.00-30-A-2024, in accordance with the ethical norms of the relevant committee on human experimentation i.e. The Helsinki Declaration of 1975, as revised in 1983. The data was collected and analyzed by single observer who was a qualified dental surgeon. The sample size was calculated by the following formula keeping the power of study equal to 80% and level of significance equal to 5%.<sup>11</sup>

$$n = \frac{\left( Z_{1-\alpha/2} \sqrt{2\bar{p}(1-\bar{p})} + Z_{1-\beta} \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right)^2}{\bar{p}} \quad \text{Where } \bar{p} = \left( \frac{p_1 + p_2}{2} \right)$$

(Sample Size determination in health studies version 2.0.21 WHO)

P1 is the anticipated proportions of lip pattern in male (Group A) = 45 %

P2 is the anticipated proportions of lip pattern in female (Group B) = 30%

p1 – p2 is the difference between proportions = 15%

Z<sub>1-β</sub> is the desired power of study = 80%

Z<sub>1-α/2</sub> is the desired level of significance = 05%

The calculated sample size was 320. Both genders (male and female) with an age range of 20-50 years were included in the study. People without established occlusion, any history of lip trauma, lip pathology or lip surgery Individuals with known hypersensitivity to lipsticks were excluded. After obtaining informed consent lip prints were obtained from the 320 participants, 160 males and 160 females.

Cheiloscopy patterns were documented and it was allotted a serial number from 1 to 321. The patterns were recorded on a self-designed format. Demographic details of subjects which included age, gender and address and National identity Card number (NIC) were documented on the consent form. These patterns were recorded by using lip shades, ear buds, cellophane tape and scissors. Ear buds were taken and hydrated with plain water and extra moisture was taken out by squeezing the ear buds then lipstick enough for upper lip was applied on one end. A single uniform motion was used to apply lip stick on the upper lip and then on the lower lip using opposite side of the ear bud. Sticking side of a cellophane tape was used to get the lip prints, applying uniform pressure and it was removed by a single attempt. Many attempts were made by applying the shade only once and scotch tape multiple times until a clear pattern showing all details in all quadrants was visible and identification of various areas of the lips were established. It was made sure that uniform pressure was applied to the upper and lower lip and then pasted on an A4 paper which was used as a permanent record. These patterns were then carefully observed by one

observer with the help of a magnifying lens deluxe magnifier (75 mm Diameter) to study them in detail in each topographic area Figure 1, classifying them according to Suzuki classification.<sup>9</sup> All varieties of lip prints can be present on one lip the quantification of dominant pattern was calculated on numerical superiority of properties of prints in the study. Each lip print was divided into six topographic areas like upper right, upper middle, upper left, lower right, lower middle and lower left as shown in figure 1 and then they were examined by magnifying hand lenses, photographed and then they were documented by the Microsoft Office Picture Manager.



**Fig 1: Image Showing Six Topographic Areas from A Female Subject**

The data was analysed by using Statistical Package for Social Sciences (SPSS) version 27.0. Numeric data like age was presented in form of mean  $\pm$  S.D. Categorical data like type of lip prints and was presented in form of frequency. The Chi square test and Fischer's exact were used to compare the type of lip prints between males and females. Mann-Whitney U test was used to compare the number of different types of lip prints between males and females. The  $p$  value of  $\leq 0.05$  was considered statistically significant.

## Results

Our results showed that the predominant lip pattern was type III, and the least common variety was type V as shown in Table I in terms of frequency and percentages. Type I' II, IV showed statistically significant results whereas type I, III and type V pattern of lip prints were not statistically significant as shown in table I. All participants showed that no two lip patterns were same.

## Discussion

Lip prints are exclusive patterns and are unique for every human just as fingerprints are particular for everyone. There is need for contributions from oral biologists, anatomists and forensic odontologists to

**Table I : Distribution of Lip Prints Between Male and Female Study Participants (n=320)**

Pattern of Lip Prints	Males	Females	$p$ Value
Type I	140 (87.5%)	136 (85.0%)	0.516
Type I'	22 (13.8%)	37 (23.1%)	0.031*
Type II	152 (95.0%)	77 (48.1%)	< 0.001*
Type III	159 (99.4%)	160 (100.0%)	> 0.999
Type IV	138 (86.3%)	160 (100.0%)	< 0.001*
Type V	18 (11.3%)	14 (8.8%)	0.456

The  $p$  value of  $\leq 0.05$  was considered statistically significant

recognize the diverse features, patterns, prints and other landmarks in the maxillofacial domain. This study showed that patterns of the lip prints were extremely diverse in shapes and sizes yet very unique to every individual. The most common lip print found was type III in both genders. In our study only one male did not show type III lip pattern all 319 subjects had type III variety. Type IV was found in all females and 138 males.

The most frequently occurring pattern of lip prints in males was type III followed by type II, type I, type IV, type I' and type V. The most dominant pattern of lip prints in females was type III followed by type IV, type I, type II, type I' and type V. A study conducted by Alzapur *et al.*,<sup>12</sup> showed that distribution of males to females in type I was seen in 45% and 27%, type 1' in 28% and 14%, and type 2 in 10% and 35%, type 3 in 7% and 5%, type 4 in 2% and 4% and type 5 in 8% and 15% which showed that type I was the common found lip print and type V was the least common. Current study showed that variation exist among different populations in terms of most predominant pattern. In contrast to our study among the Malaysian-Chinese subjects, type V grooves were predominant when both lips were considered together, in all parts of the lower lip. A study conducted by Chadha *et al.*,<sup>13</sup> showed that type III grooves were predominant in the upper lip and all portions of the upper lip evaluated separately in this study. A review of the literature by Maheswari *et al.*,<sup>14</sup> concluded that the most frequent pattern was pattern IV among persons enrolled in it and this also supported our study.

In current study the upper and lower lip prints showed difference in distribution of the type of lip prints. A study with similar results to our study conducted by Kenneth *et al.*,<sup>15</sup> in Nigeria showed different patterns in terms of abundance yet type III was the most abundant.

In a recent study conducted by Tanoli *et al.*,<sup>16</sup> studied the inheritance pattern in 54 families of Pakistani population type I, II, III and IV were found in both males and females. Type II was most common type. Type V was least common in female, whereas type V lip print was not found in males. In our study also, the type V variety of lip prints were the least dominant.

A study conducted by Kaur *et al.*,<sup>17</sup> showed that various basic and combination patterns showed predominance in one of the genders, most of the patterns were not confined to a particular gender. A systematic review by Franco *et al.*,<sup>18</sup> revealed weak foundations for the use of lip print analysis for sexual dimorphism in forensic dentistry. Our study also revealed that there was no association of dominant patterns of lip prints with gender ( $p$  value 0.373) and no significant difference was observed in mean number of lip prints of type I, type IV and type V between the both genders.

Longadon *et al.*,<sup>19</sup> showed that all the quadrants of an individual's lip showed different types of patterns and every quadrant had multiple patterns present similar to our results. It was found that most of the lip print patterns did not comprise of only one pattern but consisted of a mixture of varying types. Hence individuals had similarity in the grooves but the detailed features of lip prints varied. BR *et al.*,<sup>20</sup> found that quality of lip prints depends on the method used and concluded that digital photography can be employed for instant and detailed result.

A study conducted by Samudhrasi *et al.*,<sup>21</sup> suggested the need for studying lip prints in depth and establishing further facts by encouraging research on cheiloscopy. Chaware *et al.*,<sup>22</sup> established that a systematic method should be adopted for the selection, formation, documentation and the final analysis of these patterns. So, it is need of the hour to explore further the applications of cheiloscopy in biometrics and society in general.

## Conclusion

Lip prints are useful tools that can be used as biometric markers in dentistry and forensic

odontology. Variations exists among prevalence of type of lip prints among genders, but every human has lip prints unique to them.

## Limitations

The study is cost effective, yet it requires knowledge and expertise for observation and analysis of individual lip patterns which consumes a lot of time. Recent digital technology can be used for quick and more precise results. Lipstick method provides reliable results but digital photography can provide more accurate results.

## Recommendations

This study opens avenues for broader research including various ethnic groups to check the prevalence in our population and maintain a record of lip prints in our community for academic and forensic reasons. The subject specialists must take responsibility for further documentation and research of lip prints in their region and associate it with other biometric markers.

## Conflict of Interest

none

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#### CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

## Use of Tele Dentistry in Orthodontic Patients During the Covid Pandemic

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## ABSTRACT

**Objective:** This study was aimed to assess the impact of tele dentistry on bracket breakage and orthodontic plaque index during the covid pandemic.

**Study Design:** An experimental study.

**Place and Duration of Study :** The study was conducted from 1st April 2020 to 1st March 2021 in the orthodontic department of Rawal Institute of Health Sciences Islamabad.

**Materials and Methods:** Seventy-six orthodontic patients were selected from the orthodontic department of Rawal institute of Health Sciences. Each patient had a minimum orthodontic plaque index of 1 and experienced at least four bracket breakages. The treating doctor provided counseling and communicated with the patients via WhatsApp messages, offering them the opportunity to reach out at any time with questions. After a period of three months, the patients were reassessed clinically. A paired sample t-test was applied to evaluate the pre and post counseling values pertaining to bracket breakage and the orthodontic plaque index.

**Results:** The findings revealed that by using tele dentistry incidence of bracket breakage reduced from 20% to 5% in most of the study participants. Similarly mean plaque index was reduced from 1.78 to 0.53 after online counselling session and WhatsApp messages over a period of three months.

**Conclusion:** This study highlights the potential of tele dentistry not only to enhance access to oral healthcare and its delivery but also to reduce bracket breakage incidents and promote superior oral hygiene in orthodontic patients.

**Key Words:** Bracket, Covid 19, Plaque Index, Tele Dentistry.

## Introduction

In the light of the challenges posed by the COVID-19 pandemic on global healthcare access, healthcare providers and their teams are grappling with the need to adapt their practices accordingly.<sup>1</sup> Dental professional bodies and decision-makers are tasked with offering The SARS-CoV-2 virus, which causes COVID-19, is abundantly found in the nasopharyngeal and salivary secretions of infected patients.<sup>2</sup> It is believed to spread mainly through respiratory droplets, as well as via aerosols and contaminated surfaces (fomites) guidance in an ever-changing landscape, drawing from current data, research, and established knowledge.<sup>3</sup>

Continuous advancements in information communication technology and the widespread availability of social media platforms have guided clinicians to creatively overcome traditional barriers in both clinical and technological workflows.<sup>4</sup>

Telemedicine has emerged as a recognized and regulated tool to address the COVID-19 pandemic, aiming to narrow the gap between healthcare services and the population. By minimizing physical contact between healthcare professionals and patients, tele medicine contributes to the containment of the virus.<sup>5</sup> Amidst shutdowns, certain dentists have implemented tele health services to offer virtual care to their patients.<sup>6</sup> This approach involves utilizing various mediums such as phone calls, photographs, and videos to facilitate the delivery of oral healthcare and oral health education services.<sup>7</sup>

For patients undergoing orthodontic treatment, daily oral hygiene presents a particular challenge. To mitigate the heightened risk of caries and gingivitis, it is imperative for patients to undergo intensified individual prophylaxis throughout the entirety of their orthodontic treatment. This involves regular

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oral hygiene check-ups coupled with motivation and instruction on oral hygiene practices.<sup>8</sup>

The landscape of orthodontic practice has been significantly altered by the COVID-19 pandemic. Stringent infection control measures, minimal aerosol generation, and a focus on minimal-touch dentistry are crucial in preventing contamination within the orthodontic operator. During this period, only emergency orthodontic procedures should be administered to patients, while strictly adhering to regulatory guidelines.<sup>9</sup> The concept of Dentistry 4.0 represents the fourth revolution in dentistry, leveraging modern technologies to enhance patient care.<sup>10</sup> Numerous messenger and video-calling applications are currently available, offering opportunities for hospitals and dental clinics to engage with their patients.<sup>11</sup>

Implementing virtual triage through the utilization of photos, videos, and video-calling capabilities can prove invaluable in distinguishing and prioritizing genuine orthodontic emergencies requiring immediate attention in clinics from issues that can be managed with home remedies and deferred without clinic visits.<sup>12</sup> As we navigate the current landscape, it's imperative to shift focus from solely addressing urgent dental needs towards strategizing the resumption of routine dental care. This entails devising innovative approaches to cater to patients who may be hesitant to visit dental offices in the foreseeable future, amidst the ongoing uncertainty surrounding the pandemic timeline.

The objective of this research was to advocate for the effective utilization of tele dentistry in orthodontic care during crisis scenarios like the COVID-19 pandemic, as well as its ongoing integration into non-crisis situations. This paper was planned to provide insights supporting the adoption of tele dentistry as a promising avenue for orthodontists, in reducing bracket breakage and improving oral hygiene.

## Materials and Methods

This experimental study was conducted during the peak time of Covid pandemic from 1st April 2020 to 1st March 2021 in the orthodontic department of Rawal Institute of Health Sciences RIHS Islamabad. Permission was taken from the ethical review Board of RIHS on 03/02/2020 under letter number RIHS/IRB/D/23/003. Purposive sampling was done, and 80 patients were selected who fulfilled the

inclusion criteria of minimum four bracket breakages with orthodontic plaque index value of 1. Informed consent was taken from every patient. Patients above 12 years of age were included in the study. Syndromic and highly non-compliant patients were excluded. The researchers along with four house officers collected the data. Confidentiality and the privacy of patients were diligently upheld throughout the course of the study, employing measures that encompassed the destruction of any potentially identifying data gathered during the tele dentistry session, including mobile phone numbers and photographs, after the assessment's conclusion. Prior to the study's initiation, all participants were dispatched an informed consent form via WhatsApp, wherein they were required to respond with the term 'accept' accompanied by their name, thereby granting informed consent. These records were retained as substantiation of their willingness to take part in the study. Four patients failed to respond to the consent request, resulting in their exclusion from the study, thus yielding a sample size of 76 patients. The patients were given appointments after every twelve weeks instead of routine four weeks to reduce the number of patients, thus reducing workload as a measure of precaution during the pandemic. It was found that most of the patients reporting for routine adjustment during pandemic had an increased number of bracket breakages along with poor oral hygiene because of the longer gap in appointments. So, a study was designed to know the impact of tele dentistry counselling on bracket breakage and plaque index during this span of time. Inclusion criteria was set as adolescent patients undergoing orthodontic treatment from last twelve months and reporting with minimum number of at least three bracket breakage. Orthodontic Plaque index (OPI) score was also assessed in the same patient with a minimum OPI score of 1. Every patient was reappointed after three months. During this time a video call once a month and What's app message related to oral hygiene instructions twice a month was scheduled as a reminder to improve oral hygiene leading to lesser number of bracket breakage. Thus, a total of three video calls by the treating doctor and six What's app messages on oral hygiene and eating habits were scheduled as a reminder for the patient. During the video call

patients were examined and instructed and motivated about eating habits, brushing timing and techniques. Furthermore, patients were authorized to submit intraoral photographs through WhatsApp, and seek advice from the treating orthodontist promptly, in the event of any emergencies. When the patient reported after three months for routine adjustment, the number of bracket breakage and plaque index score was again recorded to assess the change. The Orthodontic Plaque Index (OPI) constitutes a specialized metric tailored for patients undergoing treatment with fixed orthodontic appliances. The dental arch is subdivided into sextants, and the OPI primarily targets the region proximal to the bracket. Plaque accumulation is assessed on a scale ranging from 0 to 4, with consideration given to various tooth surfaces adjoining the bracket base, including the mesial, distal, occlusal/incisal, and cervical aspects.

In our study OPI was recorded in upper anterior sextant only. The OPI scoring criteria was as follows: 0: No plaque deposits on the tooth surfaces surrounding the bracket base 1: Plaque deposits on one tooth surface at the bracket base 2: Plaque deposits on two tooth surfaces at the bracket base 3: Plaque deposits on three tooth surfaces at the bracket base 4: Plaque deposits on four tooth surfaces at the bracket base and/or gingival inflammation indicators (plaque deposits near the gingiva do not necessarily have to be present).<sup>8</sup>

The initial measurements, termed BR1 for bracket breakage and PI1 for the orthodontic plaque index, were recorded prior to counseling. Subsequent measurements, labeled BR2 for bracket breakage and PI2 for the orthodontic plaque index, were taken following counseling sessions. Statistical analysis was conducted utilizing SPSS 21 software. Frequency of bracket breakage (BR1, BR2) was assessed in pre and post-counseling sessions. A paired sample *t*-test was used to assess the disparity between the pre and post-counseling values for orthodontic plaque index (PI1, PI2).

## Results

In our study, we analyzed a sample size of 76 participants, comprising of 51 females (67.1%) and 25 males (32.89%). The mean age of the sample was 16.7 years. The frequency of bracket breakage is detailed in Table 1. Notably, in 26 cases (30%) of

bracket breakage (6 brackets) were reported during the pre-counseling period. However, this frequency decreased significantly to 1 bracket (5%) in 28 cases following counseling via tele-dentistry. The mean plaque index in our sample was initially recorded at 1.78 before the administration of oral hygiene instructions through tele dentistry. This value decreased to 0.53 after a three-month counseling period, which included guidance sessions and WhatsApp messages (Table II). A paired sample *t*-test (Table III) was conducted to compare pre- and post-counseling values for orthodontic plaque index. The results were statistically significant, with a *p*-value of 0.001. The confidence interval for bracket plaque index ranged from 1.011 to 1.489.

Only two patients reported with wire poking and were seen in an emergency setting to have the wire cut. The most common issue reported by patients, accompanied by pictures, was bracket breakage, accounting for 55 cases (73%) of complaints, followed by ligature decay 15 cases (20%). Additionally, nearly 49 patients (65%) expressed concerns about treatment delays.

**Table I: Frequency of Bracket Breakage in Pre and Post Counselling Session with Tele Dentistry**

Pre-Counselling Session		Post Counselling Session with Tele Dentistry	
Number of cases assessed n=76	Frequency of bracket breakage Br1 in each case	Number of cases assessed n=76	Frequency of bracket breakage Br 2 in each case
15	4 (20%)	14	0 (0%)
12	5 (25%)	28	1 (5%)
26	6 (30%)	20	2 (10%)
10	7 (35%)	8	3 (15%)
8	8 (40%)	6	4 (20%)
5	9 (45%)	nil	nil

**Table II: Descriptive Statistics for Age, Bracket Breakage and Plaque Index (N= 76)**

	Minimum	Maximum	Mean	St. Deviation
Age (Years)	12	43	16.71	6.224
Br1	4(20%)	9 (45%)	_____	_____
Br2	0 (0%)	4 (20%)	_____	_____
PI1	0	3	1.78	.932
PI2	0	2	.53	.599

Br1: Pretreatment bracket breakage

Br2: Post treatment bracket breakage

PI1: Pretreatment plaque index

PI2: Posttreatment plaque index

**Table III: Paired sample T test for Plaque Index**

		Mean	Std Deviation	Std Error Mean	Confidence Interval		T	Df	Significance	
					Upper	Lower			One Sided P	Two sided P
Pair	pl1-pl2	1.250	1.047	.120	1.011	1.489	10.406	75	<.001	<.001

## Discussion

Our research highlights that delays in orthodontic appointments, as seen during the COVID-19 pandemic, are related to an increased frequency of bracket breakage and higher plaque indices. It is important to note that the implementation of WhatsApp and tele dentistry counselling methods significantly reduced these issues, lowering bracket breakage from 30% to 5% and the plaque index from 1.78 to 0.53. The average age of our sample population was 16.8 years. Consistent with the findings of Ammar et al.<sup>13</sup>, we observed a higher incidence of bracket breakage among teenagers compared to adults. Adolescents are more likely to have elevated levels of supragingival plaque and a higher prevalence of gingivitis than adults, which supports the selection of the 12-18 years age range for our study.

Teleconsultation, telediagnosis, and telemonitoring are elements of tele dentistry that have the potential to engage patients undergoing orthodontic treatment, which often requires cooperation from the patient.<sup>14</sup> Similar to this, our study revealed that using WhatsApp messages and videos strategically can motivate patients to alter their eating habits and improve their oral hygiene, resulting in a significant decrease in broken brackets and an improvement in the orthodontic plaque index.

In a study conducted in Genova, it was determined that participants who received post-treatment reassurance through communication (text message and calls) exhibited greater compliance in oral hygiene than the control group patients.<sup>15</sup> Similarly, another study conducted in Pakistan demonstrated a reduction in plaque index during orthodontic treatment in the text message group, thereby proving its efficacy in enhancing oral hygiene.<sup>16</sup> These findings align with our results, which illustrate that tele dentistry methods can improve oral hygiene and bracket failure in orthodontic patients. However, unlike our study, Naveda et al found that during the COVID-19 quarantine, orthodontic patients exhibited a low frequency of orthodontic appliance interference in daily life, and most of them reported

maintenance of oral hygiene habits.<sup>17</sup> This behavioral difference can be attributed to the age difference, as the patients in our study were predominantly teenagers. As for bracket breakage, another study revealed that 15.42% (n = 54) of all patients had  $\geq 1$  bracket bonding failure during the COVID-19 pandemic.<sup>18</sup> Chugh et al found in their study that the maximum number of patients reported bracket debonding four months after the lockdown. Nearly half of the patients reported debonding of at least one of the brackets.<sup>19</sup> This is likely due to the indefinite postponement of orthodontic treatments and the impact of lockdown conditions, which may reduce the motivation of orthodontic patients.<sup>20</sup> Under such conditions, orthodontic patients may consume foods that are not allowed in their diet, leading to poor oral hygiene and the loss of orthodontic appliances, which may slow down the treatment process. In a study on Brazilian orthodontists conducted during the COVID-19 pandemic, the primary reasons for orthodontic appointments were bracket breakage (67.6%), arch wire issues (60.5%), and problems with tubes and/or bands (44.1%). Among these, stainless steel fixed appliances emerged as the most prevalent type associated with breakage and unscheduled visits.<sup>21</sup> Similarly, in our study, bracket breakage was identified as the primary concern among patients, accounting for 73% of cases, followed by ligature decay of 25%.

Our study's limitation is that the time between orthodontic appointments, eating and brushing habits could not be maintained uniformly in all patients. Furthermore, there is a need for additional research on the etiology of bracket breakage, as it emerged as a significant concern among patients in our study. Upcoming research endeavors ought to involve larger sample sizes, as our study's inclusion of only 76 patients may prove insufficient to draw definitive conclusions, especially in the climate of uncertainty and challenge.

## Conclusion

This study underscores the potential of tele-dentistry to not only improve access to oral



healthcare and its delivery but also to reduce incidents of bracket breakage and promote better oral hygiene among orthodontic patients. Although tele-dentistry may not completely replace traditional methods, it serves as a valuable adjunct to the compromised dental system during this unprecedented health crisis.

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**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

## Effect of Duration and Number of Subjects on Students' Performance in Junior Clerkship

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## ABSTRACT

**Objective:** In the current study we evaluated the impact of the number of examination subjects and the length of teaching duration on student performance in the fourth professional examination.

**Study Design:** Comparative cross-sectional study.

**Place and duration of Study:** Shifa College of Medicine, Islamabad from 1<sup>st</sup> Jan 2021 to 30<sup>th</sup> June 2022.

**Materials and Methods:** The study enrolled 220 students from the batches of year 2021 and 2022. We analysed the effect of duration of the clinical teaching (2 years vs. 3 years) and the effect of number of examining subjects in professional examination (3 subjects vs. 4 subjects) on the students' final scores. The data was analysed by SPSS version 23.0. The mean scores and standard deviation for theory and objectively structured clinical exam (OSCE) were compared using independent sample t-test. The results were also compared for the number of failing students and high scores (> 80 %) between two study cohorts. The *p* value of  $\leq 0.05$  was considered statistically significant.

**Results:** No statistically significant difference was found in the scores of ophthalmology theory /OSCE, gynaecology & obstetrics (OB/GYN), ENT theory and pediatric OSCE. The mean scores were significantly better in OB/GYN OSCE (*p* value < 0.015) and ENT OSCE (*p* value < 0.001) after 2 years of teaching/ four subjects in professional examination respectively. Pediatrics theory results showed a decline after two years of teaching as compared to three years of teaching (*p* value < 0.001).

**Conclusion:** The number of years of clinical teaching and the total number of subjects in professional examination have no impact on students' academic performance in fourth professional examination.

**Key Words:** Assessment, Clerkship, Gynae, Pediatrics, Quality improvement.

## Introduction

The clerkship model of medical teaching has shown to enhance students' knowledge, skills, attitude and professionalism. They demonstrate their ability to care for patients in hospital setting as well as at community-based clinics.<sup>1, 2</sup> Medical education department of the institutes decides the curriculum and optimal time for clerkships in collaboration with national regulatory body.<sup>3</sup> While each medical school has a slightly different setup, core clinical year rotations are essentially the same everywhere. Shifa

college of medicine (SCM) has a 5 years MBBS programme. The Integrated modular curriculum is delivered over the first 3 years of the MBBS programme, and clinical rotations are introduced in 3<sup>rd</sup> year for integration of clinical teaching with modular curriculum. The junior clerkship starts in 4<sup>th</sup> year and progresses to senior clerkship in 5<sup>th</sup> year.

Medical educationists and clinicians have been calling for modifications in curriculum design of undergraduate medical teaching.<sup>4,5</sup> Since its introduction, clerkship programme is under constant reforms as influenced by internal or external factors. However, there are many challenges at each step of making these reforms.<sup>6,7</sup> The family medicine clerkship and elective clinical rotations were introduced to the 5<sup>th</sup> year MBBS curriculum in the year 2022 after developing learning outcomes of all the clinical disciplines. This curricular modification in the 5<sup>th</sup> year mandated changes in 4<sup>th</sup> year clerkships schedule as well. Before 2022, the final year MBBS professional examination was conducted for the disciplines of OB/GYN, pediatrics, medicine and

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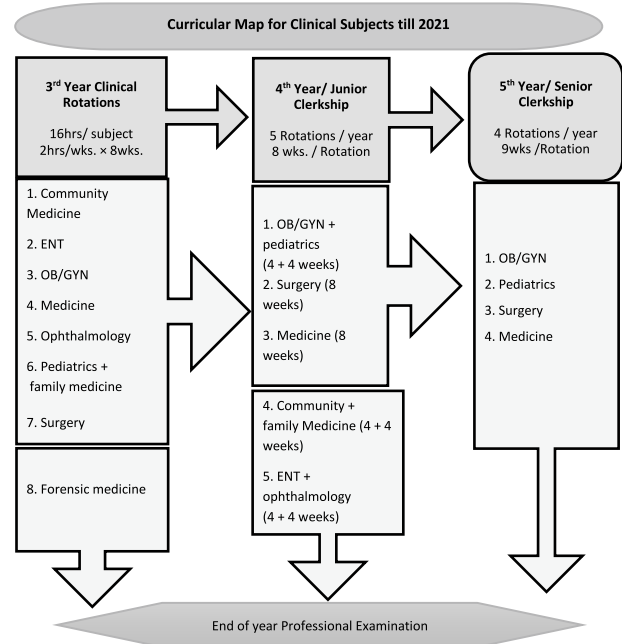
surgery. The 4<sup>th</sup> year MBBS students took 4<sup>th</sup> professional examination in 3 subjects (ophthalmology, ENT and community medicine) as shown in Figure 1. Starting from 2022, the students in 4<sup>th</sup> year MBBS were required to appear in professional exams for ophthalmology, ENT, OB/GYN and pediatrics. Community medicine was added to the year 3 curriculum (Figure 2). The number of subjects, duration and professional examination for clinical subjects before and after year 2022 are shown in Figure 1 and 2 respectively.

Apparently, the curricular shift decreased the overall duration of study for OB/GYN and pediatrics from 3 years to 2 years. It did not decrease the total number of contact hours for these subjects. After a lot of brainstorming and collective efforts of clerkship directors, students and medical educationists, the institutional learning outcomes (ILOs) were revised for above mentioned clerkships. With an increase in number of examining subjects in 4<sup>th</sup> year and a reduction in an overall duration for pediatrics and OB/GYN clerkships to 2 years, both the students and faculty felt a definite stress. The literature has described the pressure of time constraint, heavy workload and fear of failure as main sources of stress for medical students. Padmashiri *et al.*,<sup>8</sup> reported overall increase of anxiety scores in students during examination period. Sujatha *et al.*,<sup>9</sup> also reported similar findings.

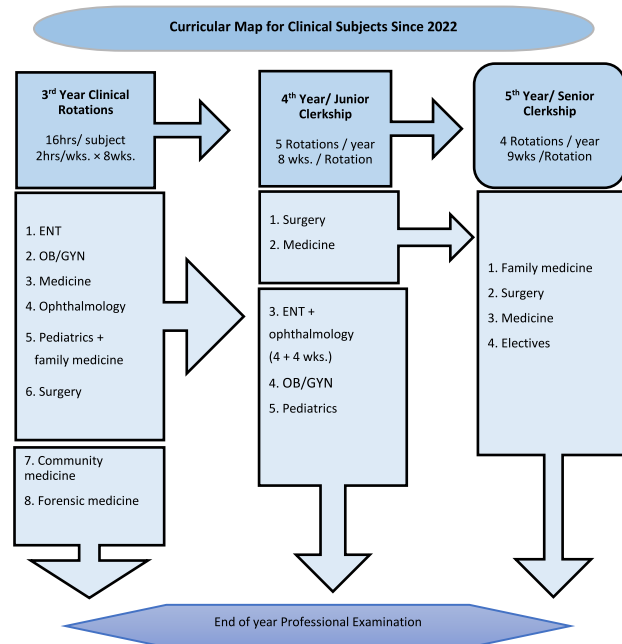
In the present study, we compared the scores and success rate of students appearing in exams for OB/GYN and pediatrics with a 3 year teaching programme of clinical rotation, junior and senior clerkship vs. 2 years teaching programme of clinical rotation and junior clerkship. We also compared the students' performance with the increased number of examination subjects in 4<sup>th</sup> professional examination. Currently, the literature, exploring the effect of shortened clerkship duration on student performance is lacking from this part of the world. So, a research was planned to evaluate the impact of the number of examination subjects and the length of teaching duration on student performance in fourth professional examination.

## Materials and Methods

This cross-sectional study (IRB # 366-21) was conducted at Shifa Tameer-e-Millat University/Shifa College of Medicine (SCM) from 1<sup>st</sup> January 2021 to



**Fig 1: Clinical Clerkship and End of Year Examination Model Year 2021**



**Fig 2: Clinical Clerkship and End of Year Examination Model Year 2022**

30<sup>th</sup> June 2022. The results of the 4<sup>th</sup> professional examination of students from the batches of 2021 and 2022 were retrieved from examination department. All the students (220) from both batches were included. For the batch of 2021, professional examination included 3 subjects: ophthalmology, ENT and community medicine. For

the batch of 2022, the 4<sup>th</sup> professional examination included 4 subjects: ophthalmology, ENT, pediatrics, OB/GYN.

The theory and OSCE scores of ophthalmology, ENT, pediatrics and OB/GYN were compared between the two batches.

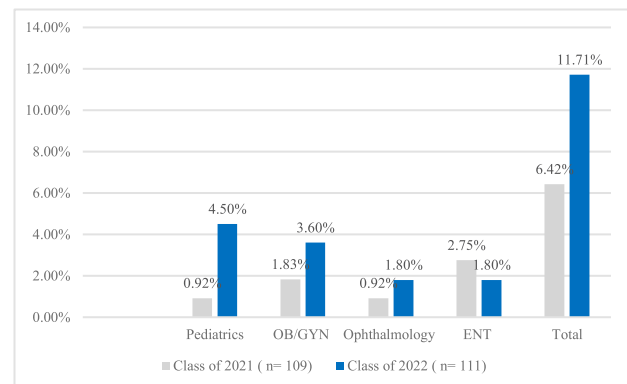
The students' scores were analysed by SPSS version 23.0. The Leven's test for equality of variance was used to measure homogeneity of variance between groups. The data was checked for normal distribution by using Kolmogorov-Smirnov test. The independent sample t-test was used to compare the means for normally distributed data (pediatrics theory & OSCE, ophthalmology theory and ENT OSCE) and Mann-Whitney U test was used if the scores did not have a normal distribution (OB/GYN theory & OSCE, ophthalmology OSCE & ENT theory). The Fisher's exact test was used to see if significant difference exists in overall pass/fail rate. The Chi square test was applied to see if significant difference exists between two batches in terms of high scores > 80 %, pass and fail rates. The *p* value of  $\leq 0.05$  was considered statistically significant.

## Results

The total number of students was 220. There were 109 students in the batch of 2021, and 111 in batch of 2022. The mean age of students in the batch of 2021 was  $22.87 \pm 0.630$  years while for the batch of 2022, it was  $23.17 \pm 0.884$  years. In cohort of 2021, males were 49 (45%) and females were 60 (55%). While in the batch of 2022, males were 42 (38%) and females were 69 (62%). While analysing the effect of duration of clinical teaching on the student scores, scores for pediatrics and OB/GYN were compared. The student's scores in professional examinations for the two academic years are shown in Table I. The mean scores of the batch of 2021 were significantly better ( $p = 0.001$ ) in pediatrics theory as compared to the batch of 2022 and the upper range for scores was also better for these students. The Chi square test showed significant difference in pediatrics theory and there were more ( $n=13$ , 11.9%) students with >80 % scores in the batch of 2021 vs none in the batch of 2022 i.e.  $p$  value < 0.001. (Table II). In OB/GYN OSCE students showed better mean scores after 2 years of teaching.

While analysing the effect of number of examining subjects on the students' final scores, scores of

students with 3 subjects in 4<sup>th</sup> professional examination versus 4 subjects in 4<sup>th</sup> professional examination, no statistically significant difference was found in the scores of ophthalmology theory, OSCE and ENT Theory. The mean scores of the batch of 2022 ( $64.25 \pm 7.03$  vs  $74.16 \pm 6.38$ ) were better in ENT OSCE ( $p$  value < 0.001). (Table I). The Fisher's exact test showed no significant difference in overall pass/fail rate in theory and OSCE in ophthalmology, ENT, pediatrics and OB/GYN. The Chi square test showed significant difference in the students' performance in ENT OSCE,  $p < 0.001$ . There were more students with >80 % scores ( $n=19$ , 17.1%) in the batch with four examining subjects. (Table II). The total number of failures (11.71% vs 6.42%) was higher in the batch of 2022 as compared to the batch of 2021. The failure rates in the batches of 2021 & 2022 are shown in Figure 3.



**Fig 3: Failure rates in batch of 2021 & 2022**

## Discussion

A lot of need based changes are being called in undergraduate curriculum by the medical educationists and clinicians.<sup>4</sup> Hence, the clerkship programmes are under continuous reform. This study is an audit of a major reform in the curriculum of 4<sup>th</sup> and 5<sup>th</sup> year MBBS which was a new experience for both the students and faculty.

The students showed better mean score in OB/GYN OSCE with 2 years of teaching as compared to the batch with 3 years of teaching ( $p$  value = 0.015) Table I. One of the factors for improved results with restructured curriculum could be increased student patient interaction in almost all clerkships. We obtained comparable results in OB/GYN theory examination. Edward *et al.*,<sup>10</sup> reported different results. They described that the students' scores

**Table I: Student's Scores in Professional Examinations (n=220)**

Subject	Batch of 2021 (n= 109)	Batch of 2022 (n= 111)	K-S test	P* value	Subject	Batch of 2021 (n= 109)	Batch of 2022 (n= 111)	K-S test	p* value
	3 yrs. teaching	2 yrs. teaching				3 exam Subjects	4 exam Subjects		
<b>Pediatrics Theory</b> Mean $\pm$ SD, Median, Range	71.66 $\pm$ 7.88 72.06 45.63 – 87.67	65.01 $\pm$ 6.61 65.04 45.45 – 78.09	0.200	<b>&lt;0.001</b>	<b>Ophth Theory</b> Mean $\pm$ SD, Median, Range	67.43 $\pm$ 6.82 67.58 48.90 – 81.30	69.14 $\pm$ 7.03 69.63 43.95 – 82.24	0.063	0.069
<b>Pediatrics OSCE</b> Mean $\pm$ SD, Median, Range	68.11 $\pm$ 7.04 68.70 50.0 – 83.8	69.36 $\pm$ 7.13 69.98 48.75 – 82.78	0.503	0.192	<b>Ophth OSCE</b> Mean $\pm$ SD, Median, Range	66.75 $\pm$ 7.01 66.85 48.54 – 81.26	67.93 $\pm$ 5.57 68.26 47.30 – 80.13	0.048	0.279
<b>OB/GYN Theory</b> Mean $\pm$ SD, Median, Range	127.01 $\pm$ 12.72 128.41 95.62 – 151.87	126.18 $\pm$ 13.11 127.60 73.72 – 149.47	0.038	0.879	<b>ENT Theory</b> Mean $\pm$ SD, Median, Range	69.20 $\pm$ 7.86 70.15 45.30 – 84.05	70.71 $\pm$ 7.85 71.98 41.46 – 85.44	0.019	0.155
<b>OB/GYN OSCE</b> Mean $\pm$ SD, Median, Range	141.59 $\pm$ 15.59 143.84 97.81 – 178.01	146.66 $\pm$ 11.38 148.08 109.05 – 172.69	0.000	<b>0.015</b>	<b>ENT OSCE</b> Mean $\pm$ SD, Median, Range	64.25 $\pm$ 7.03 64.65 47.50 – 78.90	74.16 $\pm$ 6.38 74.80 56.60 – 86.40	0.100	<b>&lt;0.001</b>

\*The *p* value less than .05 was considered statistically significant.

\*Summary: t-test Independent sample/ Mann-Whitney U Test, K-S: Kolmogorov- Smirnov, ophthalmology: Ophth

**Table II: Comparison of Student's Performance in Professional Examinations**

Disciplines	Batch of 2021 (n= 109)	Batch of 2022 (n= 111)	Fisher's exact test <i>p</i> -values	Batch of 2021 (n= 109)	Batch of 2022 (n= 111)	<i>p</i> values Chi- square test
	<50% score	<50% score		> 80 % score	> 80 % score	
Pediatrics Theory	1 (0.92%)	5 (4.50%)	0.212	13 (11.9%)	-	<b>&lt;0.001*</b>
Pediatrics OSCE	-	2 (1.8%)	0.498	6 (5.5%)	5 (4.5%)	0.354
OB/GYN Theory	1 (0.92%)	4 (3.6%)	0.369	-	-	0.190
OB/GYN OSCE	1 (0.92%)	-	0.495	9 (8.3%)	7 (6.3%)	0.508
Ophthalmology Theory	1 (0.92%)	1 (0.9%)	1.000	1 (0.92%)	4 (3.6%)	0.409
Ophthalmology OSCE	1 (0.9%)	1 (0.9%)	1.000	1 (0.9%)	1 (0.9%)	1.000
ENT Theory	-	2 (1.8%)	0.498	9 (8.3%)	8 (7.2%)	0.360
ENT OSCE	1 (1.8%)	-	0.244	-	19(17.1%)	<b>&lt;0.001*</b>

\*The *p* value less than .05 was considered statistically significant.

were lower in subject examination when duration of the OB/GYN clerkship was decreased from 8 to 6 weeks.

Regarding pediatrics theory examination, the mean scores of the batch with 2 years teaching were less as compared to 3 years teaching, (*p* value < 0.001). In contrast to our observation Lindsey *et al.*,<sup>12</sup> did not find any statistically significant difference (*p* value <

0.322) in USMLE Step 1 scores in pediatrics between groups with traditional vs. shortened teaching duration.<sup>11</sup> According to literature, such curricular modifications involving changes in one whole year of teaching, can decline students' performance. Moreover, each student of the class cannot be expected to retain 100 percent of knowledge from lectures.<sup>13</sup> The self-study habits, revision of the



syllabus and ability of clinical application of knowledge varies from student to student.<sup>14, 15</sup> The students' maturity, reasoning skills, and understanding of the subjects and clinical correlation of knowledge increases with spending a whole year in clinical rotations in various disciplines.<sup>16</sup> In pediatrics we had 5 students who were unsuccessful in theory exam in shortened duration cohort ( $p$  value  $<0.001$ ). Based on the results of our study, the learning objectives for pediatrics were modified with better integration of pediatrics, family medicine and internal medicine component. Recently, the University of Michigan had published a study of their clinical clerkship curriculum changes. The study showed that a 25% decrease in clerkship length resulted in no significant difference between student performance.<sup>16</sup> Some studies show no difference or even better student satisfaction and clerkship evaluation scores for shortened curricula.<sup>17</sup>

In pediatrics OSCE, the results were comparable for both batches ( $p$  value = 0.192). The OSCE evaluates the clinical skills of students.<sup>18</sup> Our group of students were given extra classes /sessions for practice of clinical skills. Hence, individual student-patient interaction was increased in pediatric clerkship, resulting in improvement of skills and persistent performance in spite of decreased duration. Many other, individual student trends are also influencing their exam performance like strong motivation, aptitude for study and spending less time in social networking.<sup>19</sup> Similar trend in performance of the cohorts in pediatrics and ophthalmology OSCEs appears to be due to the focus of OSCE on core clinical conditions. Our institution's robust pre-clerkship clinical skills course, case-based discussion, and time spent in clinical patient care enabled our students to perform better in OSCE. Additional factors may be the variety of physicians with whom students interact, observe and learn through different practices and teaching approaches.<sup>20, 21, 22</sup>

There was no change in students' scores in ophthalmology with 3 or 4 subjects in professional examination. The reason for this could be that there was no change in duration of ophthalmology clerkship.

In ENT OSCE 13 students scored  $>80\%$  ( $p$  value  $<0.001$ ) which was the highest number of students achieving top scores in a subject in that year. This

distinction could be due to interest in the subject.<sup>23</sup>

Our study has certain limitations. We compared scores of only one academic year. Comparison of scores of two or more successive years might give a broader perspective of the curricular change. We recommend that further studies should be conducted including student's perspective about shorter duration of clerkship and subjects of interests. Another limitation which can be addressed in future studies is catering for the confounding factors like faculty training and factors affecting student's stress and interest for a speciality.

## Conclusion

The number of years of clinical teaching and total number of subjects in professional examination have no impact on students' academic performance in fourth professional exam.

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#### CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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## ORIGINAL ARTICLE

**Healthcare Professionals' Attitudes, Challenges, and Potential Solutions for Conducting Research: A Mixed-Method Study**Sidra Tul Muntaha<sup>1</sup>, Farhan Hassan<sup>2</sup>, Afifa Kulsoom<sup>3</sup>, Sadaf Asma<sup>4</sup>, Yasira Abbasi<sup>5</sup>**ABSTRACT**

**Objective:** To explore healthcare professionals' attitudes toward research, identify key barriers they face, and uncover potential solutions for overcoming these obstacles, as proposed by the professionals themselves.

**Study Design:** Exploratory Sequential Design Mixed method study.

**Place and Duration of Study:** It was conducted from 15th April 2022 to 15th Oct 2022 among health professionals working in tertiary care hospitals of Rawalpindi and Islamabad.

**Materials and Methods:** A total of 276 junior to mid-level health professionals were included through nonprobability consecutive sampling. The quantitative part was done using a structured close ended questionnaire regarding research barriers. The SPSS version 24.0 was utilized for statistical analysis of quantitative data.

**Results:** In this study a total of 276 health care professionals were included. Male and female doctors were 144 (52.2%) & 132 (47.8%) respectively. Regarding attitude of research 121 (43.8 %) participants believed that research promotes critical thinking and 116 (42.0%) considered it most helpful to improve patients' care. 133 (48.2%) participants attended research training of minimum 8 hours. The common barriers to research as identified by health professionals are absence of support especially technical guidance, less time allotted for research, less funding opportunities, no proper mentoring or supervision, lack of knowledge and skills about research.

**Conclusion:** Health professionals face several research barriers, including a lack of technical support, time, funding, mentorship, and research skills. The absence of a research culture and logistical support further complicates the situation, leaving them overwhelmed and without time for research. Addressing these challenges is essential for enabling impactful research.

**Key Words:** Barriers, Health Research, Health Care Professionals, Junior Faculty, Solutions.

**Introduction**

Research is considered a systematic assessment, that includes research proposal development, testing hypothesis and evaluations, with the aim to add to the generalizable body of knowledge.<sup>1, 2</sup> There is an increasing need for the utilization of evidence-based medicine in clinical practice and that requires good

quality scientific research too.<sup>3</sup> This demands proper research knowledge and experience as well as time, financial assistance, and a team of scientists.<sup>4,5</sup>

Many studies have been done previously on medical students as well as postgraduate trainees to find out their interest in health research and barriers while conducting their research work.<sup>6</sup> A study conducted by Auf Al et al.,<sup>7</sup> on teaching faculty stated that main barriers in research are lack of funding, financial incentives followed by work stress. Lack of allocated time, statistical, supervisor and research skill were also main barriers in research. Another study on health care workers concluded that doctors have a positive attitude towards research but they find it to be a stressful task as well.<sup>8</sup> A study conducted by Asaar A et al; on doctors analyzed that lack of time, insufficient research training, improper mentorship, and deficient statistical support are the main barriers to research.<sup>9</sup> Khalaf AJ et al., reported that 76.5% of the respondents mentioned that the time allowed

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for conducting research is not appropriate and is one of the most significant barriers. They also pointed out that no financial (63.0%) and statistical support (50.0%) is available for facilitating the research process.<sup>3</sup>

The rationale of study was that it is the need of an hour to find out the barriers and their relevant solutions in the process of research, to fill the gap and create a research conducive environment in health institutions. Scientific gap in literature still prevails because studies on research barriers are not only lacking nationally but also internationally. Since there is a lack of evidence on research barriers and possible solutions especially considering doctors perceptions, there is a need to conduct further research to reach some positive conclusions. Moreover, there is paucity of evidence generated through in-depth qualitative exploration of perceptions of doctors regarding solutions to barriers and their suggestions to overcome these barriers. So, we conducted this study with the aim of identifying the attitude of health care professionals of different institutes belonging to different specialties. This study was aimed at identifying barriers faced by health care professionals while conducting research. The novelty of this study is that it aimed at in-depth exploration of the ideas and suggestions of health care professionals to overcome the health research barriers to fill the gap in literature to some extent. This study addressed the gaps and barriers to research and recommends suggestions to overcome the barriers thus promoting research. The Objective of study was to explore healthcare professionals' attitudes toward research, identify key barriers they face, and uncover potential solutions for overcoming these obstacles, as proposed by the professionals themselves.

### Materials and Methods

An Exploratory Sequential Design Mixed Method study was conducted from 15<sup>th</sup> April 2022 to 15<sup>th</sup> October 2022. Junior to mid-level health care professionals (House officers, medical officers, Registrars and Assistant professors) working in tertiary care hospitals of Rawalpindi and having some prior experience of conducting health research were included. Undergraduate medical students and post graduate trainees were not included in this study. Sample size was calculated to be 276 using

WHO sample size calculator and following statistical assumptions: Confidence level=95%, Alpha error = 5, Relative precision = 5%, Anticipated proportion mentioning insufficient research time allotted = 76.5%.<sup>3</sup> Approval from ethical committee was taken via letter no 0-83-22/09/2022. Nonprobability consecutive sampling technique was employed to select the sample. Quantitative data was collected by administration of pre-determined and validated closed ended questionnaire. Permission from the primary author to use the pre-validated questionnaire was sought through email. A written informed consent was taken from all participants. The identity of all participants in terms of their names and institutes was kept confidential. The questionnaire was based on demographic details like age, sex, professional experience, degree, designation. The second part was based on questions regarding attitude and barriers of research. It was measured on a Likert scale (1-5 points) where the higher score showed a positive attitude and more barriers. The survey questionnaire was filled out online by all participants. Data was entered and analyzed with statistical program SPSS version 24. Descriptive statistics were calculated for quantitative numerical variables such as mean and standard deviation for age and professional experience. The categorical variables like gender, attitude towards research and possible barriers were measured as frequency and percentages. The demographic characteristics were associated with positive attitude and possible barriers towards health research. ANOVA was applied to compare attitudes towards research based on age, years of practice, designation and qualification.  $P < 0.05$  was taken as significant.

Qualitative part of the data was collected through In-Depth Interviews (IDIs) and Focus Group Discussions (FGDs). A total of 3 FGDs and 10 IDIs were conducted. An open-ended questionnaire was developed to assess the ideas and perceptions of respondents towards possible solutions in conducting health research. The methods of data collection during focus group discussion included audio and tape recording, note-taking and participant observation. The study investigator moderated all the interviews herself using a semi structured data collection form (open ended questionnaire). Among participants of

1<sup>st</sup> FGD there were 3 Medical officers (MOs), 2 Registrars and 1 Assistant Professor. In 2<sup>nd</sup> FGD there were 6 MOs and in 3<sup>rd</sup> FGD there were 6 Registrars. Similarly, the individual interviews were conducted using the same data collection form from 3 House officers, 3 Medical officers, 2 Registrars and 2 Assistant Professors. All interviewees belonged to the same population on whom the quantitative survey was done. For qualitative interviews and FGDs verbal consent was taken from interviewees before recording their perceptions and views regarding barriers and possible solutions to health research. For the qualitative part, notes and recordings of the 10 interviews and 3 focused group discussions were converted into transcripts. A Five steps thematic analysis was done as: organization of notes and recordings as transcripts, reading and review of transcripts, development of codes from transcripts, conversion of codes into themes and subthemes, review the themes. Triangulation was done for validation of qualitative data. For this purpose a qualified medical educationist and a content specialist from an independent medical institute were referred to. They were involved in thematic analysis in all 5 steps. They confirmed and ensured the obtained data in the form of themes and subthemes that almost matched their perceptions.

## Results

In this study a total of 276 health care professionals were enrolled. Males and females were 52.2% & 47.8% respectively. Most of the respondents were in the age groups of 25 to 30 and 31 to 35 years. Four groups of years of practice were made as shown in Table I.

Information about involvement in Research Activities, independently conducting Research and Research Trainings are shown in Table II.

Attitude of Health Care Professionals towards Research are shown in Table III.

Barriers faced By Health Care Professionals in Conducting Research Shown in Table IV.

Perceptions of health care professionals towards research based on gender difference revealed that there is no significant difference in the male and female attitude towards the research work as  $t=1.21$ ,  $p>.05$ . Both males and females' attitude towards research was positive and they believed that research is helpful in many ways.

**Table I: Respondents Demographic Information (n=276)**

Variables	Groups	Number	Percentage
Gender	Male	144	52.2%
	Female	132	47.8%
Age	Less than 25	30	10.9%
	25 to 30	97	35.1%
	31 to 35	74	26.8%
	36 to 40	51	18.5%
	41 to 45	13	4.7%
	46+	11	4.0%
Years of Practice	less than 5	152	55.1%
	5 to 10	75	27.2%
	11 to 15	25	9.1%
	More than 15	24	8.7%
Designation	House officer	44	15.9%
	Medical officer	101	36.6%
	Registrar	48	17.4%
	Assistant professor	26	9.4%
	Other*	57	20.7%
Highest Qualification	MBBS /MD	173	62.7%
	Diploma	10	3.6%
	MCPS	16	5.8%
	FCPS	58	21.0%
	Other	19	6.9%

Others\*: General Physicians and General practitioners.

**Table II: Information about involvement in Research Activities, independently conducting Research and Research Trainings (N=276)**

Question Statements	YES n (%)	NO n (%)
Presently involved in any research activity	116 (42.0%)	160 (58.0%)
Conducted a research by your own	144 (52.2%)	132 (47.8%)
Attended any research training so far at least 8 hours	133 (48.2%)	143 (51.8%)

**Table III: Perceptions of HCP Towards the Significance of Conducting Research**

	1 Not Helpful n (%)	2 Somewhat Helpful n (%)	3 Helpful n (%)	4 Very Helpful n (%)	5 Most Helpful n (%)
Promotes critical thinking	5(1.8%)	7(2.5%)	58(21.0%)	85(30.8%)	121(43.8%)
Improves patients care	5(1.8%)	5(1.8%)	51(18.5%)	99(35.9%)	116(42.0%)
Helps in promotion	6(2.2%)	5(1.8%)	47(17.0%)	96(34.8%)	122(44.2%)
Helps professional enhancement	3(1.1%)	2(0.7%)	44(15.9%)	98(35.5%)	129(46.7%)
Helps to change health policy	6(2.2%)	11(4.0%)	47(17.0%)	90(32.6%)	122(44.2%)



**Table IV: Barriers Faced By Health Care Professionals in Conducting Research (N=276)**

	1 Not a Barrier	2	3 Somewhat Barrier	4	5 Maximum Barrier	Mean	SD
	n(%)	n(%)	n(%)	n(%)	n(%)		
Insufficient research allotted time	8 (2.9%)	17(6.2%)	81(29.3%)	82(29.7%)	88(31.9%)	3.82	1.044
Lack of research training and skills	3(1.1%)	12(4.3%)	48(17.4%)	77(27.9%)	136(49.3%)	4.20	.950
Lack of statistical support	3(1.1%)	9(3.3%)	41(14.9%)	85(30.8%)	138(50.0%)	4.25	.903
Lack of mentorship and teamwork	1(0.4%)	12(4.3%)	42(15.2%)	91(33.0%)	130(47.1%)	4.22	.885
Insufficient financial support	3(1.1%)	17(6.2%)	53(19.2%)	72(26.1%)	131(47.5%)	4.13	.999
Technical & logistic support like computer and internet not easily available	26(9.4%)	39(14.1%)	60(21.7%)	82(29.7%)	69(25.0%)	3.47	1.266
Lack of self interest and motivation	18(6.5%)	31(11.2%)	88(31.9%)	77(27.9%)	62(22.5%)	3.49	1.149
Lack of communication and linkages with other institutions	5(1.8%)	13(4.7%)	65(23.6%)	92(33.3%)	101(36.6%)	3.98	.978
Lack of financial incentives	4(1.4%)	17(6.2%)	55(19.9%)	76(27.5%)	124(44.9%)	4.08	1.011

Attitude towards Research Based on Age, Years of Practice, Designation and Highest degree are shown in Table V.

**Table V: Attitude towards Research Based on Age, Years of Practice, designation and highest degree**

		Mean	SD	F	p	95%CI
Age (years)	Less than 25 (n=30)	4.02	0.86	.277	0.01	3.69, 4.34
	25 to 30 (n=97)	4.02	0.72			3.87, 4.16
	31 to 35 (n=74)	4.30	0.70			4.14, 4.46
	36 to 40 (n=51)	4.30	.622			4.13, 4.48
	41 to 45 (n=13)	3.93	.660			3.53, 4.33
	46+ (n=11)	4.49	.500			4.15, 4.82
Years of Practice	Less than 5 (n=152)	4.03	.737	4.19	0.006	3.91, 4.15
	5 to 10 (n=75)	4.35	.677			4.19, 4.51
	11 to 15 (n=25)	4.19	.722			3.89, 4.49
	More than 15 (n=24)	4.36	.529			4.14, 4.59

Designation	House officer (n=44)	3.97	.709	1.448	0.218	3.75, 4.19
	Medical officer (n=101)	4.16	.710			4.02, 4.30
	Registrar (n=48)	4.28	.626			4.10, 4.46
	Assistant professor (n=26)	4.31	.612			4.06, 4.56
	Other (n=57)	4.13	.831			3.91, 4.35
Qualification	MBBS /MD (n=173)	4.11	.739	1.577	0.181	4.00, 4.22
	Diploma (n=10)	4.52	.413			4.22, 4.81
	MCPS (n=16)	3.98	.850			3.53, 4.44
	FCPS (n=58)	4.28	.665			4.11, 4.46
	Other (n=19)	4.24	.598			3.95, 4.53

Barriers faced by health care professionals towards conducting Research Based on Age, Years of Practice, Designation and Highest degree showed no significant mean score difference with  $p>.05$ .

Only mean score difference was found in various groups of designations as  $p<.05$ . The mean level (4.27) of research barriers was greater in Assistant professors compared to other professional cadres in this study. This shows that the higher the mean score, the better the attitude of the higher designation category.

### Qualitative Findings

The review and detailed observation of the transcriptions led the researcher to code the verbatim. Based on synthesis and coding of transcriptions, various themes and subthemes were drawn. The list of themes is mentioned below, and the sub-themes have been described accordingly as per the perceptions and ideas of the various respondents of the in-depth interviews.

### Discussion

In this study, a total of 276 medical doctors in their early to mid-career were enrolled. The average age was  $29.1 \pm 4.7$  years and most of the study respondents were found between 25 to 35 years in the current study. In a local study the majority of the junior faculty members of medical universities of Pakistan were male and more than or equal to 35 years of age<sup>10</sup> A comparative study from Bahrain by

**Table VI: Thematic Analysis of Possible solutions**

<b>Theme: Common barriers and probable solutions to research</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Proper training and technical assistance</li> <li>• Reducing overburden from hospitals</li> <li>• Availability of proper facilities and environment for research</li> <li>• Adequate time allocation for research</li> </ul>
<b>Theme: Research environment</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Logistics like internet and computer facility</li> <li>• Encouraging organizational culture</li> </ul>
<b>Theme: Coordination and communication</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Advocacy and awareness regarding research</li> <li>• Availability of financial support</li> </ul>
<b>Theme: Solutions for improvement of research facilities</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Technical support</li> <li>• Financial incentives</li> </ul>
<b>Theme: Capacity development</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Training workshops</li> <li>• Specialized focused classes</li> <li>• Research in medical curriculum</li> <li>• Proper research mentoring</li> </ul>
<b>Theme: Statistical and technical support</b>
<b>Sub-theme:</b>
Institutionalization of statistical support
<b>Theme: Fake data and research corruption</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Penalties for research corruption</li> <li>• Strict policy for plagiarism</li> </ul>
<b>Theme: Further suggestions for improvement of research</b>
<b>Sub-themes:</b>
<ul style="list-style-type: none"> <li>• Coordination and research channelization</li> <li>• Awareness regarding developments and advancements</li> <li>• Focus on local communities for finding local solutions to problems</li> </ul>
<b>Theme: The diseases are changing patterns and shapes</b>
<b>Sub-theme:</b>
<ul style="list-style-type: none"> <li>• Proper logistical and technical support</li> </ul>

Khalaf *et al.*, witnessed mean age of  $43.0 \pm 4.7$  years in their study respondent physicians.<sup>3</sup> Similarly, in the current study just by slight margin but the males constituted the majority (52.2%). Many previous studies have also witnessed this stratum and there is no significance of respondents' gender.<sup>11,12</sup> Most of the evidence revealed female gender preponderance.<sup>13</sup> When the attitude of doctors towards research was assessed in the current study, it was noted that research studies were helpful in doctors' professional enhancement such as promotion of critical thinking, support policy change

and beneficial for patient care. Many previous research studies on this topic had similar findings. A study by Khalaf J *et al* also found that junior doctors had a positive attitude towards research.<sup>3</sup> Another undergraduate medical student's-based study witnessed that most of the students agreed that research is beneficial in their profession, they had an overall positive attitude towards research and believed that it is relevant to their profession.<sup>14</sup>

In our study most of the interviewees mentioned that there is a lack of training, non-availability of mentors and logistic support. Atreya *et al* in their study observed similar findings.<sup>15</sup> In the current study, the common barriers faced by the junior doctors were lack of multiple factors like; statistical support, research allotted time, research training and skills, mentorship, financial support or incentives. There is huge evidence regarding barriers faced by medical students and medical faculty in conducting research as witnessed by many investigators.<sup>16,17</sup> A previous study from Pakistan by Sabzwari *et al.*, also witnessed similar findings.<sup>10,18</sup> Khalaf *et al.*, also witnessed that insufficient research allotted time, lack of financial support, financial incentives and technical support in terms of statistics were the major barriers.<sup>3</sup> Another recent study by Assar *et al.*, assessed perceived barriers towards research in medical students and found out that lack of access to lab equipment, lack of time due to educational tasks, poor attention given to researchers, lack of funding, poor collaboration between different academic departments and research centers, insufficient research skills, lack of research space, lack of professor input and lack of familiarity with research studies were the key barriers as thought out by junior medical professionals.<sup>17</sup> These are similar to findings observed by our study. Pakistan is a lower middle-income country; the research budget and opportunities are also very few or rare.<sup>19</sup> One of the key barriers is lack of opportunity to conduct research and lack of supervision and mentorship skills for research in the country. Our study also observed that the solution to these limitations could be proper training of health care professionals. Capacity development in the shape of training workshops, specialized classes, and hiring of qualified and proficient research mentors could

solve this issue. Demirtas A et al and Habineza H et al found similar solution to these barriers.<sup>20,21</sup>

Following themes and subthemes were identified by qualitative part of our study:

- Proper training and technical assistance
- Decreasing burden in hospitals during duty
- Provision of proper research environment and in-house facilities
- Proper time allocation for conducting research, at least 1 to 2 hours daily or 4 to 5 hours weekly
- Communication skills, especially learning analytical and writing skills
- Financial support
- Technical support in terms of statistical support
- Developing a research culture and organizational attitude
- Proper coordination and channelization of research activities

There is no proper guidance, mentorship facility or logistical support. The supervisors are often heads of the departments, they have many other tasks like administration of wards, clinical consultations & teaching. Due to lack of research and writing capacity, many of mentors and supervisors are not well versed with research basics, so often they avoid indulging or discussing it.<sup>22</sup> The solution for this limitation could be proper training and grooming of health care professionals in their mid and advanced level of training, especially, with focused and courses and hands on regarding scientific research planning, conduct and analysis as well as writing skills.

The issue of unawareness, lack of coordination and communication is affecting the already poor research status of our medical institution. Capacity development in the shape of training workshops, specialized classes, and hiring of qualified and proficient research mentors could solve this issue.<sup>23</sup>

One of the possible solutions identified in our research was proper training and technical assistance. Technical support, especially statistical issues are one of the barriers and weaknesses, there is a need to institutionalize statistical support. Thus, statistical support is crucial if one wants to conduct research and improve the overall research environment.<sup>3, 18</sup> Scientific research provides an opportunity to identify and understand emerging

threats as well as predict future challenges and issues.<sup>24</sup> Like all research this study also had a few limitations. Primary health care physicians working in primary & secondary health care weren't included.

## Conclusion

Health professionals face several research barriers, including a lack of technical support, time, funding, mentorship, and research skills. The absence of a research culture and logistical support further complicates the situation, leaving them overwhelmed and without time for research. Addressing these challenges is essential for enabling impactful research.

## Recommendations

- By conducting advocacy and refresher courses regarding research opportunities and advancements, one can solve the issue of lack of information among healthcare professionals.
- By providing technical support, especially statistical support in medical institutions and hospitals, this issue can be solved as claimed by most of the interviewees in the present study.
- To enhance and promote overall research culture, the senior faculty and mentors could be trained and sensitized regarding basics of research so that they can guide junior doctors and other colleagues while conducting research.
- An environment of teamwork and coordination for research activity needs to be institutionalized.

## Conflict of Interest

There was no conflict of interest in this study.

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**CONFLICT OF INTEREST**

Authors declared no conflicts of Interest.

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Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector.

**DATA SHARING STATMENT**

The data that support the findings of this study are available from the corresponding author upon request.

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## CASE REPORT

### Caruncular Oncocytoma in an Elderly Pakistani Female: A Case Report

Fariha Sahrish, Sahar Iqbal, Sara Masood Cheema, Mehwish Niazi, Amema Hafiz, Maira Jabbar Chaudhary

#### ABSTRACT

Caruncular Oncocytoma (CO) is a benign epithelial tumor, frequent in elderly females. Caruncular lesions are rare compared to conjunctival lesions, with a reported incidence of 0.3 to 1.1 %, and histologically proven oncocytoma are 0.3 million/year. The mitochondrial and somatic gene mutations, resulting in defective oxidative phosphorylation, are responsible for oncocytoma. This reduces ATP, resulting in a decrease in energy production, which, in compensation, increases mitochondrial content. The common complaint is epiphora, which is excessive lacrimation. CO is composed of oncocytes with fine, eosinophilic cytoplasm containing numerous mitochondria. A 60-year-old lady presented in an Ophthalmology outdoor clinic with a small, nodular, painless, reddish-colored swelling at the inner canthus of the left eye for three years. No clinical complaints and visual disability were noted. On physical examination, a mass with overlying, reddish-colored mucosa was located at the medial canthus of the left eye. Excision in Toto was done. Grossly, there was a 10 mm × 5 mm × 5 mm smooth surfaced, red-colored mass with round contours. Microscopy showed a well-encapsulated mass with overlying thinned-out stratified squamous epithelium and sebaceous glands. The underlying lesion is composed of tall, columnar, uniform cells with round to oval nuclei, fine chromatin, and abundant granular cytoplasm.

**Key Words:** *Caruncle, Oncocytoma, Mitochondria.*

#### Introduction

Caruncular Oncocytoma (CO) is a benign glandular epithelial/subepithelial tumor composed of granular abundant eosinophilic cytoplasm with para-central nuclei. Oncocytomas are also noted in other organs, i.e., thyroid gland, parathyroid gland, adrenal gland, kidneys, and gastrointestinal tract.<sup>1</sup> The most commonly reported are renal oncocytomas. Regarding head and neck region, OC are reported in lacrimal gland, salivary glands, caruncle, bulbar conjunctiva, and plica semilunaris. Caruncle is a bilateral triangular structure present distal to the medial canthus. Caruncular lesions are rare as compared to conjunctival lesions, with a reported incidence of 0.3 to 1.1 %, and histologically proven OC are 0.3 million/year.<sup>2</sup> CO, or oxyphil cell adenoma, is a benign tumor that presents unilaterally. Ninety five percent of CO is benign. Apart from caruncle, it is

also noted in the lacrimal gland, sac, conjunctiva, and eyelid margins. The mitochondrial and somatic gene mutations, resulting in defective oxidative phosphorylation, are responsible for oncocytoma. This reduces ATP, resulting in a decrease in energy production, which, in compensation, increases mitochondrial content.<sup>3</sup> The common complaint is epiphora, which is excessive lacrimation. Slit-lamp examination is the initial investigation of choice. Complete surgical resection is the treatment of choice. There is minimal chance of recurrence. CO is an uncommon tumor but should be included in the list of differential diagnosis. CO is composed of oncocytes with fine, eosinophilic cytoplasm containing numerous mitochondria.<sup>4</sup>

#### Case

A 60-year-old female patient presented in an Ophthalmology outdoor clinic. There was a small, nodular, painless, reddish-colored swelling at the inner canthus of the left eye for three years (Figure 1A). Clinically, the patient did not report any symptoms such as lacrimation, pain, discharge, or bleeding. On visual examination, visual acuity and ocular motility were unremarkable. She did not give any history of eye trauma, weight loss, or surgery was provided. On physical examination, a mass with overlying, reddish-colored mucosa was located at

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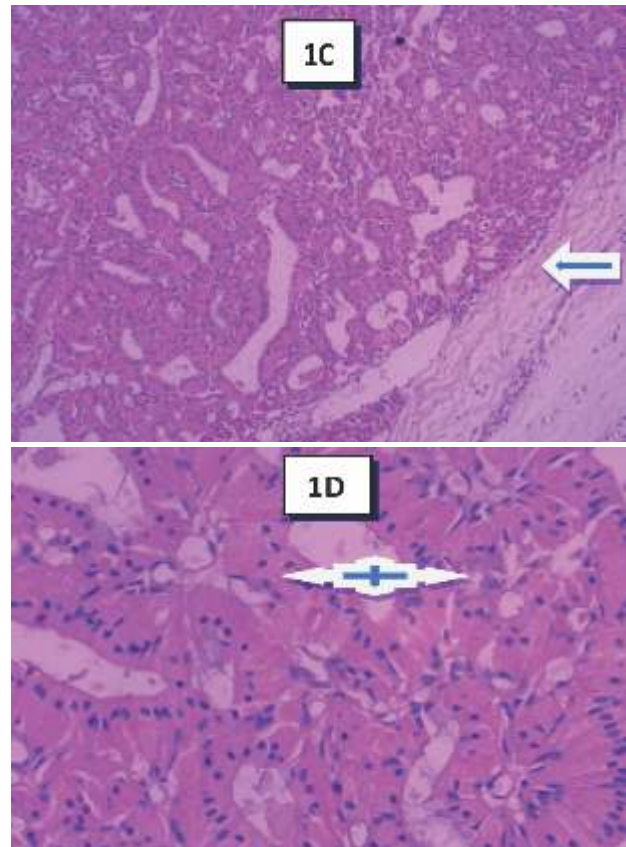
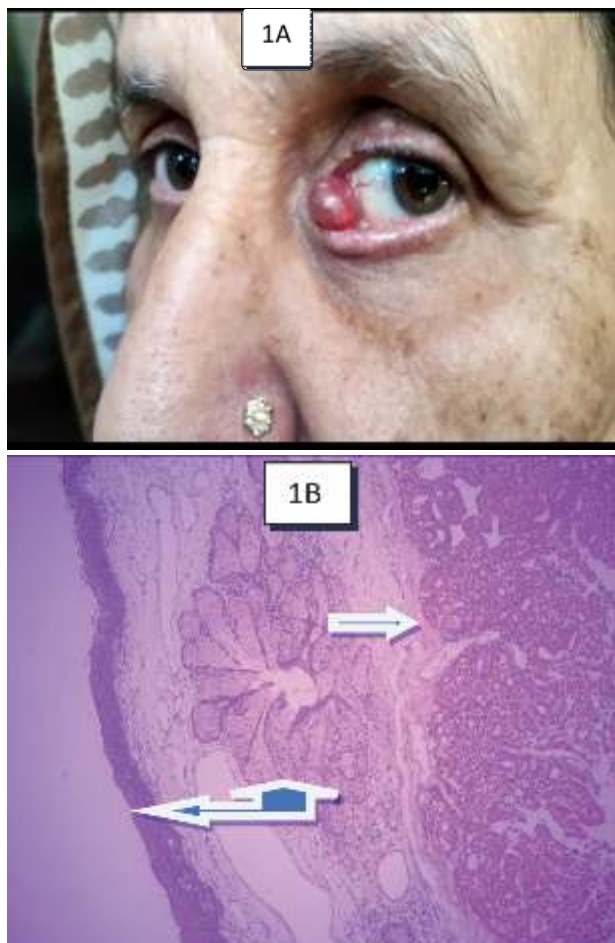
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the medial canthus of the left eye. Her baseline blood investigations were normal in range. The patient was prepared for surgery and the lesion was excised via a transcaruncular approach by giving a vertical incision in the conjunctiva between the caruncle and plica semilunaris. The mass was excised in toto and sent for histopathological examination. Gross examination showed there was a 10 mm × 5 mm × 5 mm smooth surfaced, red-colored mass with round contours. The cut surface was cystic brown. Microscopy showed a well-encapsulated mass with overlying thinned-out stratified squamous epithelium and sebaceous glands (Figure 1B). The underlying lesion was composed of tall, columnar, uniform cells with round to oval nuclei, fine chromatin, and abundant granular cytoplasm. Dark round to ovoid paracentral nuclei were noted. The oncocytes were arranged in solid cords and tubular structures with cystic spaces. Interspersed lymphoplasmacytic infiltrate was also noted. No cytological atypia, mitosis, and necrosis were found (Fig 1C&D).



**Fig 1A:** Presence of a tumor in the medial canthus of left eye. **Fig 1B:** A panoramic view shows skin (arrows) with underlying sebaceous glands and well circumscribed lesion arranged in lobules. **Fig 1C:** H&E stain shows a well circumscribed lesion predominantly arranged in glandular pattern. **Fig 1D:** H&E shows a high-power view of lesion arranged in glandular pattern. The neoplastic are large containing abundant eosinophilic cytoplasm and small round nuclei on the apical side.

## Discussion

The case of CO in an elderly Pakistani female highlights the rarity and distinctive pathology of this benign tumor, characterized by its prevalence in the elderly and its primary manifestation at the caruncle. The successful excision with no recurrence emphasizes the effectiveness of surgical intervention. Despite being rare, CO is being reported by various case reports and studies around the globe. OC are oncocytes, which are modified epithelial cells with large size and voluminous granular, eosinophilic cytoplasm. Electron microscopic examination showed a cytoplasm packed with abnormal and dysfunctional variable-sized irregular mitochondria. Clinically, differentials include squamous papilloma, lobular capillary

hemangioma, melanocytic nevi, and basal cell carcinoma.<sup>1-4</sup>

This case report from Pakistan contributes to the limited available data on CO in South Asia, where few cases have been documented. For example, Alam MS *et al.*,<sup>2</sup> and Mitra S *et al.*,<sup>4</sup> reported one and two cases over two decades, and Clemens AC *et al.*,<sup>1</sup> documented nine cases over 22 years. Our case is unilateral and solitary, consistent with reports by Clemens AC *et al.*,<sup>1</sup> Alam MS *et al.*,<sup>2</sup> Heathcote JG *et al.*,<sup>3</sup> and Fenelon EM *et al.*,<sup>6</sup> although some cases of bilateral CO have been noted. The slow growth over three years aligns with the findings by these authors. The duration of symptoms for CO typically ranges from two months to five years, with Alam MS *et al.*,<sup>2</sup> reporting a mean of 36 months and Heathcote JG *et al.*,<sup>3</sup> noting 3-4 years, while Mitra S *et al.*,<sup>4</sup> reported the earliest duration at four months. Rest of the cases are described in Table I and II for detailed demographic comparison of index case with total cases, CO cases, site of lesion, and time-period noted.

The reported average age is 68 years, and most COs

were seen at more than 55 years of age, with female preponderance. The index study showed a 60-year-old female like the one reported by AC Clemens *et al.*,<sup>1</sup> and Heathcote JG *et al.*,<sup>3</sup>. However, in contrast, Luthra *et al.*,<sup>7</sup> reported a young male patient of 30 years in 1978, and Fenelon EM *et al.*,<sup>6</sup> reported an oncocytoma of the lacrimal gland in a 4-year-old African girl. The differential diagnosis is narrowed down according to the color and size of tumors noted in the caruncle. Our study showed that the left eye's inner medial canthus is 10mm in size. Similarly, Indian author Mitra *et al.*,<sup>4</sup> reported 7 and 11 mm-sized tumors. However, one case reported by Heathcote JG *et al.*,<sup>3</sup> was 7mm, and in contrast, another case was 23mm in size. The clinical appearance of CO in the index case was a reddish round mass, similarly, reported by AC Clemens *et al.*,<sup>1</sup> however, the color and size of lesions reported do not affect the prognosis.

### Conclusion

CO are rare benign tumors frequent in elderly females. No clinical complaints, and visual disability were noted. Excision in Toto is the treatment of

**Table I: A Detailed Comparison of Index Case with Case Series with International Studies**

S.No	Author	Year of publication	Country	Age in years	Gener	Site of tumor
1	Fenelon <i>et al</i> <sup>6</sup>	2015	Brazil	40	Female	Lacrimal Gland Presented with proptosis
2	Surakiatchanukul <i>et al</i> <sup>5</sup>	2017	USA	81	Female	Caruncle Clinically mimicking melanoma
3	Mitra <i>et al</i> <sup>4</sup>	2018	India	72 and 80	Female and male	Caruncle Cystic and solid with papillary configuration Solid with central degeneration
4	Heathcote <i>et al</i> <sup>3</sup>	2022	USA	70 and 87	Male Female	Left Caruncle Mainly cystic Right Lacrimal sac Solid and cystic

**Table II: A Detailed Comparison of Index Case with Case Reports with International Studies**

S. No	Author	Year of Publication	Country	Centre	Total Cases	Oncocytoma Cases	Time Period in Years	Gender Female: Male	Site of Tumor	Significant Findings
1	Levy <i>et al</i> <sup>10</sup>	2008	Israel	Single center	42	3	17	2:1	Caruncle	Pigmented lesions
2	Solar <i>et al</i> <sup>8</sup>	2009	Canada	Single center	42	3	15	2:1	Caruncle	-----
3	Shields <i>et al</i> <sup>11</sup>	2016	USA	Multicenter	5002	6	40	51:49	Lacrimal Glands	-----
4	Alam <i>et al</i> <sup>2</sup>	2002	India	Single Center	87	1	20	49:51	Caruncle	-----
5	Clemens <i>et al</i> <sup>1</sup>	2002	Germany	Single Center	82	9	22	4:5	Lacrimal Gland and Caruncle	5 Solid and 4 Cystic Morphologies

choice with no recurrence. Given the rarity of CO, the timely and accurate reporting of these lesions is paramount. Our case report adds to the limited literature available on these benign tumors.

### Conflict of Interest

Authors declared no conflicts of Interest.

### Grant Support and Financial Disclosure

Authors have declared no specific grant for this research from any funding agency in public, commercial or nonprofit sector

### Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon request

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#### GRANT SUPPORT AND FINANCIAL DISCLOSURE

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#### DATA SHARING STATMENT

The data that support the findings of this study are available from the corresponding author upon request.

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# JOURNAL OF ISLAMIC INTERNATIONAL MEDICAL COLLEGE (JIIMC)

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