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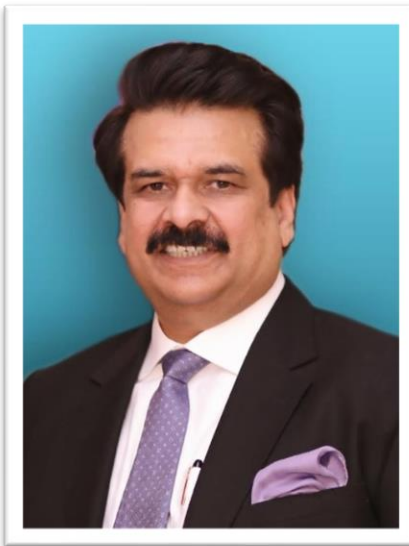
# JOURNAL OF HBS M&DC

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

## Message From Chief Editor



**Dr. M. Riaz Shahbaz Janjua**

**Chairman: HBS M&DC**

Allah TWT says in Quran that “Saulayheen” shall have command on Earth. Most of the scholars have translated “Sualayheen” incorrectly. Actual meaning of “Sualaheen” according to the Arabic lughat (Dictionary) is a people who are competent and proficient or who possess the capability. We, as health professionals, are gifted of being “Saulayheen” as are capable of gaining scientific knowledge by carrying out research.

I believe that it is on the shoulders of today’s practicing doctors and teachers, to learn the essential skills necessary to be able to carry out research in an effective clear, methodical and time effective manner. Effective literature review, filling the gap in existing research with new information gained by one’s own research endeavors and its documentation is mandatory to conduct a result-oriented-research.

Launching of the Journal of HBSM & DC is a step towards fostering research and providing a new platform to publish good quality research papers based on empirical or scholarly research work encompassing the fields of Medicine and Dentistry. Editorial board of JHBSMDC is comprised of our faculty members well known in their specialties nationally and internationally. Each member is committed to work with utmost dedication in order to bring this journal at par with internationally renowned impact factor journals.

I pray and hope that this journal will benefit people in the field of Medicine and Dentistry and shall unravel a chain of research questions with innovative ideas compelling the researcher to think out of the box.

I am very thankful to all the members of editorial board for their hard work in stream lining the matters of publication. I am also grateful to our national and international reviewers for giving their consent to be a part of this noble cause. I can never ignore the role of authors who contributed to the journal.

Above all I can never thank Allah TWT enough for His Fazal and Rehmatulil Alameen, Sarwar e Kainat Hazrat Mohammad S.A.W. (may peace be upon him) for his Rehmat as it is my faith that crossing this milestone could not have been possible without these blessings.

May Allah TWT make everyone contributing to this journal most capable and may He bless you all with the best of capabilities.

Thank You



# Journal of HBS M&DC

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# Robots are here!

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Dear Readers,

Let me start by thanking the entire HBS journal team for their untiring efforts to publish this volume of journal. They have worked day and night to perform a marvelous job. We hope that you would benefit from this new, excellent and intellectual work.

Not so long ago, we were watching movies like “Terminator” and we loved it. That was just a beginning of an era of robot movies. We saw a barricade of similar theme movies, especially in last couple of decades. Movies like judgement Day. Iron Giant. RoboCop. The Matrix. Pacific Rim. I robot and so forth. We all enjoyed them, but at that time, had not a glimpse of idea that one day it would be an actuality, that too in our lifetime. Then it was merely a dream, a fantasy, a hot movie topic, but guess what, now it’s a reality.

All this has become possible by Artificial intelligence. A new intelligent computer that could think act and work, like and perhaps better than humans.

It’s a breakthrough not just for computing, engineering, and other scientific ordeals but also plays marvel in medicine. All data is entered, analyzed and processed by a computer which takes decisions based on information. This is not just an ordinary computer, but a new superfast machine that could discern, a very accurate, credible and logical decisions.

In last few years the use of AI technology has already revolutionized a lot of our medical work. You can now, not only diagnose a patient with far more accuracy, but also prescribe medicines without fear of drug interaction. Investigations can now yield much more accurate conclusions, after deciphering x-rays, Ct scans and MRIs. The accuracy of picking up a positive finding on radiological workup has reached almost 98%. In future apart from CT/MRI, all Mammograms, Histopathological slides, Contrast studies, Angiograms etc. etc., would be computer analyzed. More so it does not have that human factor, I missed it, forgetfulness or bias in it, making it extremely unerring. Doctors would just countersign the decisions to avoid a negligible

chance of so called “computer error”, possible due to error in data entry.

Medical students, students, teachers also find AI as extremely useful tool. They can now solve lot of their queries, answer difficult questions and solve equations. A lot of AI tools are now available very easily by internet. In fact, for every educational task, a separate tool is now available. Below is the list of some of common tools available either free or at a very nominal cost.

- a. Chatbots (ChatGPT, Claude 2, Bing AI)
- b. Content creation (Jasper, Copy.ai, Anyword)
- c. Video creation and editing (Descript, Wondershare Filmora, Runway)
- d. Image generation (DALL·E 3, Midjourney, Stable Diffusion)
- e. Knowledge management and AI grounding (Mem, Notion AI Q&A, Personal AI)
- f. Task and project management (Asana, Any.do, BeeDone)
- g. Transcription and meeting assistants (Fireflies, Airgram, Krisp)
- h. Slide decks and presentations (Decktopus, Beautiful.ai, Slidesgo)
- i. Automation (Zapier)

But there is the other side of coin as well. Students have already started using there AI software’s for medical writing like doing assignments, research and other intellectual work. This plagiarized work is difficult to detect even with modern similarity index softwares. Not only this, but the students would stop doing cognitive and creative work and would rely more on digital technologies. Will this tool make doctors dull or smart? well only time will tell. However, the ethical use of AI is a big challenge both for teachers, publishers and academicians.

Yet the era of AI, superfast computers and robots have started and we all have to be well versed with these new armamentaria. Robots have been possible only by AI and may replace many human positions in near future. AI software’s can help surgeons perform mush more and difficult surgeries quiet safely and intelligent robots are now used for surgical procedures and operation theaters.

Who knows, in few years' time you would enter the hospital and would be greeted by a Robo Nurse. She would hurl you in examining room to be seen by a Robo Doc.

How about that! just imagine. The era has started, friends.

Regards

Professor Muhammad Idrees Anwar.  
MBBS, FRCS, DCPS(HPE), MHPE.  
Editor

# Correlation of Serological Markers with Haematological Parameters in Diagnosis of Dengue: A Single Centre Study at a Tertiary Care Hospital of Pakistan

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## Abstract

**Objective:** To correlate the serological markers with hematological parameters in the diagnosis of dengue fever.

**Background:** Dengue is a viral disease spread by *Aedes Aegypti* mosquitoes. It is most common in Southeast Asia. In recent times yearly epidemics of dengue fever has put a great burden on our health care system. The World Health Organization (WHO) has classified symptomatic dengue as dengue fever with or without warning signs and severe dengue. Early diagnosis of the disease and management can save countless lives in this curable infectious disease.

**Methodology:** It is a prospective observational study from HBS General Hospital, Islamabad. 102 patients who presented with fever and tested positive for NS1 antigen and/or IgM dengue antibodies and fulfilled the inclusion criteria over a period of one year were included. The demographic data along with serological and hematological results were recorded.

**Conclusion:** The most common serological test in dengue infection is NS- 1. Thrombocytopenia, leucopenia, monocytosis and reversed neutrophil lymphocyte ratio are important hematological clues in diagnosis of dengue fever. Thrombocytopenia is most severe in NS- 1 positive group as compared to antibody positive groups.

**Key words:** Dengue fever, hematological profile, thrombocytopenia, leucopenia.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Dengue virus measures 50 nm and has 11kb long genome. It is an sRNA virus. It contains three structural genes which encode capsid protein, membrane protein, and envelope protein. It also contains seven nonstructural genes which encode seven types of proteins. It is a mosquito borne viral infection and *Aedes aegypti* and *Aedes albopictus* families of mosquito are its vectors.<sup>1</sup> Dengue infection usually runs its course but some cases can be life threatening and mortality has been frequently reported.<sup>2</sup>

Dengue infection has been declared as the most rapidly spreading mosquito borne disease. The global incidence of dengue infection has significantly increased and a 30-fold rise has been reported in the last 50 years. Not only the incidence but a changing trend in the disease

distribution is also noted in the recent years. The disease seems to have spread to new countries and more cases has been reported from the rural areas than before.<sup>3</sup> approximately every year about 50 million cases of dengue infection are reported with almost 2.5 billion at risk population reside in dengue endemic countries. Almost 70% of cases are from Asia. The disease is endemic in more than 100 nations. The increasing trend is evident from the fact that 44,585 cases were reported from India in 2020 and in two years this figure has risen to 193,245 cases in 2022. It means the prevalence of dengue fever increased more than three times in India. Similarly, Pakistan and Bangladesh has reported a seven and nineteen times, respectively increase in the cases of dengue fever in the last two years.<sup>4</sup>

In laboratory dengue virus infection can be confirmed by identifying the virus, viral nucleic acid, antigens or

antiviral antibodies.<sup>5</sup> The most commonly employed test for dengue viral diagnosis in first 4–5 days is detection of viral antigen. In 50% of patients IgM antibodies become detectable by days 3-5 of the diseases. Their levels steadily increase to 80% by day 5 and by day 10 th IgM antibody can be detected in 99% of the cases. The peak level of IgM antibodies is detected about two weeks after the onset of symptoms. After that it gradually decrease to undetectable levels in the next 2–3 months. Low titers of serum IgG becomes detectable at the end of the first week of illness and then they increase thereafter. The serum IgG is detectable after several months, and probably even for life.<sup>6,7</sup>

Many diagnostic methods are available for DENV detection like Polymerase Chain Reaction (PCR), dengue NS1 and antibody detection by enzyme-linked immunosorbent assay (ELISA) or rapid diagnostic test (RDT).<sup>8</sup>

In dengue fever the hematological parameters vary every day.<sup>9</sup> In this study we aim to correlate the serological markers with hematological parameters in dengue fever which will facilitate in the diagnosis of dengue infection, its risk categorization and will aid in timely management via the use of rapid serological tests.

## Methodology

This cross-sectional observational study was conducted at HBS General Hospital, Islamabad. 102 seropositive dengue patients from the year 2022 were enrolled in this study. Nonprobability consecutive sampling technique was used.

### Inclusion Criteria:

Male and female admitted patients of all ages with positive dengue serology tests. (Patients with serologically dengue NS1 antigen and/ or dengue IgM antibody positive result).

### Exclusion Criteria:

1. Patients with other hematologic diseases, chronic diseases.
2. Patients who are on chemotherapeutic regimens and who are immunocompromised.
3. Patients with fever but no diagnosis has been established.
4. Dengue-infected patients with evidence of harboring another infection.

Admitted patients presenting to HBS hospital, fulfilling the inclusion criteria, were enrolled in the study after taking approval from the hospital's ethical committee.

The study details were discussed with the patients and both verbal and written consent was taken. Most cases were admitted on the third day of the onset of the fever. 3ml of venous blood was collected in an EDTA tube by a clean venipuncture using a disposable syringe to analyze blood counts in a fully automated hematology counter (Sysmex Kx21/Medonic), while centrifugation was done at 3000rpm for 10 minutes. The hematological parameters from the daily CBC reports were recorded. The hospital stay for the admitted patients was 4 days minimally. After which the number of patients decreased each day due to discharge and day 7 data is available for 25 admitted patients who were initially enrolled in the study. NS- 1 and IgM antibody testing was done by rapid test method.

All data was entered and analyzed in SPSS version 21. Frequency and percentage were calculated for the qualitative variables like gender etc while mean and range were calculated for quantitative variables such as hemoglobin, hematocrit, WBC count, and differential leucocyte count. Data is represented as figures and tables.

The confidentiality and privacy of the patients was insured by not disclosing their personal information to anybody but those who are directly involved in this research project.

## Results

A total of 1743 cases were diagnosed as dengue infection in 2022 on the basis of positive serology, out of which 102 patients were enrolled in this research project. There were 70 (64%) males and 32 (29%) females. Table1 shows the age groups of the participants.

Age group	Frequency (percentage)
10-20	4 (3.9%)
21-30	14 (13.7%)
31-40	18 (17.6%)
41-50	35 (34.3%)
51-60	19 (18.6%)
61-70	0 (0%)
71-80	3 (2.9%)

57.9% (59/102) patients tested positive for NS1 antigen, 27.2% (28/102) were dengue IgM antibody positive while both tests came out positive in 14.9% (15/102) patients. Out of 102 (12.7%) dengue positive cases, 13 were of severe dengue infection. The common presenting complaints were headache, nausea, vomiting, loss of appetite, rash, arthralgia and myalgia. Gastrointestinal symptoms like diarrhoea, abdominal pain and sore throat were also common.

The rise and fall of the several hematological parameters were observed over a period of 7 days after admission. Most cases were admitted on day 3 of the onset of the fever. Mean values of all haematological parameters were calculated according to the day of fever and findings are represented in the Table II

and 32(29%) female patients. Similar demographic profile is reported by Qamash et al in the district Swabi of Pakistan with more cases in males and the most common age group involved was 21-30 years.<sup>11</sup>

Most patients were diagnosed with dengue infection because of positive NS 1 serology i-e 59/102 (57.7%).

Day of fever	No of admitted patients	Complete blood count (CBC) Parameters			
		Leukopenia (<4x10 <sup>3</sup> /uL )	Thrombocytopenia (<150x10 <sup>3</sup> /µl)	Neutrophil count (<50%)	Monocytosis
3	102	38 (37.2%)	102 (100%)	25 (24.5%)	23 (22.5%)
4	102	50 (49%)	102 (100%)	37 (36.2%)	31 (30.3%)
5	102	56 (54.9%)	102 (100%)	56 (54.9%)	45 (44.1%)
6	102	53 (51.9%)	102 (100%)	87 (85.2%)	57 (55.8%)
7	71	44 (61.9%)	71 (100%)	52 (73.2%)	47 (66.1%)
8	44	38 (86.3%)	44 (100%)	42 (95.4%)	29 (65.9%)
9	25	23 (92%)	25 (100%)	11 (44%)	14 (44%)

Haematological parameter	NS 1 positive N= 57.9% (59/102)	IgM positive N= 27.2% (28/102)	NS 1+ IgM positive N= 14.9% (15/102)
TLC	4.9 (2.0- 8.4)	4.3 (1.3-8.4)	3.6 (1.7- 6.2)
RBC	4.5 (4.0- 6.2)	5.4 (4.0- 11.2)	5.4 (5.3- 5.6)
HB	13.2 (11.5- 14.4)	14 (12.9- 16.2)	12.7 (12.1- 13.6)
HCT	39.8 (35- 47.7)	44.6 (33.1- 48.8)	41.2 (31- 39.6)
Platelets	61.4 (9.8- 110)	84.6 (45- 183.6)	81.1 (42.8- 119.3)
Neutrophils	37.8 (29- 48)	51.4 (45.3- 67.8)	42.6 (32- 55)
Lymphocytes	49.9 (36- 67.8)	32.4 (26.2- 41)	52.3 (49.8- 75)
Monocytes	8 (3.4- 6.6)	11.9 (3.4- 20)	6.2 (3.8- 10)
Eosinophils	4.4 (2.6- 7.0)	4.6 (2.5- 8.0)	3.3 (2- 6)

13 out of 102 (12.7%) dengue infected patients were classified as severe dengue infection and presented with symptoms like abdominal pain, bleeding and persistent vomiting. All enrolled patients were admitted for a period of 4 days. After which 26 were discharged and 5 were referred to other hospitals. By day 9 there were 25 admitted patients. The enrolled patients were divided in three groups according to their serology as NS-1 positive, IgM positive and NS- 1+ IgM positive. The mean values of each haematological parameter of all the admitted days was calculated and is represented along with range for that parameter in each group in Table III.

## Discussion

Symptomatic dengue infection in most cases presents with low to moderate grade fever. But in almost 5% cases the disease is life-threatening disease and it is classified as "Severe dengue". In the last fifty year the global incidence of dengue fever has significantly increased and it poses a substantial health and economic burden.<sup>10</sup>

In the present study most cases were of the age group was 41-50 years (34.3%) with 70 (64%) male patients

Habib MB et al have also reported a frequency of 77% positive NS-I cases with a male predominance and the most commonly affected age group in their study was 31-45-year.<sup>12</sup>

The hematological parameters studied showed one consistent feature and that was thrombocytopenia and leucopenia, which was present in all patients throughout the study duration i-e 7 days. A rising trend of platelets was observed from day 6, and the recovery of platelets occurs before a rising trend is seen in TLC. In our study a reversed neutrophil to lymphocyte ratio was observed from day 6 to 8. Monocytosis was present in > 60% of the cases in our study. Diseases like other viral infections, typhoid fever, tuberculosis, malaria, HIV infection, malignancy or pyrexia of unknown origin are also associated with monocytosis. It is not specific to dengue infection. Its cause is believed to be the monocytes and macrophages involved in phagocytosis and antigen presentation to T helper cells.<sup>13</sup> Sivathanu et al have reported monocytosis in 91% cases of dengue infection in their study on 67 children.<sup>14</sup>

Chaloemwong J study from Thailand in 2018 also reports that thrombocytopenia and leucopenia in dengue patients in the start of fever with recovery of these

parameters from day 5 of the fever. They have reported a higher monocyte percentage from day 1 to 4 and neutrophil to lymphocyte ratio was noted to reverse on day 6 to 9 of fever in their study.<sup>15</sup>

The role of non structural antigen NS1 is pivotal in the pathogenesis of dengue infection. It causes vascular leak through binding toll like receptors and damaging the endothelial glycocalyx. Some studies show the role of NS 1 in activating compliment membrane attack complex C5b-C9 on the endothelial cells to destroy viral infected cells but at the same time it cause release of anaphylotoxins like C3a- C5a which promotes inflammation.<sup>16</sup> The human immunity has an important role in the pathogenesis of the disease. Dengue infection is most severe when the virus is being cleared by the host immune system and not with the peak viral load. Some antiNS1 antibodies can cross-react with endothelial cells and cause apoptosis, thus creating a form of autoimmune response. Anti NS1 antibody also causes endothelial cells to produce IL-6 and IL- 8, both of which are found to be elevated in patients with severe dengue infection.<sup>17</sup> In our study the hematological parameters did not differ much in the three serological groups. The IgM positive group showed maximum hematocrit and hemoglobin levels. Antigen as well as antibody takes part in endothelial cell damage and plasma leakage which in turn causes hemoconcentration; an important hallmark of dengue infection. Dengue virus has an avidity to infect hepatocytes. Hepatocyte apoptosis releases soluble substances in blood which also causes consumption deficiency of platelets. Most severe thrombocytopenia in our study was seen in the NS- 1 positive groups. (mean value  $61.4 \times 10^3/uL$  compared to  $84.6 \times 10^3/uL$  and  $81.1 \times 10^3/uL$  in other two groups)

A large scale study conducted in Nepal in 2023 analyzed the serological, biochemical and hematological parameters in dengue infection. Their results are comparable to our study. The hematological parameters did not differ much in the three groups. The NS -1, IgM positive and NS- 1+ IgM positive groups showed thrombocytopenia and leucopenia. But monocytosis was seen in NS- 1 positive group mostly which in our study is in IgM positive group. Same goes for lymphocytosis which in our study is more pronounced in NS- 1 and IgM positive group than the other groups.<sup>18</sup> A study in 2018 by Joshi A et al showed highest values of Hb, and RBC count in cases with positive antibody as compared to antigen alone. But in contrast to our study thrombocytopenia in their study was more severe in antibody positive cases than NS- 1 positive cases.<sup>19</sup> In contrast to our study, Mishra et al showed lowest platelet counts in patients who are NS- 1+ IgM positive.<sup>20</sup>

It is currently not clear if anti NS1 antibodies against different regions of NS1 antigen play roles in the defense or disease progression of dengue fever in humans. According to the current available data it seems that multiple factors which include viral load, patient immune status, NS1 antigen, anti-NS1 antibodies, infecting serotype, and genotype may all contribute in the pathogenesis of dengue infection and the development of severe disease. The nature and mode of these interactions is not fully understood yet and there are many grey areas.

## Conclusion

The most common serological test in dengue infection is NS- 1. Thrombocytopenia, leucopenia, monocytosis and reversed neutrophil lymphocyte ratio are important hematological clues in diagnosis of dengue fever. Thrombocytopenia is most severe in NS- 1 positive group as compared to antibody positive groups.

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<sup>1,6</sup>Substantial contributions to the conception or design of the work;

<sup>2,3,4,5</sup>the acquisition, analysis, or interpretation of data for the work & Final approval of the version to be published

<sup>1,6</sup> Drafting the work or revising it critically for important intellectual content.

# A Single Centre Retrospective Study to Assess the Frequency of Congenital Anomalies in a Tertiary Care Hospital

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## Abstract

**Objectives:** The objective of our study was to determine the frequency and types of congenital fetal anomalies among pregnant patient in tertiary care hospital.

**Methodology:** This is descriptive, cross sectional retrospective study conducted in obstetrics and gynecology and radiology department of HBS General hospital Islamabad from January 2021 to January 2023. It includes all booked and unbooked antenatal patients

**Results:** Over a period of two years total 3723 antenatal patients included in our study out of which 2.01% were diagnosed with congenitally structurally malformed fetuses. Commonly observed congenital abnormality in our study was CNS anomaly which was observed in 26 patients followed by congenital cardiac defects (16 patients), renal abnormality in 8 patients, and skeletal anomalies in 6 patients and respiratory tract anomalies in 2 patients.

**Conclusion:** Congenital anomalies are a major cause of stillbirth and infant mortality. Patients history of medical disorders, previous anomalous baby, consanguinity, advanced maternal age and drug history have strong association with congenital fetal anomalies.

**Key words:** (Pregnant women, second trimester, antenatal, anomaly scan.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Congenital anomalies are normally considered as structural and functional anomalies<sup>1</sup>, which were further classified as major and minor congenital anomalies. Due to recent advances antenatal care and medical technology lot of improvement is seen in detection of congenital anomalies in 3<sup>rd</sup> world countries.

Anomaly scan during second trimester of pregnancy has been commonly practiced in our health care settings. Every pregnant woman is advised second trimester USG between 18- 22 weeks from sonologist.<sup>3</sup> Anomalous babies are of great concern for health care professional<sup>2</sup> and is a source of psychological trauma for parents.

WHO fact sheet 2016 showed 303,000 neonatal deaths occurs every year due to congenital anomalies.<sup>4</sup>

In European countries different studies conducted showed increasing incidence of congenital heart disease and decreasing trends in limb defects<sup>2</sup>. In Pakistan mostly studies showed increasing incidence and NTD<sup>5-12</sup> with prevalence ranging for 1.4% to 7%.

Cousin marriages, increase in maternal age, folic acid deficiency, diabetes, thyroid disorders are also considered as risk factor associated with congenital anomalies.<sup>13,14,15,16,17,18,19</sup>

The purpose of our study is to determine frequency of congenital anomalies among general population during 2<sup>nd</sup> trimester USG. Although number of studies done on it but lot more work is required in this aspect.

## Methodology

This study was conducted on pregnant patients of OPD and IPD of gynae department of HBS general hospital

Islamabad in collaboration with radiology department over period of 2 years. This was retrospective study, data was retrieved from the computer. All patients with gestational age of more than 20 weeks were included in the study. Detailed history of all the patients including maternal age, parity, gestational age, consanguineous marriage and family history of anomalous baby was also taken. USG machine Aloka IPFI701B of 3.5Mtlz multifrequency curvilinear transducer was used, allocated time was 30 minutes. Associated comorbidities were not considered in our study as it was retrospective.

Ethical approval was taken from internal review board of HBS medical and dental college Islamabad. Informed consent was taken from all the patients included in our study as per our hospital policy. According to the results of USG we fully inform our patient regarding type of anomaly and its prognosis.

## Results

Over a period of 2 years, number of pregnant patients included were 3732, out of which 2.01% were diagnoses with congenital anomalies, commonest abnormality which was observed in our study was central nervous system (CNS) anomalies and is observed in 26 patients. Among CNS anomalies anencephaly was most common and hydrocephalus was second most common. (Table I)

Second commonly identified birth defect were cardiac anomalies in which we had VSD in 6 patients and cardiomegaly and septal wall defects in rest of the patients. Cardiac anomalies were identified in 16 patients. We entered rest of the anomalies in miscellaneous group which includes skeletal anomalies in 6 patients, renal anomalies in 8 patients and tracheal atresia in 2 patients. Most of the pregnant patients with congenital anomalies were of 25 – 30 weeks. Out of all 94 patients has first cousin marriage.

Type of anomaly	Numbers	%
<b>CNS</b>	27	
Anencephaly	11	15
Hydrocephaly	09	12
Others	07	09
<b>CVS</b>	16	
VSD	06	08
Cardiomegaly and septal wall defects	10	13
<b>Miscellaneous</b>	32	
Skeletal	06	08
Renal	08	11
Tracheal atresia	02	03
Others	16	21
Total number (%)	75	

## Discussion

In our study, frequency of congenital abnormalities was about 2.01% which is quite comparable with other studies done in our part of the world<sup>13,20</sup>. Same results were found in two other international studies conducted in Brazil by Al media et al, which showed prevalence of about 2.4%. Second study conducted in Europe by Mohammed A et al, which showed prevalence of 2.5%<sup>16,21</sup> Another study conducted in India by Sarkar S et al, which showed incidence of 2.2%.<sup>17</sup>

Most congenital anomalies identified in our study were between 20-30 weeks of pregnancy although ideal time is between 18-22 weeks when most of the structural anomalies were identified. Association between cousin marriages and congenital anomalies is evident in many studies<sup>11,12</sup> which is also seen in our study.

Most of the studies correlate congenital anomalies with established diabetes and GDM and with gender of fetus which is not considered in our study<sup>12,17</sup>. These studies showed 7.5% patients have anomalous fetus along with gestational diabetes mellitus (GDM) and among gender of fetuses, congenital anomalies were more frequent in male fetuses.

CNS anomalies were commonest in our study which was followed by cardiac anomalies. The results of our study are almost similar to studies conducted in Pakistan<sup>2</sup> and other neighboring countries<sup>8,11,12,22</sup> The most common CNS anomaly found in our study was anencephaly followed by hydrocephalies and meningocele. Our local studies done previously support our results of CNS anomalies being commonest<sup>8,9,12</sup>

Results of our study is comparable with previously done national and international studies, but in our study, consanguinity was the only comparable factor.

As it's a retrospective study so other associated risk factors were not compared which should be considered in future.

## Conclusion

Congenital anomalies are a major cause of stillbirth and infant mortality. Patients history of medical disorders, previous anomalous baby, consanguinity, advanced maternal age and drug history have strong association with congenital fetal anomalies.

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<sup>2,3,4</sup> The acquisition, analysis, or interpretation of data for the work;

<sup>1</sup> Final approval of the version to be published;

<sup>1,5,6</sup> Drafting the work or revising it critically for important intellectual content.

# A Retrospective Single Centre Study on Histopathological Analysis of Cholecystectomy Specimens in a Pakistani Tertiary Care Centre. Is the Spectrum Changing?

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## Abstract

**Objective:** To study the histopathological spectrum of gallbladder diseases and compare the occurrence of gallbladder carcinoma in relation to age, gender and weight of the patient.

**Methodology:** This Cross-sectional study, retrospective study was conducted at Histopathology department of HBS General Hospital and Pakistan Institute of Medical Sciences (PIMS). Study was conducted over a period of 6 months from October 2021 to March 2022. Cholecystectomy specimens from either gender irrespective of their age received at the Histopathology Department of HBS General Hospital and Pakistan Institute of Medical Sciences (PIMS), over a period of 6 months were included in the study. The specimens were fixed in 10% formalin for 24 to 48 hours before sectioning. Then every specimen was macroscopically evaluated followed by cutting of the tissue into three representative sections, one each from the fundus, body and neck of the gallbladder along with the lymph nodes if provided. Hematoxylin & eosin staining was done & specimen. The clinical data from pathology reports and slides were retrieved from the archives and were microscopically examined by the histopathologists and the results were recorded.

**Results:** Mean age of the patients was  $54.58 \pm 18.04$  years. Females were more than the male patients. There were 315 (77.4%) female patients as compared to 92 (22.6%) male patients. The mean weight of the patients was  $60.18 \pm 5.11$  kg. The most common finding was chronic cholecystitis in 303 out of 407 total cases (85%) followed by acute cholecystitis in 27 cases (9.8%). There were 16 cases (3.9%) of incidental carcinoma and three cases (0.7%) of dysplasia.

**Conclusion:** A spectrum of histopathological diagnosis is observed in cholecystectomy specimen. The inflammatory gallbladder lesions are the most common pathology and the commonest diagnosis is chronic cholecystitis but the doctors cannot rely on the clinical or radiological diagnosis of gallbladder diseases. Histopathological analysis of all gallbladder specimens is necessary for the detection of carcinoma as the frequency of gallbladder cancer appears to be on the rise in our population which is significantly more common in females.

**Keywords:** Carcinoma, Gallbladder, Cholecystectomy specimens

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Gall bladder diseases are frequently seen in clinical practice. Patients may be asymptomatic or manifest symptoms including acute biliary colic, fever, and jaundice. The most common malignant tumor of the biliary system and the sixth most common cancer of the

gastrointestinal tract are gallbladder cancers. Globally, the incidence of gall bladder carcinoma varies significantly by area, and this variation is correlated with the prevalence of cholelithiasis.<sup>1</sup>

Globally gallstones affect 10% to 15% of the adult population and constitute a major health problem. The healthcare burden of gallbladder disease has steadily

increased by 20% in the last three decades.<sup>2</sup> The prevalence of cholelithiasis in Asian population is 3-4%. In China it is reported as 3%, 6% in Northern India and 5% in Taiwan. Data from Pakistan showed that in the Southern Sindh region of Pakistan, the prevalence rate is 4 percent among men and 14.2 percent among women. This study conducted in Hyderabad, Sindh showed the frequency of gallbladder carcinoma in cholecystectomy specimens to be 4%.<sup>3</sup> Another local study from Armed force Institute of Pathology (AFIP), Rawalpindi showed a frequency of gallbladder cancer as 1.55% in 10,549 cholecystectomy specimens.<sup>4</sup>

Gallbladder carcinoma also presents with symptoms of chronic cholecystitis. Gallbladder carcinoma is a common malignancy in the extra-hepatic biliary tract, with females being the most common patients. The disease has a specific ethnic group predisposition, with more cases reported in native Americans, Bolivians, and northern Indian populations.<sup>2</sup> The most common age group involved is 50-70 years old. Risk factors for gallbladder carcinoma include gallstones, cholecysto-enteric fistula, porcelain gallbladder, ulcerative colitis, adeno-myomatosis, polyposis coli, gartner syndrome, and anomalous connections between the common bile duct and pancreatic duct. Histopathological evaluation of every cholecystectomy specimen is recommended to diagnose gallbladder carcinoma, which can be treatable in early stages but has a poor prognosis when advanced disease is present.<sup>5</sup>

The rationale of this investigation was to find out the frequency of different gallbladder diseases and to find out the incidental finding of gallbladder carcinoma when it is not clinically suspected. This stresses the need for histopathological examination of all routine cholecystectomy specimens so as to guide treatment planning.

## Methodology

This Cross-sectional study, retrospective study was conducted at Histopathology department of HBS General Hospital and Pakistan Institute of Medical Sciences (PIMS). Study was conducted over a period of 6 months from October 2021 to March 2022. Nonprobability Consecutive Sampling technique was used. The calculated sample size is 407 for a population proportion of 3.64%<sup>6</sup> for gallbladder carcinoma, keeping the confidence interval at 95% & margin of error at 1.82%. Sample size was calculated by the WHO calculator.

### Inclusion Criteria:

1. All elective cholecystectomy specimens of either gender.

2. All age groups undergoing cholecystectomy.

### Exclusion Criteria:

1. Diagnosed as tumour on Ultrasound or CT scan.
2. Grossly autolyzed tissue specimen which could not be processed technically.

Cholecystectomy specimens in 10% buffered formaldehyde were received in the histopathology department. Hematoxylin & eosin staining was done after routine processing. Cases with complete clinical data from pathology reports; mentioning age, gender and benign ultrasound diagnosis were included in the study. Slides were retrieved from the archives and were microscopically examined by the histopathologists and the results were recorded.

The data was analyzed with the help of SPSS version 21. The descriptive statistics like mean & standard deviation was calculated for age. For categorical variables like gender & carcinoma of the gallbladder frequency was calculated. The data was stratified according to age, gender & weight. Post stratification chi-square test was applied to investigate the effect of these factors with various independent variables. Results are represented as tables and graphs. P value < 0.05 was taken as significant.

## Results

There were 407 cases of cholecystectomy included in the study. Females were more than the male patients. There were 315 (77.4%) female patients as compared to 92 (22.6%) male patients. The mean age of the patients included in the study was 54.58 ±18.04 years, with 62.4% of them being over 50 year. The mean weight of the patients was 60.18 ±5.11 kg, with 56.8% having a weight below 60 kg. There were 274 (67.5%) cases of laparoscopic cholecystectomy, 40 cases (10%) open cholecystectomies and in 91 cases (22.5%) laparoscopic procedure was changed to open cholecystectomy.

Histopathological examination of gall bladder specimens showed a variety of microscopic diagnosis as shown in Figure 1 and microscopic features of some cases are shown in figures 2, 3, 4.

Table I show the distribution of gallbladder carcinoma in relation to age, gender and weight of the patient. A significant p-value > 0.05 was identified when gender was compared with the occurrence of gallbladder cancer. All cases of cancer occurred in females and none in the males.

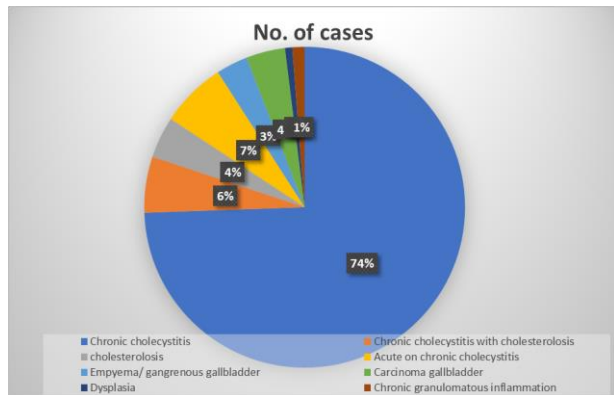


Figure 1. Frequency of different histopathological diagnosis in 407 cholecystectomy specimen. (n=407)

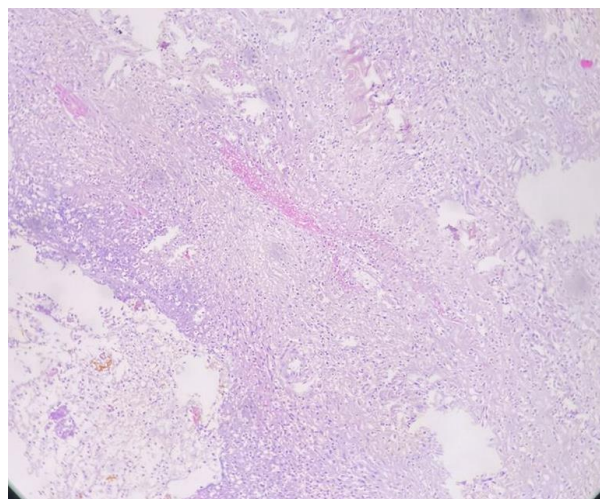


Figure 2. H&E (100x) Acute on chronic cholecystitis showing neutrophils with lymphocytes and plasma cells throughout the wall of gallbladder with mucosal necrosis

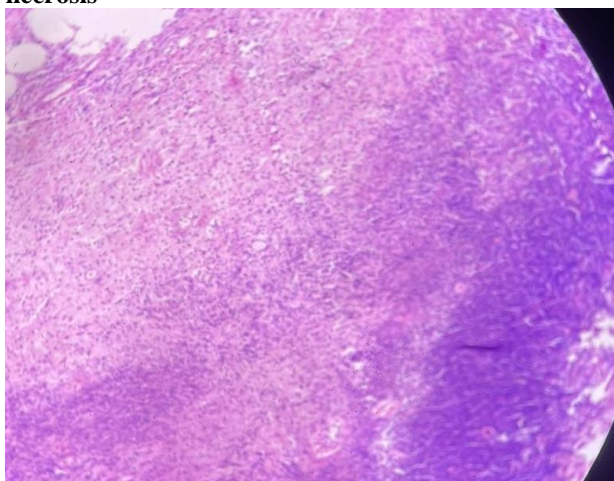


Figure 3. H&E (100x) Empyema gallbladder showing sheets of neutrophils with necrosis in the wall and sloughing of mucosa. This case was associated with pus in the gallbladder lumen.

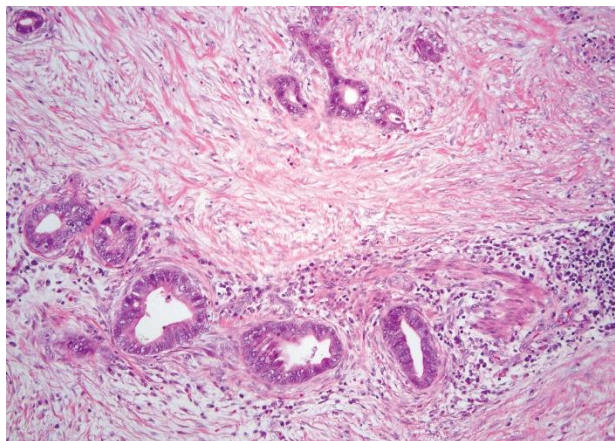


Figure 4. H&E (100x) Carcinoma gallbladder showing glands with open lumina and subtle atypia associated with desmoplasia and invasion.

**Table I: Comparison of gall bladder carcinoma with the age, gender, weight of the patient (n=407)**

Age (in years)	Carcinoma of gall bladder			P-value
	Present	Absent	Total	
≤50	5 (3.3)	148 (96.7)	153 (100)	0.593
>50	11 (4.3)	243 (95.7)	254 (100)	
Total	16 (3.9)	391 (96.1)	407 (100)	
<b>Carcinoma of gall bladder (Gender)</b>				
Male	0 (0)	92 (100)	92 (100)	0.027
Female	16 (5.1)	299 (94.9)	315 (100)	
Total	16 (3.9)	391 (96.1)	407 (100)	
<b>Carcinoma of gall bladder (weight)</b>				
≤60	8 (3.5)	223 (96.5)	231 (100)	0.578
>60	8 (4.5)	168 (95.5)	176 (100)	
Total	16 (3.9)	391 (96.1)	407 (100)	

### Discussion

One of the most commonly encountered cases in general surgery are the diseases of the gall bladder.<sup>5</sup> The most frequent of these illnesses is cholecystitis, which is mostly caused by cholelithiasis, or the formation of gallstones.<sup>6</sup> Age, female gender, metabolic syndrome, and obesity are all risk factors for the diseases of the gallbladder.<sup>7</sup>

In our study females were 315 and made up 77.4% of the examined cases. Several studies have documented this female predominance. No studies have been reported to our knowledge to show male predominance.<sup>8</sup>

The mean age of the cholecystitis patients in the current study is 54.58 ±18.04 years, ranging from 28 to 69 years. This is comparable to that reported by Gavriilidis P et al. in 2022, in which the median age was 64 years and the affected age range was 33 to 87 years.<sup>9</sup>

In 74.4% (n=303) of the patients, the diagnosis was chronic cholecystitis, whereas in the 6.6% (n=27) cases it was acute cholecystitis.<sup>5</sup> Park Se et al reports acute

cholecystitis and acute on chronic cholecystitis in 14-30% of cholecystectomy specimens.<sup>10</sup>

Postoperative histopathological evaluation of the excised GB specimens reveals a vast spectrum of underlying pathologies. With the changing lifestyles and other environmental factors this spectrum seems to be evolving. Almas T et al in 2020 have published a study at Maroof hospital, Islamabad. In their study the most common diagnosis was chronic cholecystitis (78.7%) while acute and acute on chronic cholecystitis was 18.7% cases. This is comparable to our study but they have reported 32.8% cases of cholesterols which is much higher than that of our study (10.7% cases). The carcinoma was reported in 0.2% cases in their study which is much less than that of our study (3.9% cases).<sup>11</sup> In international and local studies chronic cholecystitis is the most common pathology of gallbladder and every type of gallbladder disease is more common in middle age females.<sup>9,10,11</sup> The number of cases of acute on chronic cholecystitis is also comparable with that reported in literature. But our study shows less number of cases of cholestrololosis than most other studies.<sup>11, 12, 13</sup>

Due to significant pathology that may be present even in a specimen that seems to be normal, the Royal College of Pathologists advises sending all removed gallbladder specimens for histopathology. The incidence of gallbladder cancer is low but it carries high mortality and morbidity.<sup>14</sup> This dreary prognosis of the gall bladder carcinoma is caused by the great biological aggressiveness of this carcinoma, the lack of accurate biomarkers, the scarcity of screening methods, as well as the late appearance of symptoms, even at an advanced stage. Also, the gallbladder cancer may not always produce a mass and is often not identified on routine ultrasound. Patient may also have only symptoms of cholecystitis.<sup>15</sup> The gallbladder cancer incidence in Asia's is high (8.1 per 100 000) compared to that in the western nations (0.7 per 100 000). The variations in genetic predisposition, environmental exposure to certain chemicals, and regional intrinsic risk of carcinogenic agents are likely to be responsible for these geographic variances in tumor occurrence.<sup>16</sup>

Gall bladder carcinoma is largely asymptomatic until it metastasizes or reaches a late stage.<sup>17</sup> In the current study, sixteen cases (3.9%) gall bladder carcinoma were unintentionally found over the course of study. Several other studies have reported incidental carcinomas in gallbladder samples that are routinely evaluated. Alabi A et al. in 2021 reported incidental gallbladder cancer as a rare entity, with an incidence ranging between 0.19 and 5.5% of all the cholecystectomies.<sup>18</sup> Pyo JS has published a meta-analysis of incidental gallbladder carcinoma in

2020 and has reported incidence rate as 0.6%. Their findings were based on data from 51 related studies.<sup>19</sup>

The distribution of gallbladder carcinoma in relation to age, gender and weight of the patient was also done in the present study. It was noted that all cases of cancer occurred in females and none in the males ( $p=0.029$ ). Nascimento JHFD et al has reported that all types of gallbladder pathologies are significantly more common in females.<sup>20</sup> Halaseh SA et al reports incidental gallbladder carcinoma to be present in 0.3 to 3% of routine cholecystectomies and the cancer risk is 2-6 times higher in females than males. The T1a stage of cancer carries a significantly better prognosis than stage T1b and T2. They also stress the need of histopathological analysis of all cholecystectomy specimen as clinical and ultrasound diagnosis may not be totally reliable.<sup>21</sup>

A study from Lahore by Iqbal S. et al in 2022 showed that out of 214 simple or laparoscopic cholecystectomy specimens, there were three (1.4%) cases of incidental gallbladder carcinoma exclusively found in females.<sup>22</sup>

A large-scale study from Rawalpindi Armed Force Institute of Pathology in 2023 showed the frequency of incidental gallbladder to be 0.9% in 995 cholecystectomy cases. The cancer was mostly found in females and the age group involved was 51 to 60 years. They also stressed the need of histological examination of all cholecystectomy cases.<sup>23</sup>

The local studies report the frequency of gallbladder cancer as 1.4%, 0.9%, 1.5% with high incidence in females. These studies were conducted in Punjab.<sup>4,19,20</sup> Gallbladder cancer in a study from Sindh is reported as 4%.<sup>3</sup> The present study is conducted in a tertiary care hospital of Islamabad suburbs with patients from Punjab and Khayber Pakhtunkhuwah. The frequency of incidental gallbladder cancer was 3.9% which is higher than that reported in other studies from Punjab. This points out an increasing trend of gallbladder cancer in our population and stresses the need for histopathological analysis of all cholecystectomy specimen.

## Conclusion

A spectrum of histopathological diagnosis is observed in cholecystectomy specimen. The inflammatory gallbladder lesions are the most common pathology and the commonest diagnosis is chronic cholecystitis but the doctors cannot rely on the clinical or radiological diagnosis of gallbladder diseases. Histopathological analysis of all gallbladder specimens is necessary for the detection of carcinoma as the frequency of gallbladder

cancer appears to be on the rise in our population which is significantly more common in females.

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- 1,5,6 Drafting the work or revising it critically for important intellectual content.

# Association of Peripheral Pro-inflammatory Cytokines with Depressed Suicidal Patients: A Hospital-based, Cross-sectional Study

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## Abstract

**Background:** Acute-phase reactants, such as C-reactive protein (CRP), along with pro-inflammatory cytokines like interleukin-6 (IL-6) and interleukin-1 $\beta$  (IL-1 $\beta$ ), exhibit elevated levels in the peripheral bloodstream during acute mood episodes. In both major depressive disorder and bipolar disorder, instances of suicidal behavior, encompassing suicidal idealization and self-harm attempts, are linked to intensified depressive exacerbation. This study investigates the correlation between pro-inflammatory markers in patients experiencing major depressive episodes (MDE) with or without inclinations toward suicidal tendencies.

**Methods:** The study comprised a sample of 75 outpatients categorized into three equally sized groups. These groups consisted of patients experiencing major depressive episodes with accompanying suicidal behavior, individuals undergoing MDE without any indications of suicidal tendencies, and a control group of mentally healthy individuals. Pertinent demographic information was gathered, and levels of pro-inflammatory markers, namely CRP, IL-6, and IL-1 $\beta$ , were quantified in the serum. The data were analyzed using the Statistical Package for the Social Sciences version 22.

**Results:** Through bi variate analysis, it was observed that all three markers exhibited a noteworthy correlation with the subgroup experiencing MDE alongside suicidality (Pearson correlation coefficient  $\leq 0.01$ ). However, this correlation was not evident within the MDE subgroup lacking suicidal tendencies. Utilizing a one-sample t-test, it was established that both MDE groups displayed a substantial association (with a two-tailed significance level of  $\leq 0.01$ ) with the inflammatory biomarkers. Notably, the mean difference in biomarker levels was considerably more pronounced in the subgroup characterized by suicidality.

**Conclusion:** Suicidal behavior, encompassing both ideation and attempts, exhibits an independent association with a pro-inflammatory state, irrespective of the severity of the linked depression. This relationship warrants further investigation to identify reliable biomarkers to enhance prediction and facilitate more effective management of suicidal behavior.

**Keywords:** suicide, major depression, inflammation, C-reactive protein, cytokines.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

A major depressive episode (MDE), as defined by the Diagnostic and Statistical Manual – 5th Edition (DSM-5), is experiencing persistently low mood for a continuous period of 2 weeks. There is both subjective

and objective evidence of depressed mood, and this is often accompanied by loss of pleasure in day-to-day activities or anhedonia<sup>1</sup>. Associated symptoms substantiating the MDE diagnosis are excessive feelings of guilt and self-blame, lack of energy, decreased appetite and weight loss, insomnia, recurrent suicidal

ideation and suicidal behavior. The latter symptoms signify that depression is severe and life-threatening, and immediate measures must be instituted to treat such cases<sup>2</sup>. MDEs occur in both unipolar major depression (MDD) and bipolar disorder (BD) and imply a severe disturbance in mood<sup>3</sup>. Research in the past couple of decades has consistently shown the relevance of inflammation, both in the periphery and center, as an essential factor in the pathogenesis of mood disorders<sup>4</sup>. Numerous original studies, systematic reviews and meta-analyses published in the recent past indicate an inflammatory imbalance in MDD and BD.

The principal findings are a rise in pro-inflammatory factors like C-reactive protein (CRP), pro-inflammatory cytokines and chemokines and a decrease in anti-inflammatory cytokines during acute episodes. With successful treatment, these abnormalities are significantly reversed, and during euthymic periods, inflammatory factors may return to normal<sup>5</sup>. Furthermore, there is evidence that severe depressive episodes associated with suicidal thinking/behavior are marked by more significant disturbance in the inflammatory milieu, with large rises in the inflammatory markers<sup>6</sup>. This finding implies that while affective episodes are associated with a pro-inflammatory state, this anomaly is markedly exaggerated in suicidal subjects<sup>7</sup>.

With this background, our study intended to investigate key inflammatory markers in mood disorder subjects presenting as outpatients. We recruited consecutive patients with major depressive episodes and classified them as presenting either with or without suicidal behavior (suicidal ideation  $\pm$  acts of self-harm). We measured levels of CRP, interleukins (IL) 6 and 1 $\beta$  in the peripheral blood of the study subjects and compared them with healthy controls.

The study aimed to examine whether there was any association between inflammatory factors and suicidality. We also examined the study subjects' demographic characteristics to further delineate the sample and clarify our findings.

## Methodology

**Study design and location:** This observational, cross-sectional study was conducted from 01/10/2022 to 31/05/2023 at the Outpatients unit of the Department of Psychiatry, HBS General Hospital, a tertiary care teaching hospital of HBS Medical & dental College, Islamabad. The study protocol was approved by the Ethical Review Board of the medical college.

**Sample collection:** A non-probability, convenience sampling technique was utilized and consecutive patients presenting with a major depressive episode were enrolled after obtaining written consent. Patients, both males and females, aged between 18 to 65 years were included in the study if they were suffering from a DSM-5-defined MDE as determined by history and mental state examination. Hamilton Rating Scale for Depression (HRSD) was instituted to all cases to determine the severity of depression and those subjects admitting to suicidal ideation/behavior were also administered the Suicidal Risk Scale by Beck.

Patients were excluded if they concomitantly suffered from chronic inflammatory conditions like rheumatoid arthritis, systemic lupus erythematosus, acute infections, and systemic disorders (diabetes mellitus, uncontrolled hypertension, and metabolic syndrome). Similarly, patients on long-term treatment with non-steroidal anti-inflammatory analgesics were excluded, and lastly, pregnant females were also not enrolled in the study. In this manner, 25 subjects of MDE with suicidal ideation/behavior and 25 MDE cases without this condition were selected to participate in the study. The control group consisted of 25 healthy adults with no previous psychiatric history. A semi-structured proforma with demographic details was administered to all participants.

**Blood collection and laboratory techniques:** After following the necessary precautions for phlebotomy, venipuncture was performed and 5 ml of blood was collected from each participant. Serum was separated at 4o C by centrifugation for 10 minutes, transferred to Eppendorf micro-centrifuge tubes, and stored at - 80o C until biochemical analysis. The latter was performed on the following biomarkers:

1. IL-1 $\beta$
2. IL-6
3. CRP

Serum IL-1 $\beta$  and IL-6 were analyzed using human sandwich enzyme-linked immunosorbent assay (ELISA) kits provided by Elabscience and the detection range for both cytokines was from 7.81 to 500 pg/ml. briefly, the assay procedure was as following:

100 $\mu$ l of serum was added to each well of the micro ELISA plate, liquid was removed and 100 $\mu$ l of biotinylated detection antibody was added and the mixture incubated for 1 hour at 37o F. After the solution was aspirated and washed 3 times, 100 $\mu$ l of avidin horseradish peroxidase specific for human IL-1 $\beta$  and IL-6 was added and incubated for 30 minutes. It was then

aspirated and washed 5 times. This was followed by the addition of 90µl of the substrate reagent and the mixture was incubated for 15 minutes. Latter 50µl of the stop solution was added, optical density read at 450 nm spectrophotometrically, and results were immediately calculated.

CRP was analyzed by Avitex-CRP latex particles coated with antibodies to human CRP. When serum containing detectable levels of CRP was added, clear agglutination was observed within 120 seconds.

Statistical analysis; This was performed by Statistical Package for Social Sciences (SPSS) Version 22, copyright IBM Corporation, Armonk, NY, USA, 2013.

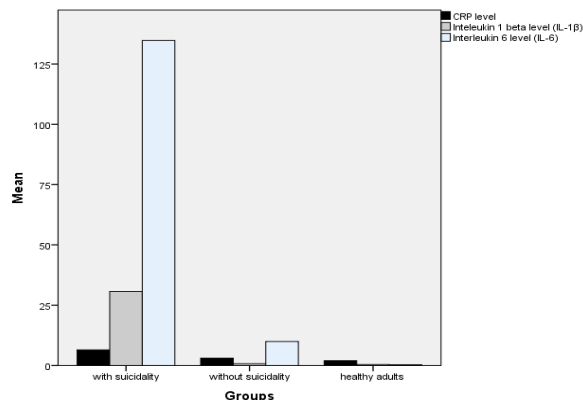
### Results

Descriptive statistics were applied to demographic variables. For continuous variables like age, mean and standard deviation were calculated, while for categorical variables such as gender, marital status, etc., frequency and percentage were computed. The mean and standard deviation for HRSD scores for the 3 groups were also determined (Table I).

Variable	Group	n	Mean ± SD
Age (years)	<b>Total</b>	75	33.32±8.93
	With suicidality	25	28.44±6.70
	Without suicidality	25	37.80±8.10
	Healthy controls	25	33.72±8.93
HRSD score	<b>Total</b>	75	19.36 ± 10.61
	With suicidality	25	26.96 ± 5.59
	Without suicidality	25	25 ± 6.15
	Healthy controls	25	6.12 ± 1.50
Gender	<b>Total</b>	75	Percentage
	Male	39	52.0%
	Female	36	48.0%
	<b>With suicidality</b>	25	
	Male	11	44.0%
	Female	14	56.0%
	<b>Without suicidality</b>		
	Male	13	52.0%
	Female	12	48.0%
	<b>Healthy controls</b>		
Male	15	60%	
Female	10	40%	
Marital status	<b>Total</b>	75	
	Married	58	77.3%
	single	17	22.7%
	<b>With suicidality</b>		
	Married	16	64.0%
	Single	09	36.0%
	<b>Without suicidality</b>		
	Married		
	Single	23	92.0%
<b>Healthy controls</b>			
Married	02	8.0%	
Single	19	76.0%	
Single	06	24.0%	

Occupational status	House wife	20	26.6%
	Student	20	26.6%
	Teacher	12	16.0%
	Own business	10	13.3%
	Miscellaneous	13	17.3%

With respect to the relationship between pro-inflammatory markers (CRP, IL-1β and IL-6) and the 3 groups studied (MDE cases with suicidality, MDE cases without suicidality and healthy controls) (Figure 1).



**Figure 1. Graphical representation of correlation.**

Classical bivariate analysis was performed on pro-inflammatory markers and the study groups using Pearson correlation coefficient. With p value significant at ≤ 0.01 (2-tailed) it was found that the biomarkers were significantly correlated to the MDE cases with suicidality but not to the other group i.e. MDE subjects without suicidality (Table II).

Study group	CRP	IL-1β	IL-6
<b>MDE cases with suicidality</b>			
Pearson correlation coefficient	<i>p</i> < 0.01	<i>p</i> < 0.01	<i>p</i> < 0.01
<b>MDE cases without suicidality</b>			
Pearson correlation coefficient	<i>P</i> > 0.01	<i>P</i> > 0.01	<i>P</i> > 0.01

**Note:** CRP – C-reactive protein; IL-1β – interleukin 1 beta; IL-6 – interleukin 6; MDE – major depressive episode.

The means of the inflammatory markers were analyzed using the one sample T- test (2-tailed significance ≤ 0.01) and the results are given in Table III.

<b>Table III: Pro-inflammatory markers – comparison of means with one sample <i>t</i>-test.</b>						
	<i>t</i>	df	Sig. (2-tailed)	95% CI of the difference	Mean Difference	
					Lower	Upper
<b>MDE cases with suicidality</b>						
CRP level	6.024	24	0.000	6.48000	4.2663	8.6937
IL-1 $\beta$ level	3.823	24	0.001	30.710400	14.13161	47.28919
IL-6 level	4.289	24	0.000	134.761480	69.92025	199.60271
<b>MDE cases without suicidality</b>						
CRP level	6.363	24	0.000	3.04000	2.0539	4.0261
IL-1 $\beta$ level	2.818	24	0.001	0.730160	0.19547	1.26485
IL-6 level	8.651	24	0.000	9.957680	7.58213	12.33323
<b>Healthy controls</b>						
CRP levels	Standard deviation is 0 so results cannot be computed					
IL-1 $\beta$	2.391	24	0.025	0.421200	0.05768	0.78472
IL-6	1.553	24	0.134	0.317280	- 0.10438	0.73894

## Discussion

The mean age of the patients presenting with a major depressive episode and suicidality was 28.44 years, while this was 37.80 years in the MDE group without suicidality (Table 1). This finding that younger people were at greater risk for suicide was somewhat similar to that reported in an epidemiological study in Malaysia, another Asian country<sup>8</sup>. In that study participants between the ages of 16 and 24 had higher risk of suicidal behavior (OR: 2.6, 95% CI 1.08 – 6.2). The reasons for this association can be multifarious, including co-morbid anxiety disorders, melancholic depression or mixed features. Additionally, the social support network may not as robust as in the older, middle-aged cohort<sup>9</sup>. The mean HRSD score in the group with suicidal tendencies was 26.96, somewhat higher than the comparison group in which the mean HRSD score was equal to 25. Greater severity of depression may play a mediating role between hopelessness and suicidal ideation and this mechanism may be contingent on the level of impulsivity. This alludes to the fact that a higher degree of depression accompanied by impulsiveness precipitates attempts at self-harm<sup>10</sup>. Table 1 also shows that females predominate in the group with suicidal ideation or behavior (56% versus 44%). This is in line with reported findings from studies with large sample sizes in which it has been shown that the female gender is a risk factor for suicide attempts<sup>11</sup>.

Figure 1 illustrates the relationship between the pro-inflammatory factors and the three study groups. It demonstrates that all three biomarkers are significantly raised in MDE cases with suicidal ideation  $\pm$  behavior, while this increase is much less in MDE subjects without suicidality. In the case of healthy controls, it can be seen that the biomarkers barely rise above the baseline. Indeed, this is a well-replicated finding and a review of the extant literature supports the proposition that suicidal

ideation and behavior are independently associated with a significant rise in inflammatory factors. A well-cited study showed that MDD patients with severe suicidal ideation or high lethality suicide attempts had a statistically significant increase in the inflammatory markers, and this rise was independent of the degree of depression<sup>12</sup>. Another noteworthy study is instrumental in understanding the role of inflammation in suicidal behavior. It measured serum IL-2, IL-6 and TNF- $\alpha$  in patients with recent suicide attempts, depressed subjects without suicidality and healthy controls. The study found increased levels of pro-inflammatory cytokines, namely IL-6 and TNF- $\alpha$  and decreased concentration of IL-2 (anti-inflammatory cytokine) in suicide attempters compared to the other two groups. The results remained statistically significant after adjusting for potential confounders like age, sex, body mass index, degree of depression, medication use, etc<sup>13</sup>.

To further clarify our findings, we performed bivariate analysis of the inflammatory biomarkers with the study groups (Table 2). Using Pearson correlation co-efficient, we were able to show that CRP, IL-1 $\beta$  and IL-6 were significantly associated ( $p \leq 0.01$ ) with the MDE group with suicidality but this relationship did not achieve statistical significance in the case of MDE subjects without suicidality (Table 2). This is a noteworthy discovery and review of the extant literature revealed that it is in line with published research. A recent study measured high sensitivity C-reactive protein (hsCRP) levels in inpatients admitted after attempted suicide, patients with suicidal ideation, and non-suicidal psychiatric controls. The investigators were able to demonstrate an apparent inflammatory gradient assessed by CRP levels from recent suicide attempters, cases with suicidal ideation and psychiatric controls in the inpatients setting<sup>14</sup>. This showed a linkage between pro-inflammatory state and suicidal behavior in the background of the real world in severely ill psychiatric

patients. In an interesting study, inpatients aged 15-30 years were assessed for multiple measures including serum cortisol as determined by hair cortisol concentration, serum CRP and mRNAs for inflammatory factors including TNF- $\alpha$ , IL-1  $\beta$ , glucocorticoid receptor, etc. In this young cohort, the researchers were successful in differentiating suicide attempters from ideators on the basis of the above biomarkers with a distinct biological profile in both the hypothalamic-pituitary- adrenal axis and the inflammatory pathways<sup>15</sup>.

Finally, we analyzed our results by comparing the means of the inflammatory factors in each of the study groups and the conclusions are provided in Table 3. The mean values were very high in the group of MDE cases with suicidality as compared to the other group, however it remained statistically significant (2-tailed significance  $\leq 0.01$ ) in both sub-groups. It implies that while pro-inflammatory markers are associated with depressive episodes without suicidal ideation or behavior, this linkage is robust in the presence of suicidality. In a pioneering study Kim and colleagues were the first to show differences in the balance of cytokines in MDD patients with and without suicidal behavior. Their sample included MDD patients with recent suicide attempts, major depression cases without suicidal tendencies and normal controls. They used mitogen stimulated whole blood and measured the levels of several pro and anti-inflammatory cytokines. Their study revealed a

distinctive immune response, with the non-suicidal patients showing increased IL-6 production, a Th1/Th2 imbalance and a shift to Th1 lymphocytes, while suicidal MDD cases had decreased IL-2<sup>16</sup>. To conclude the discussion, a worthwhile study must be mentioned which measured plasma kynurenine (KYN) levels in three groups of participants, namely MDD cases with or

without recent suicide attempts and healthy volunteers. KYN levels varied across groups ( $F=4.03$ ,  $df= (2,58)$ ,  $p=0.023$ ) and this was higher in the suicide attempter subgroup compared with non-attempters ( $t=2.105$ ,  $df=58$ ,  $p=0.040$ ), while the latter did not differ from healthy controls ( $t=0.418$ ,  $df=58$ ,  $p=0.677$ ). In post hoc analysis, KYN but not tryptophan (TRP) was associated with attempt status, and only suicide attempters showed a positive correlation of the cytokine activation marker neopterin with the KYN:TRP ratio, signifying that KYN production may be influenced by inflammatory processes among suicide attempters<sup>17</sup>.

## Limitation

While interpreting the results of this study the following caveats must be considered:

- (1) The sample size is small and studies with greater number of participants are required to replicate the findings.
- (2) The design is cross-sectional; only prospective, cohort studies can confirm the results presented here.
- (3) As serum cytokine levels are still not performed routinely, there are technical and financial barriers in conducting such studies, so that many investigators face severe difficulties in carrying out their research work.

## Conclusion

In order to discover biomarkers for the better prediction and treatment of suicide, researchers have been trying for decades to elucidate its neurobiology; the fact is that only in the past decade has the focus shifted to inflammatory factors. In this respect, different teams of investigators have been successful in showing the connection between suicide and inflammation, and this has mainly been achieved by measuring the peripheral values of pro-inflammatory markers. A picture is emerging indicating the central role of inflammatory abnormalities in developing suicidal behavior. The present study demonstrates this fact emphatically and is instrumental in further extending the published findings. It is hoped that valid biomarkers will be discovered that would assist in the overall management of suicidal behavior in psychiatric patients.

## List of Abbreviations

C-reactive protein (CRP), interleukin-6 (IL-6), interleukin-1 $\beta$  (IL-1 $\beta$ ), major depressive episodes (MDE), Diagnostic and Statistical Manual – 5th Edition (DSM-5), bipolar disorder (BD), Hamilton Rating Scale for Depression (HRSD), Statistical Package for Social Sciences (SPSS), high sensitivity C-reactive protein (hsCRP), kynurenine (KYN), tryptophan (TRP)

## Acknowledgements

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<sup>4,5,6</sup>the acquisition, analysis, or interpretation of data for the work & Final approval of the version to be published

<sup>1,2,6</sup> Drafting the work or revising it critically for important intellectual content.

# Survey of Cholera Outbreak and its Resistant Pattern in Baluchistan, Pakistan

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## Abstract

**Objective:** The present study was conducted to identify the isolates of *V. cholerae* as causative agent for the current outbreak in Baluchistan and evaluate the antibiotic sensitivity profile of the isolates

**Methodology;** This cross-sectional study was conducted at Bolan Medical Complex Hospital Quetta from March to October 2022. Suspected stool samples were received with a history of untreated severe diarrhea within 12-hour duration from different affected districts of Baluchistan Province, Suspected colonies were subjected to gram staining, biochemical analysis and antibiotic susceptibility testing was performed by the Kirby-Bauer disk diffusion method.

**Results;** Total 483 diarrheal samples were collected out of which 43 (8.90 %) were positive and 440 (91.09%) were negative. The all isolates 100 % were resistant to common antibiotics were whereas Ciprofloxacin 100%, Levofloxacin 100% and Azithromycin 100% were sensitive to all isolates.

**Conclusion:** Culture methods and biochemical identification of *V. cholerae* from suspected cholera may be helpful. There needs to be systematic surveillance of commonly used antibiotics and encourage the design and implementation of the cholera control policy advised by the WHO

**Key Words:** Cholera, Outbreak, *Vibrio cholerae*, Baluchistan.

**Conflict of Interest:** None

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## Introduction

Cholera is a serious life-threatening infection that has very high morbidity and mortality.<sup>1</sup> Cholera is an acute disease of the intestine spread by contaminated water and food with the Gram-negative facultative anaerobic rod-shaped *V. cholerae* bacterium. This illness is characterized by severe diarrhea like rice water stool with rapid loss of fluid and salts.<sup>2</sup> *V. cholerae* O1 and O139 serogroups are the causative agents of the secretory diarrheal disease. On the basis of O antigen of cell wall of lipopolysaccharide, certain subgroups of *V. cholerae* O1 are further distinguished into two biotypes, classical and ElTor on the basis of phenotypic traits.<sup>3</sup>

Cholera outbreaks were categorized in endemic or epidemic by occurrence.<sup>4</sup> In cholera endemic population, out breaks usually repeated seasonally where children below five years of age are more vulnerable due to the lack of sufficient pre-existing natural anti-cholera immunity.<sup>5</sup> *V. cholerae* has proven to be transmitted via fecal-oral rout, commonly observed around the world,

the infection is now specifically confined to developing countries.<sup>6</sup> The leading factor in the occurrence of cholera is the deficiency of clean drinking water and the absence of suitable hygienic facilities. Compromised sanitary conditions like over population, townships with inadequate health facilities, momentary refugee camps or internally displaced people, and humanitarian or environmental predicaments with disturbed settlements are also said to be major risk factors.<sup>7</sup>

The cholera disease remains endemic in around ninety-six countries, causing about 2.86 million cases and 95,000 deaths a year.<sup>8</sup> Pakistan has a high prevalence of cholera, with Baluchistan appearing to be a key area of the disease spread. Over 13000 suspected cases of cholera have been recorded in the province of Baluchistan. This is an unusual spike in the disease, according to the latest reported, there might be ten deaths in the affected districts of Khuzdar and Zhob, making a serious risk to public health.<sup>9</sup> In Baluchistan province cholera is specially found constantly which add up a

large number of deaths every year. Episodic diarrhea repeatedly happened by drinking contaminated water resulting in malnutrition causing an increase in the susceptibility to disease.<sup>10</sup>

The leading causes of death from severe diarrhea are dehydration, electrolyte disorders and related complications.<sup>11</sup> Cholera is treated with rehydration and antibiotics. Antibiotics are used as a supplement in rehydration therapy because they help reduce the duration of the disease and vibrio excretion, as well as help in prophylaxis to prevent epidemics.<sup>12</sup>

## Methodology

This study was conducted for characterization of *V. cholerae* in department of Pathology (Microbiology) Bolan Medical Complex Hospital Quetta from March 2022 to October 2022.

All ages and both genders were included in this study. Total 483 fecal / rectal swab samples were collected from different affected districts (Zhub, Dera Bugti, Khuzdar, Loralai, Lasbela and Nasirabad) of Baluchistan where cholera outbreak reported. Suspected stool samples collected with a history of untreated severe (rice water) diarrhea of less than 12 hour's duration and with evidence of significant dehydration. Patients who had taken antibiotics were excluded from the study. Cary-Blair (Oxoid, Hampshire —U K) transport medium was used for collection of stool samples and immediately transported to Microbiology laboratory for further analysis.

The suspected stool samples were cultured primarily in alkaline peptone water with a pH of 8.0 and 2% NaCl, (Oxoid, UK) followed by incubation at 37 °C for six hours for the detection of *V. cholerae* from suspected diarrheal samples. Later the culture was streaked on thiosulfate citrate bile salts sucrose agar (TCBS). Incubated the plate for 24 hour at 37°C. Growth of *V. cholerae* colonies were gram stained and the characteristic observation of bacterial isolates were based on standard methods. Selected colonies were further cultured on medium such as nutrient agar for its non-selective characteristics at 37°C for 24 hours, biochemical tests such as oxidase, catalase, indol, Simmon citrate utilization tests and motility tests were performed. The antimicrobial susceptibility of *V. cholerae* isolates to different antibiotics was performed on Mueller Hinton agar (MHA) by Kirby-Bauer agar diffusion method.<sup>13</sup> The antibiotics panel testing includes Ampicillin (10µg), Nalaxic acid (30µg), Sulfonamides-Trimethoprim (251.25+23.75µg), Tetracycline (30µg), Doxycycline (30µg), Ciprofloxacin (5µg), Ceftriaxone

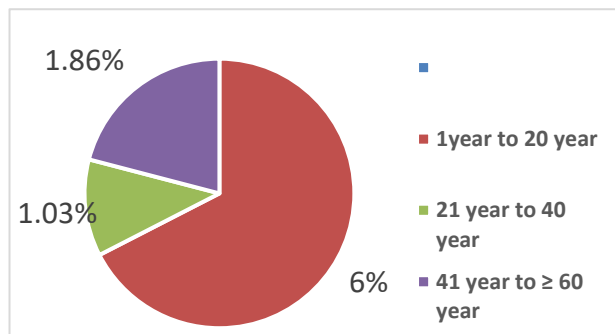
(30µg), Amikacin (30µg), Levofloxacin (5µg) and Azithromycin (15µg) according to guidelines of the clinical laboratory standards.<sup>14</sup>

A SPSS version 22 was used to analyze the data. The P value was calculated through *t*-test and 0.05 or lower was considered significant. The frequency of the antibiotic susceptibility test was also analyzed by using SPSS. Ethical approval for the research was obtained from the ethics review committee of Bolan Medical Complex Hospital Quetta (No. Supdt: /BMCH/465/49/2022) and was carried out in accordance with the Helsinki Declaration

## Results

All 483 suspected diarrheal samples, out of which 8.90% were positive during cholera outbreak. *V. cholerae* O1 serotype Ogawa as responsible for current outbreak which affected six districts Zhob, Dera bugti, Khuzdar, Loralai, Lasbela and Nasirabad of Balochistan province as shown in Table- I.

Affected districts	Percent Positive	P. value
Zhub	2.07%	< 0.001
Dera Bugti	1.86%	
Khuzdar	1.65%	
Loralai	1.44%	
Lasbela	1.0%	
Nasirabad	0.82%	



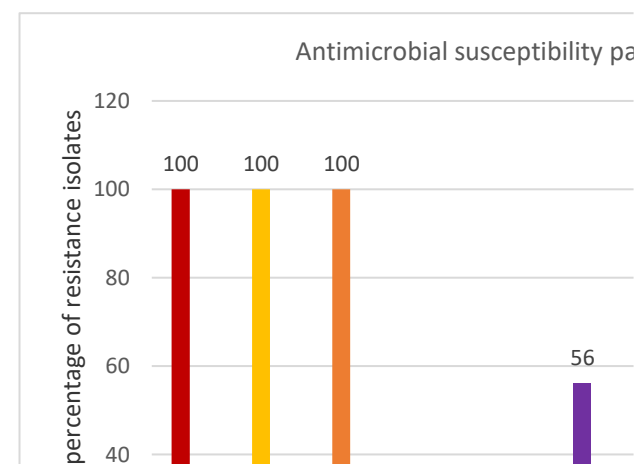
**Figure I. Cholera Incidence in different age groups.**

Variable	Percent Positive	P value
<i>V. cholerae</i> O1 serotype Ogawa	8.90%	< 0.001
Gender Male	5.17%	
Gender Female	3.72%	

## Discussion

The cholera outbreak continues to be a very important public health problem in Baluchistan, where access to safe water and sufficient hygiene is still challenging. Due to high-occurrence diseases, health care workers are unreachable due to remote areas and overworked, which

often increases the possibility of cholera outbreaks in these environments. In 1994 there was cholera outbreak occurred in Sibi in which *V. cholerae* O139 were isolated.<sup>15</sup>The *V. cholerae* serotype O1 Ogawa has been isolated from other district of Balochistan in 2005.<sup>10</sup>



\*AMP = Ampicillin, NA = Nalidixic acid, SXT= Sulfonamides-Trimethoprim

AK = Amikacin, CRO = Ceftriaxone, DO = Doxycycline, TE= Tetracycline, CIP = Ciprofloxacin, LEV = Levofloxacin, AZM = Azithromycin

**Figure- II. Antibiotic resistance against *V. cholerae* isolates.**

The age wise result showed that cholera infection was higher in individuals below 20-years-old patients as compared to above 20 years age group as shown in Figure I. Motaweq, (2018)<sup>16</sup>Hasoon et al., (2012)<sup>16</sup> reported that the *V. cholerae* affected all age population but the incidence rate was more in 1- 20-years population. Furthermore, gender wise *V. cholerae* infection was high in male patients as compared to female patients as shown in Table II. The gender wise associated findings of the current study were similar to those reported previous.<sup>18</sup> and the same case were with gender-related findings.<sup>19,20,16</sup>

The susceptibility of all isolates to ten commonly used antimicrobial agents as shown in Figure 1.2. The results showed that 100% isolates were resistance to Ampicillin, Nalidixic acid and Sulfonamides-Trimethoprim. Our finding was almost similar as described by Wang et al., (2012).<sup>21</sup>In current study Tetracycline was 93.18% resistance to isolates but previous study by Karki et al., (2010)<sup>22</sup> contrast to our study the isolates were found to be 100% sensitive to Tetracycline.

*V. cholerae* isolates were 56% resistance to Doxycycline. All *V. cholerae* strains were identified as susceptible to Doxycycline (100 %) by Gupta et al., (2016)<sup>18</sup> However, in contrast to our study.

This study revealed the resistance with Amikacin, Ceftriaxone as 24%, 14% respectively. On the contrary, in a study conducted in Iraq 2017<sup>16</sup>, strain of *V. cholerae* was found to be resistant to Amikacin (41%) and Ceftriaxone (93.3%). In the present study, the isolates showed utmost sensitive to Amikacin. High sensitivity of *V. cholerae* isolates to Amikacin has also been reported by many other researchers (Uppal et al., 2017; Kumar et al., 2014; Taneja et al., 2003).<sup>23, 24,25</sup>

All isolate were 100% sensitive to Ciprofloxacin, Levofloxacin and Azithromycin. Previous study by (Sack et al., 2006; Igere et al., 2020)<sup>26,27</sup> 100% percent sensitivity was observed for Levofloxacin, ciprofloxacin and Azithromycin which was similar to the findings of our study.

## Conclusion

In this study, commonly believed methods for isolation, detection, and characterization of *V. cholerae*, provided more extensive knowledge of the epidemiology of *V. cholerae*. We concluded that serological identification is also a good tool. We also conclude that *V. cholerae* O1 persist in developing country and is still causing incidence of cholera.

This study confirmed that *V. cholerae* has resistance to many antibiotics utilized in hospitals. The antimicrobial susceptibility pattern of the *V. cholerae* is essential to start an appropriate treatment.

**Recommendation:** Safe water supply and adequate sanitation, hygiene are the important factor for the control and prevention of cholera infection. Encourage the design and implementation of the cholera control policy advised by the WHO.

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#### Authors Contribution:

<sup>1</sup>Substantial contributions to the conception or design of the work;

<sup>2,3</sup>The acquisition, analysis, or interpretation of data for the work;

<sup>1,2</sup>Final approval of the version to be published;

<sup>1,2,3</sup> Drafting the work or revising it critically for important intellectual content;

# Knowledge, Attitude and Practices of Hand Hygiene Among First year and Fourth year MBBS Students; A Cross Sectional Comparative Study

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## Abstract

**Objective:** To compare the knowledge, attitude and practices of hand hygiene among first- and fourth-year medical students of HBS Medical and Dental College Islamabad.

**Methodology:** A cross sectional descriptive comparative study was carried out in HBS medical and dental college Islamabad from June to August 2022(n=200). The survey was based on self-structured closed-ended questionnaire about hand hygiene practices among the participants.

**Results:** We identified 90% of first year and 98% of fourth year medical students with adequate knowledge, attitude and practices about hand hygiene.

**Conclusions:** The majority of first and fourth year MBBS students had updated information regarding hand hygiene knowledge, attitude and practices.

**Key words:** Hand hygiene, Knowledge, Attitude, Practices, Medical students.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Hand hygiene is the most effective way to control infectious diseases in every day life<sup>1</sup>. A survey suggested that decreased compliance may be related with forgetting to wash the hands before and after contact with any dirty object. In developing countries one of the commonest causes of morbidity are hand transmitted infections.<sup>3</sup> The burden of disease is even greater in developing countries, having limited resources such as South East Asia and Pakistan<sup>3</sup>. In short; Adequate hand hygiene practices prevent the risk of transmission of disease.

In clinical practice this is the simplest and most cost-effective method to decrease the chance of infection transmission<sup>4</sup> as the health care providers can quiet easily transmit serious infections to their patients and jeopardies their life.<sup>5</sup> If water is not available a simple rinsing of hands with antiseptic agent is recommended.<sup>6</sup> If these measures are not followed, it can adversely affect human health and poses a serious risk of

communicable diseases<sup>7</sup>. Infection prevention in clinical settings require a multidisciplinary approach. As apart from hands, the equipment and other vectors can transmit pathogenic microorganism<sup>8</sup>. In Wuhan, China during late December 2019 the Covid 19 outbreak was result of poor compliance to such practices.<sup>9</sup> Many studies have reiterated the transmission through contaminated hands and importance of hand hygiene.<sup>10,11,12</sup> These simple measures can reduce significantly and put a stop to many nosocomial infections. These can decrease the infection rate can be as high as 40%<sup>10,11,12</sup>. hence the compliance to protocols and disinfectants is much needed. lack of compliance could be due to shortage of time and poor knowledge. Also, skin irritation may occur but use of irritant and unhygienic products.<sup>13,14,15</sup>

WHO also recommends health care providers to follow these protocols strictly and adopts a Ayliffe technique which consists of six steps. Another WHO criteria for hand washing is called "5 moments of hand hygiene."<sup>16,17</sup>

1. The study will educate us about the knowledge attitude and practices of medical students and also how they have improved from first to fourth year.
2. Novelty of the proposal: This study will improve their existing knowledge and practices about hand hygiene.
3. Benefits to the medical field: It might be helpful in better management of hand hygiene & prevention of spread of infections.
4. Benefits to patient community: This article may be helpful to encourage the positive effects of hand hygiene among the masses.

## Methodology

This cross-sectional, descriptive, comparative study conducted between June 2022 to August 2022 at HBS medical and dental college Islamabad. Total 200 student, 100 from each class of 1<sup>st</sup> and 4<sup>th</sup> year MBBS students of HBS medical and dental college Islamabad was enrolled in this study. Non probability consecutive sampling technique was used. Analysis was carried out by using SPSS version 25.

- **Inclusion criteria:** First and fourth year MBBS students of HBS medical and dental college Islamabad
- **Exclusion criteria:** Students who were not willing to participate in the study
- Self-structured closed ended questionnaire was used.

Knowledge in Hand Hygiene, Attitudes towards Hand Hygiene, Self-reported Hand Hygiene Practices.

## Discussion

Hand hygiene is considered as the basic measure in preventing the spread of microorganisms. It is also proven to be effective method in decreasing the incidence of hospital acquired infections.<sup>18,19</sup> Although hand hygiene protocols are simple, healthcare providers hand hygiene compliance is not very good.<sup>20</sup> Our study showed that knowledge regarding hand hygiene among medical students of HBS medical college is remarkable but there is some slackness of attitude and practices. After COVID 19 pandemic the knowledge of hand hygiene has increased many folds and now almost everyone is fully aware. Unlike a study conducted at tertiary care hospital Lahore, Pakistan, which showed hand hygiene knowledge, attitude and practices of health care workers as unsatisfactory.<sup>21</sup> Boyce and Pittet have reported that the average compliance rates of hand hygiene among doctors and nurses at the University Hospital in Geneva were approximately 30-50% only.<sup>19</sup>

Despite the attained hand hygiene education, many students are still unable to carry out the hand hygiene procedure as per WHO guidelines successfully.<sup>22</sup> Our results had very similar findings just like a study done in Sri Lanka showing that in spite medical students having some knowledge regarding hand hygiene practices they still needed improvements.<sup>23</sup> Study identifies that though

Questions	1 <sup>st</sup> Year		4 <sup>th</sup> Year		Mean	SD	P value
	Yes	No	Yes	No			
Do you feel hand hygiene will reduce the risk for transmission of infections?	98%	2%	99%	1%	.000	.201	1.000
Do you feel our hand are at higher exposure to 94% germs then other part of body?	90%	10%	94%	6%	-.050	.479	.299
Do you consider hand hygiene important for overall health?	92%	8%	6%	94%	.040	.281	.158
Curriculum /Training received in order to improve hand hygiene practice?	67%	33%	35%	75%	-.760	1.138	.000
Do you feel improper hand hygiene promote hospital acquired infection 81% fourth correct answer 81 % first student correct answer.	81%	21%	81%	21%	.000	.620	1.000
Do you consider hand hygiene facilities at your work place/educational institution are adequate or not?	63%	57%	97%	3%	.320	.548	.000
Do you feel hand jewelery and artificial nails should be removed before washing hands?	63%	47%	70%	30%	.060	.679	.379
Minimum time required for proper hand hygiene to control infection?	38%	62%	43%	57%	-.220	.773	.005

## Results

The survey had a total of 200 respondents. 100 students from 1<sup>st</sup> year and 100 from 4<sup>th</sup> year. 38% male participants and 64% female's participants were included in the study. Age of the participants ranges from 18 to 24 year. The results were divided into three sections according to the group of questions being asked:

health care workers have adequate knowledge but they still do not fully practice hand hygiene.<sup>23</sup> Another research showed poor knowledge and practices and they required proper guidance and training.<sup>24</sup> A gap in knowledge attitude and practices regarding hand hygiene in the 1<sup>st</sup> year medical students was also identified<sup>25</sup>.

Questions	1 <sup>st</sup> year		4 <sup>th</sup> year		Mean	SD	P value
Do you consider washing hands a necessity or an option? (a) Necessity	94%	6%	93%	7%	.000	.318	1.000
What are barriers that keep you away from hand hygiene? (c) Lack of education.	41%	59%	22%	18%	-.300	1.840	.106
Do you fear washing hands frequently will cause excessive skin dryness/allergy? or could lead to a skin condition?	64%	36%	54%	46%	-.400	.841	.000
Which is strongest motivation of the hand hygiene practice?	36%	64%	13%	87%	.940	1.013	.000

Question	1 <sup>st</sup> year		4 <sup>th</sup> year		Mean	SD	P value
How frequently do you wash your hands (b) 2 to 5 times in a day.	53%	47%	61%	94%	.520	.627	.000
Do you wash your hand before after meal? (a) Often mis.	30%	70%	46%	54%	-.160	1.212	.190
Do you perform hand hygiene after sneezing or coughing?	40%	60%	48%	52%	-.070	.517	.179
Do you wash your hand after attending toilet?	89%	11%	73%	27%	.070	.355	.52
What do you normally practice for hand hygiene(a) Soap & water.	87%	13%	92%	8%	-.160	.564	.005
How do you wipe your hand after hand 90% washing? (a) With towel.	70%	30%	90%	10%	.590	1.156	.000
Do you feel using gloves will minimize the spread of disease and improve your hand hygiene?	78%	22%	87%	13%	.150	.609	.016
What do you observe from your senior doctor regarding hand hygiene practices? (a) Majority practice after leaving duty time.	23%	77%	53%	47%	-.030	1.840	.106
Does hospital environment encourage you to do hand hygiene?	82%	12%	76%	24%	.050	.479	.299

Unsatisfactory findings were seen among many health workers about these attributes.<sup>26</sup>

Unlike undergraduates the postgraduate trainees were quite practical about good knowledge attitude and practices regarding hand hygiene<sup>27</sup>. Our study showed knowledge improvement from first to fourth year, which was good but suggested that more emphasis is required on attitudes and practices. In short, an attitude and practice change needs enforcement rather than knowledge alone to implement WHO guidelines.

## Conclusion

This study shows that the students have good knowledge regarding hand hygiene. Although there are some deficiencies and practices in attitude and practice.

### Recommendation

Improvement is required regarding hand hygiene, attitude and practice among potential health care providers for better infection prevention in future. It is necessary to educate medical students regarding proper hand hygiene procedures on regular basis. Continuous medical education and skill-assessment should be included in their teaching curriculum, which will help to minimize the risk of preventable infections.

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#### Authors Contribution:

- 1,2 Substantial contributions to the conception or design of the work;
- 3,4,5 The acquisition, analysis, or interpretation of data for the work;
- 2 Final approval of the version to be published;
- 1,2,4 Drafting the work or revising it critically for important intellectual content.

# Oral & General Health Status of Charity Based Madrasa Students – A Cross-sectional Survey in Abbottabad Region

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## Abstract

**Objective:** To evaluate the oral and general health status of Madrassas students.

**Methodology:** A cross-sectional survey was conducted on two charity-based local Madrassas in Abbottabad City, Pakistan. Data was gathered by convenient sampling technique and the sample size was n=101 students, between the age of 3-21 years. There was n=63 female & n=38 male study subjects in total. Upon oral examination following variables were recorded; 'DMFT' scores, 'orthodontic Treatment Needs' and a questionnaire of oral hygiene practices. On the other hand, Medical Physical Examination' was performed and recorded for the same participants. Although dental and medical data was prerecorded on the structured survey form and analyzed by using IBM-SPSS v.26.

**Results:** A total of 86.1% of students were performing once daily tooth brushing and in contrast, 82.2% of participants have had a frequent intake of a sugary diet on daily bases. Meanwhile, only 20.8% of participants Visited the dentist or were taken to the dental clinic. There were 70% of participants diagnosed with carious lesions and caries scores (DMFT score) were ranged between 1 to 10, hence caries trends were more common in female participants. Meanwhile, 36.6% of subjects were reported with a high prevalence of caries along with malocclusion. 'Medical Physical Examination' results showed significant differences among most of the female students, low Body Mass Index -BMI rate, hence pallor skin symptoms commonly in males. While 51% of females have reported Scabies disease.

**Conclusions:** The overall health of underprivileged pupils were compromised. This population is neglected because of many social, environmental and economic factors, therefore more public health efforts are obligatory to promote the overall health of the targeted population.

**Keywords:** Oral hygiene, Caries, Malocclusion, DMFT, Medical physical examination, BMI, Scabies

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Health is defined by World Health Organization (WHO, 1948) as 'a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity'. Hence, oral health is also an essential part of our overall health. Unfortunately oral disease burden is increasing day by day, as WHO estimated 3.5 billion of population suffering from oral diseases globally; like dental caries & periodontal diseases.<sup>1</sup> Poor oral hygiene is leading factor to cause dental caries and periodontal diseases.<sup>2</sup> Common oral diseases such as tooth decay and gum diseases are preventable if an individual has good oral hygiene, follows regular dental visits for early diagnoses of disease and cure. Oral diseases are also prevalent and affecting children as well, however primary

prevention is feasible way to reduce burden of such diseases.

Subsequently, underdeveloped nations have been facing general health issues including communicable diseases. WHO reported, more than 200 million people effected from Scabies worldwide.<sup>3</sup> Scabies is a human skin parasitic infestation cause by 'Sarcoptes scabiei var hominis' and this skin disease is common all over the world. It is most common among population living in highly populated and tropical areas.<sup>4</sup> According to WHO the children living in poor-resource areas are affected from scabies more than 10% in world.

Body Mass Index (BMI) is a method of screening ,to estimate weight categories (high or low) of population which is a causative factor to health problems and

linked to malnutrition.<sup>5</sup>

Present study aimed to determine the oral & general health status of Madrassa’s students by assessing the Dental Caries, Malocclusion, Oral Hygiene status and complete Medical Physical Examination.

### Methodology

Madrassa in Abbottabad city, KPK, Pakistan. Madrassa is an educational-institution where Islamic education given to communities and these institutions are funded by both government and non-government philanthropist organizations .This study conducted in February 2022 by convenient sampling technique under a survey titled ‘Community Out-reach Health Awareness Program’ . A total number of 101 (male n=38 & female n=63) Madrassa students were examined for dental & medical health status. During oral examination variables were recorded; such as ‘DMFT’ for dental caries, malocclusion and verbal questioning about oral hygiene practices , frequency of tooth brushing, and daily intake of sugary diet. Also “Medical Physical Examination” of study subjects were performed by medical doctors and recorded; including BMI, Pulse rate, appearance of skin (Scabies/Pallor/Anemia), Clubbing, Koilonychia, Cardiovascular examination (CVS),Chest, GIT, C.N.S. All data were recorded on structured survey forms. Furthermore, data was analyzed by IBM-SPSS v.26.

Clubbing, Koilonychia, Cardiovascular examination (CVS),Chest, GIT, C.N.S. All data were recorded on structured survey forms. Furthermore, data was analyzed by IBM-SPSS v.26.

### Results

Total 101 study participants were screened (girls n=63 -62.4% & boys n=38 - 37.6%) between the ages of 3-21 years (mean=1.66, SD=0.515) which further categorized into 3 groups, Group1= 3 - 10 years (35.6%), Group2 =11-20 years (62.4%), Group3=21(2%).

Participants of this study were questioned about their daily oral hygiene practices. Significance results were observed in responses to; tooth brushing once daily 86.1% and only 24.8% flossing daily (mostly in the age group 11-20 years). Hence daily intake of a sugary diet was also very common (82.2%) among both male/female participants. Although only 20.8% of the study subject visited the dentist in the past and 78.2% never seek dental professional assistance.

“Dental caries scores” and “orthodontic treatment

needs” were observed during the oral examination. DMFT scores were recorded between 1 to 10 in almost 70% of participants of the study {Figure:1}, while 30% reported no carious lesion (0 = scores) in which the maximum was from the age of 11 to 20 years and the minimum was from 3 to 10 years. {Figure:2}. Female participants reported more carious teeth as a contrast to male subjects {Figure:3}

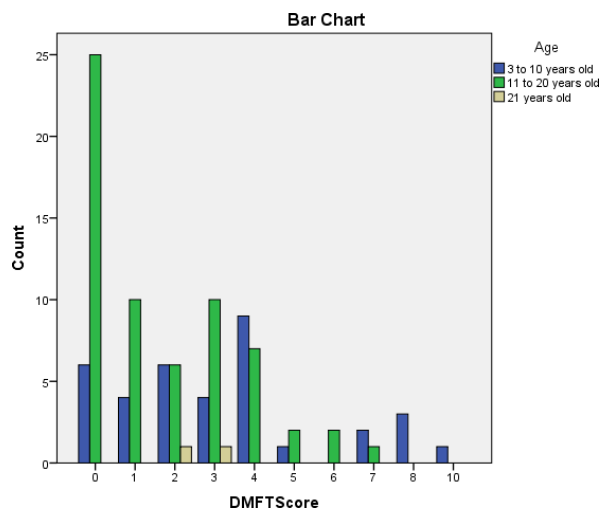


Figure 1. DMFT score.

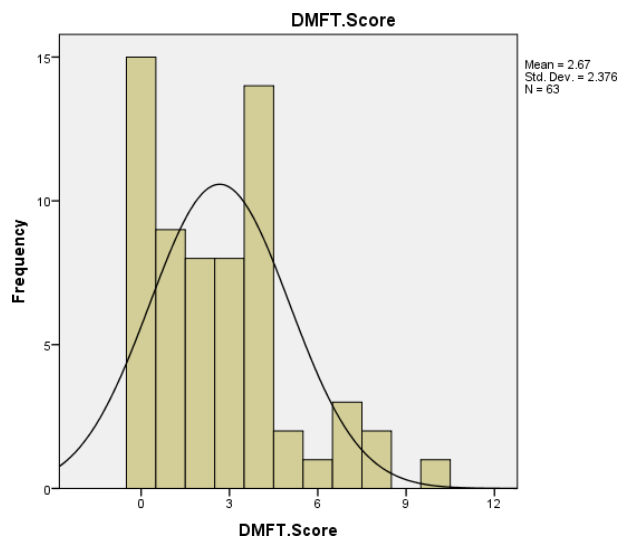
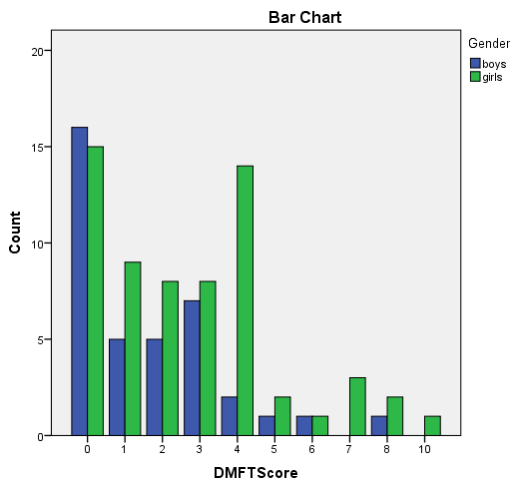


Figure 2. DMFT scores in different age groups

Although 37 (36.6%) out of 101 participants have been diagnosed with malocclusion and remaining 64 (63.4%) subjects have well-aligned dentition {Figure: 4}. Pupils who need orthodontic treatment also showed carious teeth (DMFT fluctuated scores were 1,2,3, 4,7,8 &10) which is alarming for their future oral health state and the dire need to treat caries and malocclusion.

Subsequently, a Medical Physical Examination of study participants was performed, most significant results were found a low BMI rate among 70.73% of participants (BMI-range between 10.23 to 17.90) in

which female participants were the majority (63%) showed a low BMI rate {Figure:5}. Most significant responses were recorded while examining skin; a total of 51% of females were reported with signs & symptoms of scabies disease and pallor skin was



observed among males 31.42% & 29.78% in females {Figure:6 & 7}.

Figure 3. DMFT score in male and female

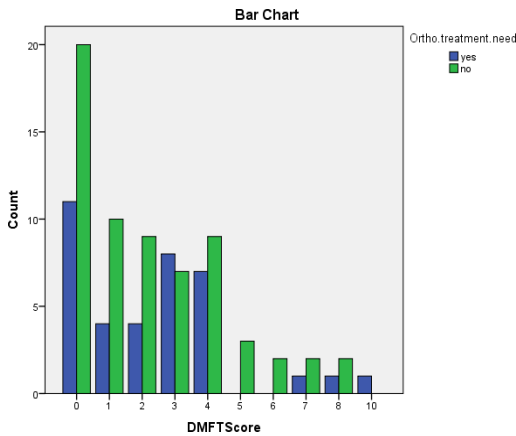


Figure 4. DMFT Score & Orthodontic Treatment Need

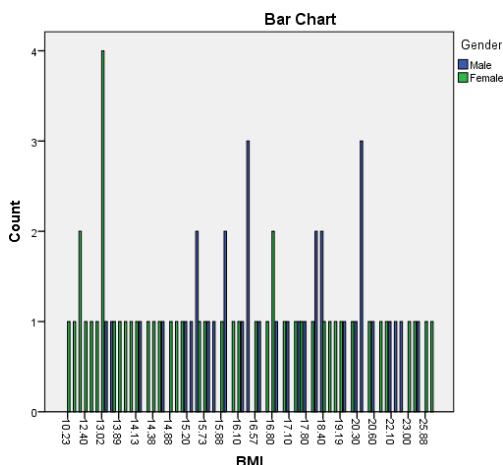


Figure 5. BMI Rate: 70.73% students have low BMI, (range from 10.23 to 17.90) in which 63% female

were showed low BMI rate

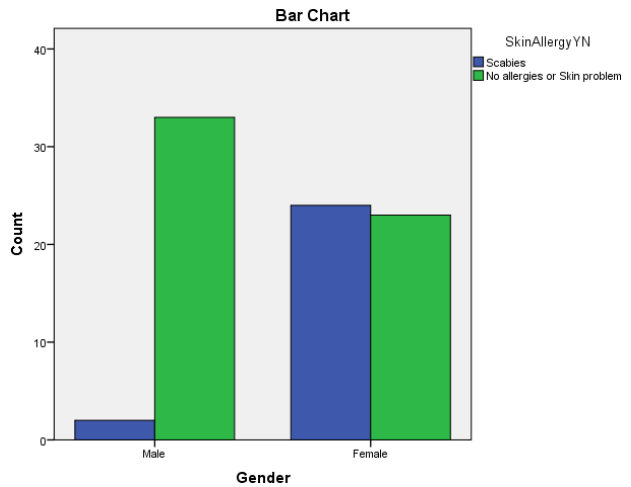


Figure 6. Scabies cases reported 51% in female & 5.7% in male students of madrassa

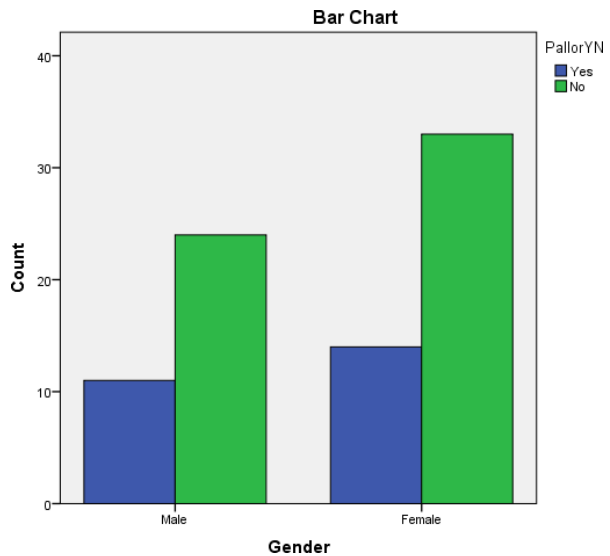


Figure 7. Pallor skin were reported 31.42% in male & 29.78% female

## Discussion

Poor population has compromised general and oral health (6). World Health Organization estimates more oral diseases among people with low socioeconomic class(7). Also treatment of oral disease is quite expensive along with scarcity of dental care services for all population in low middle income countries including Pakistan(1). The present study targeted population of a lower socioeconomic class and their oral and general health practices were poor.

Oral diseases including dental caries are more common all over the world, due to its multi factorial nature and existing health inequalities (8). Oral health related results of present study showed; 70% participants

diagnosed with dental caries with females more affected than males, and low frequency of tooth brushing daily. Precisely these findings are similar to another study done in Bangladesh namely “oral health status among Madrassa going children in selected area of Dhaka City, Bangladesh” (9). Yet another study conducted on madrassa students showed high caries rate. A study to measure caries with serum iron level showed caries prevalence similar to our study but no correlation (10). The increased frequently intake of sugary diet, inadequate brushing timing & technique is directly related to poor oral hygiene which results in carious lesions. In our study, maximum participants although had brushing frequency, once daily, but with faulty brushing technique, and more intake of sugary diet they had poor oral hygiene. The results were similar to another study held in Indonesia; “Habit of tooth brushing with the dental caries incidence”(11). The caries lesion affects the dentition more with malocclusion as results showed 36.6% students requiring orthodontic treatment. The high caries scores in malocclusion and mal- aligned were seen also at India; “Dental Caries and its Relationship to Malocclusion in Permanent Dentition Among 12-15 Year Old School Going Children”(12).

The Medical Physical Examination showed significant low BMI rate, number of Scabies cases and Pallor skin. Present study results illustrated 70.73% with low Body Mass Index- BMI in overall study participants, majority were females (63%). Causes of being underweight were many like, genetics, unhealthy eating or deficient nutrition intake, high metabolic rate, increase physical activities, or physical illness or chronic diseases. Consequences of low BMI; were osteoporosis, hair, nail & teeth problems, prone to get disease frequently, risk of anemia and many more(13). One of reasons for low BMI were increased physical activities of Madrassa students since they are performing household chores and other labor work without healthy diet. Imbalance nutritional diet due to low socioeconomic status also affects their mental health but we did not observe any mental problems. We also did not observe any sign or symptom for chronic disease/illness during “Medical Physical Examination” which might have been related to their low BMI. Nevertheless pallor skin were observed among study subjects which could also be attributed to cold weather and study done in winters(14).

We did find Scabies, which is an endemic and contagious disease spreading quickly to family members, school children or close contact groups of people. Present study reported cases 51% in female &

5.7% in male participants. A study was conducted for prevalence of Scabies in welfare home Malaysia, found 31% cases with male predominance (15). Another study in Ethiopia with malnutrition, poor hygienic life style and overcrowded population, also showed scabies with 11% of school children dropping from school. (16). A cross-sectional survey was conducted in Timor-Leste country for prevalence of Scabies with 30.6% cases. Yet another study showed participants (children) dwelling in rural areas, more affected. (17). Likewise, our study found scabies in female madrassa students, because of over populated area, small building with poor infrastructure, poor living standards, poor hygiene and living with close contacts.

This particular group of Madrassa students direly need basic life facilities and health education to fulfill their health state & wellbeing. Burden of diseases can reduce by public health intervention as stated by WHO. More public health program in collaboration of medical and dental professionals should be organized on routine basis and complete health coverage provided to uplift these low socioeconomic class.

The evidence of this study is limited due to smaller sample size and result could not be generalized. Furthermore, for complete workup, investigations should also be performed. A multicenter study with detail workup could clarify all doubts, and justify results further.

## Conclusion

The majority of these underprivileged Madrassa students were suffering from oral & general health problems. Lack of facilities and health awareness are responsible for compromised health and well-being the root cause of their poor health. Although there is dire equity to plan and should be implemented “targeted preventive approach” - health program to promote this specific population's health. As well as provide them easy and free or low-cost access to medical and dental professional care services on the local and national levels.

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#### Authors Contribution:

<sup>1,2</sup> Substantial contributions to the conception or design of the work; The acquisition, analysis, or interpretation of data for the work; Final approval of the version to be published; Drafting the work or revising it critically for important intellectual content.

# Prevalence of type-2 Diabetes Mellitus and Prediabetes using HbA1c in OGDCL Employees

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## Abstract

**Objectives:** A screening survey using glycosylated hemoglobin (HbA1c) as the screening tool, was conducted amid OGDCL employees across Pakistan to evaluate the occurrence of pre-diabetes and type 2 diabetes.

**Methodology:** This study included employees aged  $\geq 40$  years working at OGDCL's Head Office and various Oil Fields across Pakistan, all of whom subjected to HbA1c testing. Data collected were analyzed using SPSS version 26. Categorical variables were summarized as frequencies and percentages, with data presented graphically using bar charts. Chi-square tests were used to compare characteristics across three groups: non-diabetes, pre-diabetes, and diabetes. A significance level of  $p \leq 0.05$  indicated significance, while  $p \leq 0.01$  was considered highly significant.

**Results:** A total of 8689 participants were recruited in our study. Out of which 66.26% were non-diabetic and the prevalence of pre-diabetes and diabetes was found to be 12.3% and 21.5% respectively. In our study 34.7% of the participants had family history of diabetes. In diabetic people percentage (54%) of having family history of diabetes was high as compared to the non-diabetics (29%) and pre-diabetics (32.1%) individuals which created a substantial variance between the three groups. ( $p=0.001$ ) Majority 94.8% of the study participants never smoked, 3.3% were current smokers and 1.9% had history of smoking. The prevalence of current smokers was high (6.1%) in pre-diabetes as compared to the diabetes (2.0%) and non-diabetes (3.2%). A strong significant association was observed between smoking and pre-diabetes. ( $p=0.0001$ ) In our study prevalence of hypertension among pre-diabetes and diabetes was 21% and 20% respectively. ( $p=0.738$ ) The majority 57.8% of the participants in our study was overweight and the prevalence of obese was 10.7%. ( $p=0.001$ )

**Conclusions:** In OGDCL, there is much higher incidence of type 2 diabetes and pre-diabetes as was previously thought. Comprehensive approaches are required to be established to integrate screening, prevention and treatment of type 2 diabetes both at primary and secondary health care levels, at Head Office and Oil Fields. This is necessary not only for intensification of the workout put but also to cut down the medical budget.

**Key words:** Type II Diabetes Mellitus, Prediabetes, HbA1c, Glycosylated Hemoglobin

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Diabetes mellitus (DM) is not just a disease but a worldwide public health concern. This has affected masses of people of all ages, gender, and racial and ethnic groups. It is a chronic illness that causes derangement of the metabolism of all macro nutrients, which later on result in chronic progression to failure in the smooth functioning of multiple organ systems in the body, including cardiovascular, visual and renal systems.

Patients that suffer from Diabetes Mellitus have a higher incidence of IHD, HTN and dyslipidemia as compared to non-DM patients. Vascular derangements are the most considered important offenders in producing pathologies in diabetic patients.

DM is considered as metabolic disorder linked to hyperglycemia, atherosclerosis, inflammation, hypercoagulability, dyslipidemia and insulin resistance. All of the mentioned pathologies lead to endothelial dysfunction in organ systems and associated complications. Endothelial damage is the foremost player behind vascular complications in DM.<sup>1,2</sup>

Vascular pathologies related to DM lead to development of macrovascular and microvascular complications. Microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications (cardiovascular disease, stroke, and peripheral artery disease).<sup>3</sup>

The increased risk for Type 2 diabetes associated macrovascular and microvascular complications and cardiovascular disease is eminent factor which needs therapeutic attention for at a higher risk group and this demands early screening for disease. Later there could be approach to a change in life style and weight reduction. Increased body weight has been shown to be a strong predictor of the metabolic syndrome. This understanding of correlation of body weight with disease gives hope to the philosophy that treatment and prevention may seem therapeutic because it means that losing weight or stopping weight gain might decrease the hazard of a future appearance of a factor that is still not obvious.<sup>4</sup>

Diabetes mellitus (DM) is increasingly considered as a pandemic worldwide. Globally, DM is affecting health of more than 285 million people up till now. It is estimated that by end of 2030, it will reach to 438 million. This is particularly true for low resource countries, where there are limited health resources, hence becoming a huge public health problem in these countries. South Asian population is more predisposed to type 2 DM. The prevalence of DM has estimated to increase over 151% between year 2000 and 2030 in South Asian region. In year 2012, incidence of DM in adults in Pakistan was almost 6.6 million therefore being ranked the tenth largest country with this pathology. In Pakistani population, there is substantially high occurrence of diabetes leading to development of associated complications at relatively younger age.<sup>5,6</sup>

The worldwide prevalence and incidence of Intermediate hyperglycemia or borderline diabetes (a.k.a Pre-diabetes) is rising and it is estimated that by 2030 more than 470 million individuals will be prediabetic. The risk for Type 2 DM intensifies due to prediabetes, which results in the predisposition to different diabetes associated complications. However, literature specifies that Lifestyle alterations can aid in reducing or preventing diabetes progression by 40–70%. This outcome emphasizes the stipulation for early diagnosis of prediabetes and diabetes.<sup>7</sup>

Therefore, the current study, is aimed to explore both the prevalence and the causative factors of prediabetes and diabetes in OGDC so that strategies can be developed for early detection and improvement of prediabetes and to

avert development of diabetes and its associated complications.

The specific objectives of our study were to estimate prediabetes and diabetes prevalence among the OGDC employees across the country and compare the qualitative characteristics of divided three study groups of participants.

## Methodology

After approval from ethical review committee of institute, an observational and cross section study was conducted on eight thousand six hundred and eighty-nine employees aged  $\geq 40$  years, of OGDC working at Head Office and different Oil Fields all over Pakistan and was completed in 2 months. Three subgroups were made after HbA1c based diagnosed prediabetics, diabetics and nondiabetics. Non-probability convenience sampling was done. Written consent form was verbally explained and consent taken. The standard collection technique was used for Specimen's collection. A 2 ml of venous blood sample was obtained by a nurse practitioner, and transferred in a vacutainer with EDTA, labelled with the employee identification number and then shifted to the haematology lab for the analysis of glycated haemoglobin (HbA1c). Participants were weighed and interviewed for family and smoking history. Hypertension was defined as systolic BP  $\geq 140$  mmHg and/or diastolic BP  $\geq 90$  mmHg.<sup>8</sup>

World Health Organization (WHO) defines obesity as BMI  $\geq 30$  kg/m<sup>2</sup> and overweight as 25–29.9 kg/m<sup>2</sup>.<sup>9</sup>

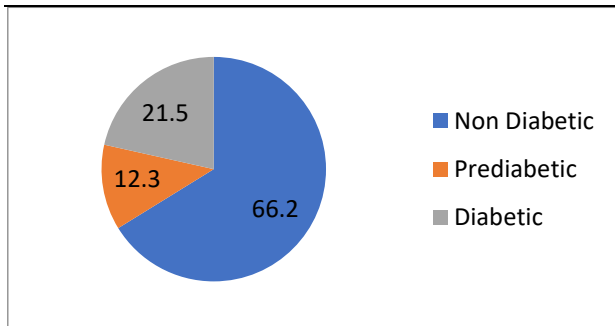
Data was analyzed by SPSS version 26, mean and standard error of mean was calculated for quantitative variables and parameters was analyzed, using student "t" test followed by ANOVA and "p" value of  $<0.05$  was taken as significant.

## Results

Eight thousand six hundred and eighty-nine, apparently healthy individuals of 40 years and above participated in the study. All participants were male. No female participated in the study. The Participants belonged to different geographical areas of Pakistan. Table I describes demographic details of the study participants. After compilation data was collectively analyzed as well as in subgroups separately. Table 1 presents baseline characteristics of the study participants by their diabetes status and for total population. A total of 8689 participants were recruited in the study. Out of which, 1866(21.5%) were diabetics, 1066 (12.3%) were prediabetics, and 5757 (66.2%) were nondiabetics. Among diabetics 54% had family history of DM, while 32% prediabetic also had family history of DM.

**Table I: Demographics of participants.**

Characteristics	Group			Total	P-value <sup>1</sup>
	Non-diabetic	Pre-diabetic	Diabetic		
Frequency (%)	5757(66.2)	1066(12.3)	1866(21.5)	8689(100)	-----
Family History	1669(29.0)	342(32.1)	1007(54.0)	3018(34.7)	0.0001**
<b>Smoking</b>					
Never	5474(95.1)	980(91.9)	1779(95.3)	8504(94.8)	0.0001**
Current	185(3.2)	65(6.1)	38(2.0)	288(3.3)	
Ex-Smoker	98(1.7)	21(2.0)	49(2.6)	168(1.9)	
Hypertension	1151(20.0)	224(21.0)	373(20.0)	1748(20.1)	0.738
<b>Weight</b>					
Normal	1842(32)	300(28.1)	597(32)	2739(31.5)	0.001**
Overweight	3339(58.0)	617(57.9)	1064(57)	5020(57.8)	
Obese	576(10.0)	149(14.0)	205(11.0)	930(10.7)	

**Figure 1: Prevalence of Diabetes and pre-diabetes.**

## Discussion

Worldwide, Diabetes mellitus is considered to be an imperative public health issue. It is a perpetually progressive global health crisis in the 21<sup>st</sup> century and approximately more than 300 million individuals may be impacted by diabetes in the year 2025. It may lead to devastating complications if remain undiagnosed and treated at early stages. This contemporary study was carried out to investigate the determinants and prevalence of pre diabetes among employees of OGDC.

In current study, status of diabetes as well as prediabetes was precisely defined by using HbA1c cutoff levels. Therefore, according to American Diabetes Association 2016, nondiabetics have HbA1c less than 5.7 there are cluster of %, prediabetes have greater than 5.7% but less than 6.5% values, and diabetics have greater than or equal to 6.5%.<sup>10</sup>

The prevalence of diabetes and prediabetes in our study, was 21.5% and 12.3% respectively which is hazardously high showing that similar results are expected in urban areas and in office workers of Pakistan. Office employees spend most of their duty hours, using computers, contributing in screen-based recreation therefore spend most of their duty timings while being seated. There are cluster of studies done in recent past reporting the predisposition of DM with sedentary jobs and life styles. Therefore, while at work taking frequent steps and walk or standing and moving the joints can be beneficial in

reducing the risk of development of DM by growing surge of energy utilization.<sup>11</sup>

Among diabetics 54% had family history of DM, while 32% prediabetic also had family history of DM. The relationship of diabetics and nondiabetics with family history is highly significant in both groups which is similar to the already established finding that incidence of diabetes is pretty much higher among individuals having diabetic first-degree relative than that of individuals without a positive family history. Fundamentally, hereditary factors interplay a decisive role in the development and progression of diabetes and hence there is a desire of understanding the causes of this disease and introducing the probable pre-emptive avoidable measures.

The finding implies that the type II diabetes risk attributed to family history is supported by distinct etiology affecting the family member and is also superimposed by the presence of diverse metabolic derangements reliant on the affected family member.

In our recent study, significant relation of hypertension was not found with our studied sub groups. All three subgroups have same percentage of hypertensive patients. Among 5757 of nondiabetics 20% were hypertensive. Among 1066 prediabetics 21% were hypertensive and among 1866 diabetics 20% were hypertensive. Hypertension and T2DM as reviewed by no of research studies are meticulously connected to each other, but the effective causative link bridging the two conditions is unclear. There exist common pathophysiological mechanisms which help prove the coexistence among Hypertension and T2DM. Insulin resistance, increased blood insulin levels leading to increased oxidative stress, and underlying inflammation.<sup>14,15</sup>

Insulin resistance leads to elevated insulin levels in blood which predisposes to hypertension development in normotensive nondiabetic individuals. Hyperinsulinemia, produces augmented sympathetic output by the nervous system, increases the activity of renin-angiotensin-aldosterone system in the kidney, and increases sodium

reabsorption by the renal tubules. Furthermore, endothelial dysfunction by increased oxidative stress leading to inflammatory response predisposes to endothelial dysfunction, decreased vascular responsiveness, increased resistance in the peripheral vasculature and metabolic derangements specifically in glucose and lipid metabolism. This changing carbohydrate and lipid metabolism finally leads to hypertension and DM.<sup>16</sup>

In our study most of subjects were unaware of their disease status, not able to document the first onset of hypertension or diabetes. Research studies have now documented that there is increased risk hypertension in inherently susceptible T2D but, no such correlation is found among predisposed hypertensives and T2D. The scientists have distinguished momentous pleiotropy in the connection between hypertension and the development of T2D.<sup>17</sup> However the biggest limitation of our study is only males were enrolled in study due to fact that majority of workers in OGDC are males.

Smoking can not only aggravate the diabetes complications such as macro- or micro-vascular disease, but has also been shown to deteriorate glucose metabolism in normal subjects and thereby may provoke the onset of type 2 diabetes. In our study most of the population was non smoker, among 8689 subjects 95% never smoked. Among 66% of nondiabetics 4.9% had history of smoking. In 1066 of prediabetics 8% have history of smoking. This significant p Value strongly supports previous research work showing relationship of type 2 diabetes with smoking. The exact mechanism for why smoking increases the risk of diabetes and worsens glucose homeostasis has not been fully explained, but the available evidence shows that smoking increases insulin resistance. Another effect of early onset of diabetes in smokers is that smoking increases inflammation and oxidative stress which damages  $\beta$ -cell function.<sup>18,19</sup> The use of e-cigarettes is increasing in our young generation day by day and research studies showed that use of cigarette and e-cigarettes have been shown to be associated with prediabetes and increased HbA1c levels in adults without a history of diabetes.<sup>20</sup>

In our study prediabetic and diabetics subjects almost 58% were overweight, showing a significant p value of 0.001. Obesity is a key underlying etiological factor for initiation of diabetes. Obesity is increasing worldwide and millions of youths and adults are imposed to risk of metabolic diseases including hypertension, dyslipidemia, liver steatosis and abnormal blood glucose regulation. In obesity, there is chronic tissue inflammation in liver, muscle, adipose tissue and pancreatic beta cells leading to abnormal CHO metabolism and hyperglycemia. The

epidemic of obesity related diabetes is a big problem in recent decades and needs to educate changes in lifestyle. Changing our lifestyle including diet and physical activity, can help prevent or decrease the prevalence of development of these diseases.<sup>21,22</sup>

## Conclusion

Diabetes mellitus is a worldwide public health problem affecting millions of people and prevalence is dangerously rising in Pakistan. The prevalence of type 2 diabetes and pre-diabetes is much higher than previously thought

### Recommendations

It is therefore essential to identify prediabetics and there is a need to educate about lifestyle modifications, targeting nutrition, exercise and weight loss which contribute to health by reducing body weight, blood pressure and blood glucose levels.

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#### Authors Contribution:

<sup>1</sup>Substantial contributions to the conception or design of the work;

<sup>3,4</sup>The acquisition, analysis, or interpretation of data for the work;

<sup>1,2,3,4</sup>Final approval of the version to be published

<sup>2,3</sup>Drafting the work or revising it critically for important intellectual content.

# Vulval Leiomyoma: A Case Report

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## Abstract

Vulval leiomyoma is a rare condition and usually misdiagnosed, with only a few cases reported till now. A 38 years old women presented with painful mass over right labia majora. Pelvic examination showed redness and swelling over right labia. An 8 x 5 cm mass of firm consistency was found near vestibule, extended above up to lower one third of vagina causing discomfort at the perineum. She had complete excision of mass. Mass sent for histopathology. Post-operative histopathology confirmed the diagnosis of vulval leiomyoma.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Leiomyoma's shows about 3.8% of benign tumors of soft tissues.<sup>1</sup> They can grow in any body part where smooth muscles are found however uterus being the commonest site.<sup>2</sup> Although vulval leiomyomas are rare and usually misdiagnosed and confused with Bartholin cysts. Here we report a case of 38 year old women who was diagnosed as vulval leiomyoma on post-operative histopathology.

## Case Series

A 38 year old woman p4 last child born 4 years back presented in outpatient department with history of painful swelling over right labia majora. She was referred from district headquarters hospital Bagh (Azad Kashmir). She had no history of trauma and recent sexual experience. The size of swelling was about 8 x 5 cm. some local doctor took biopsy from the middle of the mass and it was infected. It was quite tender with all signs of inflammation. According to patient mass appeared 4 months back and gradually increase in size and causing perineal discomfort. On examination. It was located on right labia majora and extending up to lower third of vagina. It was firm in consistency and very tender. Inguinal lymph nodes were not palpable. Rest of the pelvic examination was unremarkable.

Patient was admitted and intravenous antibiotics started to clear the infective elements and then removal of mass

was planned vaginally. Then after 48 hours of antibiotic cover the mass was completely excised under spinal anaesthesia. Vertical incision was given on inner side of labia majora, upper skin was excised from mass and free mass, hold it with vulsellum forceps and avulsed it to completely excised it from base. Hemostasis was secured, and overlying skin was closed after excision of redundant skin.

Specimen was send for histopathology. Microscopic examination showed proliferation of smooth muscle fibers with intertwined bundles and separated by congestive vessels and fibrosis without atypical mitosis. The peripheral capsule was intact. Morphological analysis showed vulval leiomyoma. Her post-operative period was uneventful. Her follow up was on 7<sup>th</sup> post-operative day with no complaint and was completely pain free.

## Discussion

Leiomyoma can be found beyond the uterus at any site containing smooth muscle cells. Vulval myoma usually involves any age group.<sup>3</sup> but more frequent in age between 35 – 40 years. So far 160 cases of vulval leiomyoma have been reported<sup>4</sup> Most patients present with painless swelling in labia.<sup>5</sup> This benign tumor remains small for a long time and grew slowly, but when it increases in size it become symptomatic.

Differential diagnosis of vulval leiomyoma includes Bartholin cyst, Bartholin abscess, lymphangioma fibroma

and soft tissue sarcoma. Usually on examination vulval leiomyoma are firm, mobile and nontender.<sup>5</sup>

Extra uterine leiomyoma's are infrequent and may be situated in sites like vagina and vulva. Ultrasound and MRI help in diagnosis.<sup>6</sup>

Surgical excision of the tumor with small amount of the normal surrounding tissues is the management of choice. Follow up after surgery is recommend because of risk of recurrence.

## Conclusion

Vulvar leiomyoma is an uncommon tumor frequently mistaken for Bartholin's cyst. The differentiation between benign and malignant variants presents a diagnostic challenge. Various techniques are employed are discern the tumor's nature, but currently, excisional biopsy stands as the preferred method and primary treatment option for such case. It is strongly advised to maintain regular follow-up after treatment.

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# Surgical Enucleation of an Uncommon Complex Odontoma: A Case Report

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## Abstract

Odontomes are developmental malformations that are produced by the growth of completely differentiated mesenchymal as well as epithelial cells responsible for the formation of odontoblasts and ameloblasts. There are two types of odontomas i.e., Complex and compound. In complex odontome, all dental tissues are present, but are disorderly organised, dissimilar to teeth. On X-ray, complex odontoma appears as a distinct radio-opacity, occasionally encircled by a thin radiolucent region. This case report presents an uncommon type of complex odontoma where patient reported with pain along with the large swelling in the posterior mandibular region without eruption. Enucleation was the treatment of choice.

**Keywords:** Odontoma, complex odontoma, mesenchymal and epithelial cells, radio-opacity, Enucleation.

**Conflict of Interest:** None

**Funding Source:** None

## Introduction

Odontomes also called as odontomas are developmental anomalies that are formed by the development of completely differentiated mesenchymal as well as epithelial cells that produce odontoblasts and ameloblasts. These are benign tumors made up of dentin and enamel but may also contain cementum and pulp<sup>1</sup>. The word odontoma (or odontome) was labelled firstly by Paul Broca, in 1867<sup>2</sup>. According to WHO (World Health Organisation), there are two types of odontomes. A deformity in which all dental tissues are present, but disorderly organised is called Complex Odontoma. While an anomaly having all of the dental tissues arranged in a more orderly manner as compared to complex odontoma is known as Compound odontoma<sup>3,4</sup>. In 2005, WHO characterised complex odontomes as irregular solidified mass that are not similar to teeth whereas compound odontomes are calcified mass that have similarity to the tooth structure<sup>5</sup>. The most common location of complex Odontoma is posterior mandibular jaw.

While compound odontomas are mostly present in the anterior region of maxilla<sup>6</sup>. Changes in the genetic make accountable for dental growth, trauma to deciduous teeth and hyperactivity of odontoblasts are included in the

aetiology of odontomas<sup>7</sup>. Conventional surgical removal of the lesion is treatment of choice<sup>8</sup>.

This case report presents an uncommon case of complex odontoma in a 17-year-old female. Informed consent was taken for publication of this report and any associated pictures

## Case report

A 17 years old female, reported to Oral and maxillofacial surgery department of a teaching hospital presented with history of mild dull pain and swelling in right lower mandibular region for 06 months. Extraoral examination showed an isolated swelling measuring 3 × 2 cm, present on the right side of mandible ranging from body of the mandible to the ramus. On palpation, the swelling was firm, hard, nonreducible and resistant to compression [Figure 1].

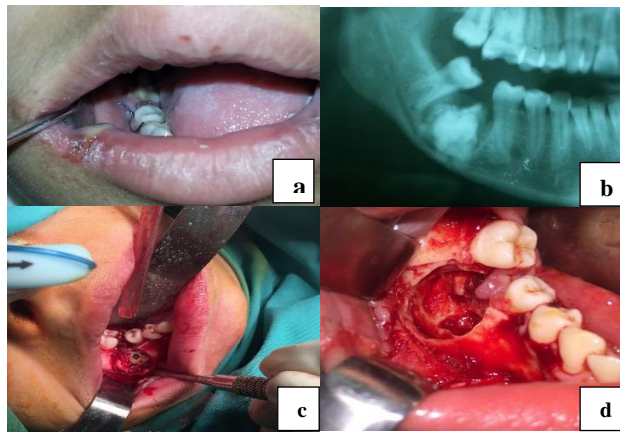
Intraoral examination revealed missing 46 and mesially tilted 47. There was expansion of buccal cortical plate in the region of mandibular right first molar. An OPG (Orthopantomogram) was done. OPG displayed a radiopaque mass surrounded by a thin radiolucent lining extending from mandibular right first premolar to right 2nd molar. The upper part of the mass was bulging towards the superior border of the mandible.



Figure 1: Frontal and Lateral view of swelling in the right lower region of the face

The provisional diagnosis of this case was complex odontoma. Surgical removal of the odontome under GA (General anaesthesia) was planned.

Routine blood tests were done. The lesion was approached intraorally and it was completely excised. The area was curettage deeply. 3-0 vicryl suture was used to close the wound. The sample was sent to laboratory for histopathological assessment. Diagnosis of complex odontome was confirmed after the lab result [Figure 2].



**Figure 2:** a: Intraoral examination showing missing # 46. b: OPG revealed a radio-opacity surrounded by a radiolucent lining c: The lesion was operated intraorally. d: The lesion was completely enucleated under GA.

The patient was observed for 7-months. Postoperative orthopantomogram showed good healing without any bony defect. There was no sign of relapse. After surgery, there was no numbness or tickling sensation on the right side of the mandible.

## Discussion

The complex odontoma shows disorderly organization of dental tissues even though complete differentiation of the

cells of the enamel organ has done. The odontoma is mostly covered by the connective tissue. An X-ray is the most accurate technique to distinguish between complex and compound odontomes as compared to clinical and manual examination<sup>9</sup>. Generally, odontomas are small and asymptomatic; but sometimes they may grow large, resulting in bone expansion. When odontomas erupt, swelling, pain and infection are most common symptoms, which happen very rare<sup>10,11</sup>. In the present case, the patient reported with pain and swelling in the lower posterior region without eruption.

On X-ray, odontomas appear as a well-demarcated radio-opaque mass along with a radiolucent cavity surrounded by a thin sclerosed lining. Radiolucent region is the connective tissue covering of a normal dental follicle<sup>12</sup>. In the present case, complex odontoma appears as a distinct radio-opacity, encircled by a thin radiolucent region on X-ray.

Surgical removal is the best possible treatment for odontomas. The purpose is to eradicate the mechanical obstruction and then probable eruption of secondary teeth. Complete removal of the lesion decreases the chance of relapse<sup>13</sup>. The lesion was also completely enucleated in this present report and did not show any recurrence during the seven months of follow up. Bone grafts are used to fill any bony defect, if found after the surgical removal of odontomes<sup>13</sup>. The present case report showed good intraoral healing without any bony fault.

Pralidoxime (2-PAM) also should be given to affect the nicotinic receptors since atropine only works on muscarinic receptors. Pralidoxime works by reactivating the phosphorylated AChE by binding to the organophosphate. However, to work, it has to be given within 48 hours of the poisoning. The agent does not depress the respiratory center and can be combined with atropine. Atropine must be given before 2-PAM to avoid worsening of muscarinic-mediated symptoms. A bolus of at least 30 mg/kg in adults or 20 to 50 mg/kg for children should be given over 30 minutes. Rapid administration can cause cardiac arrest. After the bolus, a continuous infusion of at least 8 mg/kg/hr for adults and 10 to 20 mg/kg/hr for children should be started and may be needed for several days.<sup>6-7</sup> Patients with seizures may benefit from benzodiazepines.

## Conclusion

This was a case of complex odontoma occurred rarely as compared to compound odontomas. This complex odontoma presented with pain along with the large swelling in the posterior mandibular region. Surgical enucleation was the treatment option. Postoperative

orthopantomogram showed good healing of bone without any defect and recurrence. To improve the diagnosis, treatment and prognosis, it is very important to have the adequate information and knowledge of all the jaw tumors.

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# Has Monkey Pox Got Viral: Is it time to Worry?

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## Abstract

The world has seen a worldwide rise of Monkey pox infection since May 2022 and now has reached an endemic status in Africa. This situation has spurred concerns about the risk for another viral pandemic especially after experience of COVID 19. Though only 3 cases of Mpox have been detected in Pakistan so far ,but we need to be vigilant for any future outbreak as transmission and pathogenicity of this virus is increasing day by day. By this scoping review we would like to create an awareness for public and health workers regarding etiology, routes of transmission and susceptibility. Current clinical presentations, methods for diagnosis, available treatments and active immune prophylaxis are also considered. Readiness of health authorities in Pakistan to deal with any emerging epidemic, documented as set guidelines by National Institute of Health (NIH) will be discussed briefly and few recommendations are put forward. Different search engines (Google Scholar, PubMed) and databases like SCOPUS are used for extracting relevant information. The need of this narrative review is to compile worldwide guidelines and strategies on dealing with Mpox virus as a proactive approach.

**Keywords:** Monkey Pox, Mpox, Transmission, Prophylaxis

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## Introduction

Monkeypox (Mpox) is a viral zoonotic disease by infection of "Monkey pox virus" (MPXV). It is a DNA virus, classified in genus Orthopoxvirus (Poxviridae family), same as of Variola virus implicated in small pox.<sup>1</sup> This virus was first detected during a vaccine trial in monkeys, in 1958, in Denmark so named as 'monkey pox'.<sup>2</sup> Later on it was found to be pathogenic in both animals and humans.<sup>3</sup> Human monkeypox was initially endemic in African countries by animal human transmission. In 2003 first case of human monkey pox was detected in United States (US) resulting through same route.<sup>4</sup>

In 2022 Mpox outbreak in non-endemic areas and number of rising cases forced World Health Organization (WHO) to announce a "global health emergency of moderate nature"<sup>(5)</sup>. Its nomenclature was changed to "Mpox" instead of "monkeypox" for a non-discriminatory and non-stigmatizing reason.<sup>6</sup> Till January 16<sup>th</sup> 2023 WHO reported 110 countries, areas, or territories having cases of Mpox and eighty deaths, while United states(US) having the highest number of cases.<sup>7</sup>

This is a serious concern firstly because, though global cases have declined after achieving their highest peak in August 2022 but Mpox virus has potential for mutation hence relaxation of safety measures can lead to more waves of international outbreaks. Secondly, due to globalization, social and population exchange has increased manifold so as the risk. In recent years due to extensive international travels the disease shows rapid spread across multiple countries and control has become a global challenge. Mpox outbreaks the past, especially in non-endemic countries a clear transmission chain could be traced. The infected patients exhibited a clear history of animal exposure with recent travel to area with outbreak , and/or exposure to imported cases.<sup>8</sup> But in current episode of rapidly spreading international outbreak a new human to human (social) transmissions predominantly among men, was the main source.<sup>9</sup>

In recent epidemic a concern and alarm was raised due to fact that 10% of cases of Mpox have proved fatal <sup>(10)</sup> unlike smallpox infections and more so, its spread from endemic areas to nonendemic regions. Also after eradication of small pox , Mpox is the only naturally

occurring poxvirus infection that poses threat during pregnancy.<sup>11</sup>

## SITUATION IN PAKISTAN

Three cases have been detected till May 2023 in Pakistan.<sup>12</sup> Initially (because of lack of diagnostic facilities) it was denied by the Government but then typical history and symptoms raised concern and virus was positively isolated. In Pakistan Mpox virus can become major threat as the population is more susceptible. This occurred, ever since small pox vaccine got discontinued since 1980 from EPI after WHO declared Pakistan small pox free creating a lack of pox virus immunity<sup>(13)</sup>. The objective is to acquaint health care providers and authorities, especially in Pakistan, with Mpox routes of transmission and symptoms as it presents challenges in all three domains: Inadequate capacity to diagnose, Clinical management of patients and accurate identification of exposure. Health system in Pakistan is already overburdened along with worsening economic situation. Hence, if proper actions are not taken such pandemics can prove catastrophic in future.

## ETIOLOGY

Mpox virus (DNA) enters host cells by multiple routes through direct contact, mucosal surfaces, body fluids, shedding through feces, intramuscular, subcutaneous or intradermal pathways. Inflammatory immune-mediated phagocytosis is triggered by its replication at the inoculation site which causes virus to spread to the blood, lymph nodes, tonsils, bone marrow, spleen, and other organs.<sup>14, 15</sup>

## ROUTES OF TRANSMISSION

Several modes of transmission of Mpox virus are observed: Animal-to-animal, animal-to-human, or human-to-human.<sup>16</sup> In animal-to-human transmission rodents, squirrels and non-human primates are believed to be reservoir hosts. Direct contact with an infected animal (where animal contact is frequent due to rodent infestations) or body fluids like blood, urine, saliva is the commonest mode seen in endemic manifestations. Human-to-human transmission such as exposure to large respiratory droplets, use of contaminated personal items or direct contact with skin lesions are some of the mediators for transmission.<sup>17</sup> In 2018 a health employee in United Kingdom(UK) got infected from a patient contaminated bedding.<sup>18</sup> The 2022 outbreak is different from previous endemics as more sufferers were homosexual or promiscuous men.<sup>19</sup> Center of disease control (CDC) has also identified other

routes like kissing, hugging, oral, anal and vaginal sexual intercourse.<sup>20</sup>

In current era more people are exposed to infections due to massive migration and travelling from one place to another either for religious meetings or sports event or forced migration because of natural disasters or wars<sup>(16)</sup>.

## SYMPTOMS

Typical symptoms of Mpox (before current outbreak) included fever lasting for few days followed by appearance of well circumscribed vesiculopustular rashes. These rashes are spread over different exposed skin portions of the body, such as face, mouth, and eyes, hands and feet but with maximum intensity on face. The rash progressed as macule then papule, passing to vesicle, pustule and finally crust formation and desquamation occurring 14–24 days after rash onset.<sup>21</sup> Lymphadenopathy is also very discrete symptom where lymph nodes around ear, jaw, and neck or groin region enlarge.<sup>22</sup> Nonspecific features involved headache, muscular pain, back ache associated with extreme tiredness.<sup>23</sup> The incubation period of varies from five to twenty-one days. The symptoms are very similar to small pox however the incubation period of Mpox is dependent on its route of transmission.<sup>24</sup>

WHO data collected in current outbreak exhibited asynchronous skin lesions. The prodromal symptoms and the appearance of skin lesions had no clear distinction, with only about half of cases having systemic symptoms. The genital rash and perineal lesions were seen in only 37 percent of cases. In Africa harbors a predominance of affected males (>99%) especially in individuals claiming to be homosexual, bisexual, gay, or promiscuous. Most cases in current outbreak had no travel history to endemic countries but do admit having multiple sex partners or were HIV-positive.<sup>25</sup> The current approach of 21 days quarantine (or other methods to avoid infectious contacts) is appropriate. But the different transmission routes can cause transmission hence, continued surveillance in incubation period for Mpox is mandatory.

## SUSCEPTIBILITY OF Mpox

M-pox susceptibility is dependent upon age, gender, ethnicity and medical history. The vaccination status also and exposure history also effect the susceptibility. Vaccination against Orthopoxvirus species and age-related immunity are also important considerations. HIV-positive immunosuppressed individuals, when get coinfection exhibit causes symptoms and also higher mortality.<sup>26</sup> In 2022 outbreak, males were more prone, especially gay and homosexual men, showed worse

symptoms. Those working with animals –farmers, butchers –or any connection with possible animal reservoirs make individuals more susceptible.<sup>27</sup> Since long, there has been absence of vaccination against small pox (since 1971 worldwide and 1980 in Pakistan) in the population. Hence individuals, due to lack of immunity (similar genome of M pos and small pox) have become more vulnerable to M pox <sup>28</sup>. Extremes of age (children and elderly) , pregnant females with a suppressed immune system were included in a vulnerable group<sup>(29)</sup>.

## **VULNERABLE GROUP**

### PAEDIATRIC Mpox

Children were disproportionately more affected in previous outbreaks, but had higher morbidity with relatively less mortality (up to 10% during the 1980s)(1). The authorities should be prepared to rapidly implement vaccination for them, should outbreaks involve them. Secondly symptoms of viral exanthemas, like in varicella (chicken pox) and hand foot & mouth disease can be quiet similar and confusing<sup>(30)</sup>. Hence very few children are tested for M pox and false positive results could be seen. This can result in an unnecessary and prophylactic vaccination of close contacts<sup>(31)</sup> with over burdening the health care system and unintended social stigma.

### PREGNANT WOMEN & Mpox

Pregnant woman can easily contact Mpox. This could result in a high-risk pregnancy loss, maternal morbidity or even mortality along with fetal transmission. Fetal infections lead to poor outcomes, include spontaneous abortion, stillbirth, and congenital infections thereby classifying MPXV as “TORCH” agent.<sup>32</sup> In this outbreak UK, USA, Brazil, the positive cases for Mpox reported at various stages of pregnancy, resulted in one vertical transmission to the neonate. A nine-day-old neonate developed the typical vesicular rash starting from palms and soles, and eventually spreading to face and trunk along with axillary lymphadenopathy. The PCR testing in this case confirmed MPXV.<sup>11</sup>

Symptoms in pregnant woman are more or less same as above -vesiculopustular rash—including rash localized to genital or perianal region- and lymphadenopathy.<sup>33</sup>

Various routes of transmission from mother to fetus, as suggested by researches.<sup>34, 35</sup> include vertical MPXV transmission via antenatal transplacental infection after an episode of maternal viremia, hence the virus reaches placenta through blood in uterine artery. Postnatal environmental exposure is also suggested as an alternate route.

## **DIAGNOSIS**

Diagnosis on the basis of history, symptoms and signs needs to be assisted by tests on patient specimens for case confirmation.

Conventional and real-time C-(PCR) & (RT)-PCR tests for diagnosing suspected Mpox cases is recommended by WHO as it specifically targets MPXV’s DNA taken from lesion material from active cases. This is an expensive yet very sensitive method plus needs trained technician with specific equipment.<sup>36</sup>

Serology tests measuring antibodies against Orth poxviruses including ELISA testing is not recommended alone for diagnosing MPXV<sup>37</sup> as this virus being similar to variola virus may show false positive results for Mpox where the antibodies had been developed in suspected case as a result of smallpox immunization so vaccination may interfere with serologic testing.

In Pakistan smallpox vaccination is no longer a regime of EPI program as it has been declared small pox free region in 1980.<sup>38</sup>The diagnostic facilities for M pox are unknown at present. But all health authorities have been advised as effective control and prevention measure to ensure surveillance by isolating suspected cases, providing available lab diagnostics, contact tracing and follow up of contacts to observe any early signs of infection.<sup>39</sup> Rapid identification of suspected cases, determination of source, clusters of infections and identification of risk groups are needed the most.

## **THERAPEUTIC OPTIONS**

all patients with mild symptoms require no treatment and get well without any medical treatment. They just require supportive treatments like pain killers and plenty of fluid to prevent dehydration, and are sufficient. Most of the time the supportive is coupled with symptomatic treatments along with antiviral agents. The common antiviral drugs used, act as an inhibitor for viral DNA synthesis.

The indications of antivirals use are; severe cases, pregnant or breast-feeding women, immunocompromised patients, extreme of age like pediatric population and elderly. The patients suffering from lesions near mouth, eyes and genitalia can also be prescribed antivirals.<sup>40</sup>

In cases of pregnant women presenting with lymphadenopathy and vesiculopustular rash with suspected MPXV exposure, diagnosis should be confirmed by real time PCR. At the same time varicella (chicken pox), herpes simplex and syphilis should be ruled out. In case mother is found to be positive for

Mpox the fetus should be monitored by periodic ultrasounds for further management. Real-time PCR analysis for specimens, obtained from suspected neonate immediately at delivery, should be the standard protocol.<sup>34, 41</sup>

## PREVENTION

Smallpox vaccines such as JYNNEOS™ (live-attenuated, non-replicating vaccine)-are made from weakened vaccine viruses. These vaccines like Imvamune/ Imvanex, can cross-protect humans from MPXV. This is due to similar viral infection, but its use is restricted to prophylaxis. Vaccination after show of Mpox symptoms is not recommended by CDC.<sup>2nd</sup> Food and Drug Administration (FDA) approved vaccine ACAM2000 (live-attenuated, replicating vaccine) can cause side effects because the virus replicates inside cells. Both are currently available for post exposure prophylaxis and for prevention in high risk populations like health care workers, laboratory researchers, military personnel living in combined facilities<sup>(42)</sup>. International agencies WHO and American agency FDA are planning future vaccination programs. They are also working on provision of vaccine supplies along with adjusting suitable doses and determining correct vaccination intervals.

## Conclusion

Current review has shown that Mpox infection has acquired high transmissibility and pathogenicity. There is a increase danger of a pandemic effecting a wider human populations. Strict regulatory measures are required to prevent further Mpox spread, especially among non-endemic regions of the world. Potential factors regarding susceptibility need scientific and thorough investigation. Furthermore, scientific approaches and techniques are needed to clarify the relationship among population genetics, viral genomics, disease susceptibility and public immunity. More research data is required to assess the vaccine efficacy and treatments results for Mpox to develop standard guidelines. Affected countries in partnership with local scientific and public health authorities are best venues for such researches. This report is based on cases but but has tried to acquaint general public and high-risk health workers with recognition of symptoms in general and in specific vulnerable population.

## RECOMMENDATIONS<sup>41, 43</sup>

We suggest following in Pakistan for spread of this viral infection, shaking our health care system and preventing this disease,

1. Educating community by awareness programs for:
  - Awareness of symptoms and routes of transmission including sexual practices and promoting vaccination drives.
  - Significance of detecting symptoms and self-quarantining and contact for immediate healthcare consultancy as a precautionary measure.
  - All general Physicians should be educated to be aware of Mpox infectious symptoms so as to limit the spread by identifying the contacts.
2. General precautionary measures especially in suspected areas, like COVID 19, should be implemented as Mpox transmission mode is still not fully comprehended.
3. Better to vaccinate health workers before disease exposure and educate them for all necessary precautions in all health providing facilities.
4. Social media platforms spreading misinformation about case reporting or mortalities should be strictly dealt with for the benefit of public.

In addition, designing or importing diagnostic kits, approving therapeutics against Mpox, improving pre-exposure and post-exposure vaccination are all the practices which needs government support. Pharmaceutical companies and Private sector can prove itself helpful by collaboration with government.

## FOR TREATMENTS

Considering symptomatic patients as high-risk groups antiviral treatment should be started. Antivirals against Mpox like Cidofovir, Modified Vaccinia Ankara virus, Tecovirimat and Vaccinia Immune Globulin Intravenous (VIGIV)) should be made available in a proactive way. Proper isolation facilities coupled with nutritional and psychological support for the patients with symptoms should be made.

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#### Authors Contribution:

<sup>1,3</sup>Substantial contributions to the conception or design of the work; or the acquisition, analysis, & Final approval of the version to be published.

<sup>1,2</sup> Drafting the work or revising it critically for important intellectual content.



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