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Glycemic Index and Load: Key Dietary Factors in the Pathogenesis of Insulin Resistance



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ARTICLE INFO

How to Cite:

Bashir, M. I. (2025). Glycemic Index and Load: Key Dietary Factors in the Pathogenesis of Insulin Resistance: Glycemic Index and Load. *Pakistan BioMedical Journal*, 8(8), 01-02. <https://doi.org/10.54393/pbmj.v8i8.1280>

Insulin resistance is the key parameter in development of type 2 diabetes and also leads to various metabolic disorders globally. Recent evidences demonstrate the vital role of glycemic index (GI) and glycemic load (GL) in glucose metabolism and insulin sensitivity.

The glycemic index determines how quickly carbohydrates-rich food elevate the glucose levels in the blood. While Glycemic load represents both quality and quantity of carbohydrates which are consumed. Actually, these factors are directly involved in glucose spike in the blood that is further responsible of insulin secretions. An increased demand of insulin can increase the secretions of insulin from pancreatic beta cells. Increased level of insulin (hyperinsulinemia) can cause insulin resistance. It happens when cells in the body, specifically in muscle, fat, and liver tissues show less response to insulin's signal to take up glucose from the bloodstream.

Recent studies has demonstrated through various randomized clinical trials that there is an important link between GI and insulin resistance. They showed that low GI-index foods can reduce the spike of blood glucose level which further can reduce the insulin secretions that can reduce the insulin resistance. A high GI-index diet can cause metabolic dysfunctions in non-diabetic adults [1]. Another research linked higher dietary GI and GL with increased pancreatic steatosis risk, describing the metabolic burden that high GI-index diet affect the pancreatic health [2]. Furthermore, some investigations have suggested that diets with high GI and GL could exacerbate insulin resistance, potentially very serious outcomes in inflammatory conditions such as COVID-19 [3].

All these studies recommend dietary modifications to control or reverse the type 2 diabetes. The use of low GI and low GL based diet can protect individuals from type 2 diabetes. Specifically, we have to modify our daily diet like grain flour, white rice, starchy vegetables, and sugary food. All these foods are high in GI and GL. More concerning for type 2 diabetes patients if they are taking medications but don't want to modify their diet, they are just suppressing their type 2 diabetes with medicines not cutting the roots of this dietary disorder. People got this disorder from their dining tables but for cure they rely on clinics only, it is better if they first change their food placed dining tables. May be this modification can protect them from continuous visits of clinics and pharmacies. Public health policies and clinical guidelines should incorporate the GI and GL considerations to reduce the growing burden of insulin resistance and its complications effectively.

In conclusion, understanding about GI and GL is very important in both diabetes management and prevention. These factors will open new strategies to cope the type 2 diabetes and other metabolic disorders as well. Future research should continue to design the low GI and low GL based personalized nutritional approaches for reducing the insulin resistance.



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Original Article



Effectiveness of Passive Chest Physiotherapy with and without Mechanical Percussion among in Patients Children

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ARTICLE INFO

Keywords:

Physical Therapy, Pneumonia, Percussion, Respiratory Tract Infections

How to Cite:Ali, M., Afzal, S., Rafique, N., Rafique, A., Aijaz, N., Khan, A., & Ikram, I. (2025). Effectiveness of Passive Chest Physiotherapy with and without Mechanical Percussion among in Patients Children: Passive Chest Physiotherapy with and Without Mechanical Percussion. *Pakistan BioMedical Journal*, 8(8), 03-08. <https://doi.org/10.54393/pbmj.v8i8.1219>***Corresponding Author:**Nazish Rafique
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ABSTRACT

The main cause of damage to lung tissue is pneumonia, a disorder marked by pulmonary inflammation or infection and brought on by a range of infectious agents. **Objectives:** To compare the benefits of mechanical percussions and chest physical therapy for pediatric hospital admission patients. **Methods:** A randomized controlled trial was conducted in the Pediatric Intensive Care Unit and the General Ward of Memon Medical Institute Hospital in Karachi, Pakistan. The study investigated 68 children who experienced lower respiratory tract infections and were hospitalized for an extended duration. The children ranged in age from one month to five years. The exclusion criteria encompassed patients with acute asthma, cystic fibrosis, pulmonary embolism, malignancies, rib fractures, spinal fusion, hemorrhage-prone diseases, recent neurosurgery that precluded head-down positioning, and pulmonary emboli. Participants were randomly allocated to either the experimental group or the control group. The experimental group underwent mechanical percussions during chest physiotherapy, whereas the control group received standard chest physiotherapy. The final assessments included the Modified Respiratory Distress Assessment Instrument (mRDAI), the Face, Legs, Activity, Cry, CONSOL ability (FLACC) scale, the Wang Clinical Severity Score (WCSS), oxygen saturation (SpO₂), heart rate, and respiratory rate (RR). Evaluations were conducted both before and after the chest physical therapy session. **Results:** Improved FLACC ratings, a lower heart rate ($p < 0.05$), and better scores on the mRDAI all point to notable changes in the experimental group following the intervention. **Conclusions:** Mechanical percussions in chest physical therapy clearly improved heart rate, the mRDAI, and the FLACC.

INTRODUCTION

The main cause of damage to lung tissue is pneumonia, a disorder marked by pulmonary inflammation or infection and brought on by a range of infectious agents, including bacteria, viruses, fungi, and foreign objects aspirated into the lungs [1, 2]. Particularly vulnerable to the high frequency and death rate of pneumonia in low-income countries are children [3]. Every year, pneumonia takes the lives of as many as 4 million people, most of them are young children [4]. In children and adolescents, 329,380 instances of pneumonia were diagnosed over eight years. In this specific setting, the male-to-female ratio is 1:0.8,

meaning that for every male, there are roughly 0.8 female [5, 6]. According to official data, pneumonia claims the lives of 80,000 babies and children under five every year [7]. Southeast Asia and Africa are the most affected regions by pediatric pneumonia; each year, an estimated 61 million cases of one illness and 35 million cases of another condition in children under five are reported [8-10]. Breathing becomes more difficult as a result of respiratory infections that generate an accumulation of respiratory secretions in the airways. These infections also exacerbate symptoms by increasing airway resistance [11]. Pneumonia



is typically diagnosed by looking for symptoms like fever, fast breathing, flared nostrils, coughing, shortness of breath, inward movement of the lower chest wall, and low oxygen saturation. Because of its accuracy, clinical recommendations call for chest radiography as the best method for screening pneumonia [12, 13]. Using techniques like postural drainage, chest percussion, and chest vibration, conventional chest physiotherapy (CPT) helps to clear airways and increase effective coughing [14]. CPT opens muco-ciliary clearance, lowers airway resistance, and increases respiratory efficiency. Children who find it difficult to cough clean their airways could benefit much from postural drainage, vibration, and percussion [15]. Respiratory physiotherapy can change reactions related to exercise, metabolism, respiratory muscle weakness, heart rate, breathing patterns, and oxygen absorption kinetics. It can enhance respiratory compliance in children aged zero to three years [16]. Children's chest physical therapy seeks to increase gas exchange and lower airway resistance, therefore facilitating simpler and more comfortable breathing [17]. LEGA, a mechanical apparatus developed by Formedic Technology SDN BHD in Malaysia, has been utilized as an adjunct for CPT [11]. Challenges associated with juvenile pneumonia can be alleviated by enhancing healthcare personnel's comprehension, implementing effective airway clearance techniques, and ensuring adequate drainage placement [18, 19]. This study provides significant new insights into the management of thoracic issues in pneumonia patients. A survey must be done to address the lack of understanding of the comparative advantages of manual versus mechanical percussions and the need for proper drainage placement in pediatric chest physiotherapy for pneumonia. It is significant to highlight that, to the best of our knowledge, no prior studies on the efficacy of manual vs mechanical percussions and drainage placement in pediatric pneumonia patients have been carried out locally in Pakistan.

Despite the widespread use of chest physiotherapy in pediatric pneumonia, limited local evidence exists comparing the effectiveness of mechanical versus manual percussion techniques in hospitalized children. The uncertainty regarding which method provides superior clinical improvement creates a gap in standardized pediatric respiratory care. Therefore, this study aimed to compare the effectiveness of passive chest physiotherapy with and without mechanical percussion on respiratory outcomes among hospitalized children.

METHODS

From September 2022 to March 2023, a single-blinded randomized controlled experiment was conducted at

Memon Medical Institute Hospital in Karachi, Pakistan, in the Pediatric General Ward and Intensive Care Unit. The Iranian Registry of Clinical Trials, with the identification number IRCT20220804055615N1, and the Institutional Review Board (IRB) of Memon Medical Institute Hospital (IRB/MMIH/2022/07) approved the study protocol. Sixty-eight children were included by a non-probability purposive sampling technique, and randomization was conducted using the sealed envelope method. Sample size was calculated through open epi tool by using the Face Legs Activity Cry CONSOL ability (FLACC) scale, and the confidence level was set at 95%, effect size was 0.5, power was 80% and the type I error rate was set at 5% (alpha level, 0.05) [11]. In all, 68 kids between the ages of one month and five years' old who had prolonged hospital stays for lower respiratory tract infections were included in the research. Excluded from the trial were patients with certain disorders such as cystic fibrosis, acute asthma, pulmonary embolus, malignancies, spinal fusion, hemorrhage-prone conditions, recent neurosurgery that prohibited the head-down position, and rib fractures. Those who met the inclusion criteria were divided at random into two groups: 34 patients made up the experimental group, and 34 more made up the control group. Whereas the experimental group received manual chest physiotherapy in combination with mechanical percussions, the control group underwent routine chest physiotherapy. Results were evaluated using the FLACC scale, Wang Clinical Severity Score (WCSS), Modified respiration Distress Assessment Instrument (mRDAI), oxygen saturation (SpO₂), heart rate, and respiration rate (RR). These assessments preceded and followed the session of chest physiotherapy. Every patient involved in the study signed a written informed consent before the examination. Carefully defined inclusion criteria ensured that the selected study participants were only qualified patients. All evaluations were conducted at baseline and subsequent to the intervention. Group A (Experimental) had all 34 members positioned comfortably to offer thorough lung coverage during mechanical percussion chest physiotherapy. LEGA, a mechanical device, is used in this study. The bed levels were adjusted to guarantee appropriate body mechanics, therefore facilitating the effective application of the mechanical percussion technique at a level of 20–50Hz. The section on percussion was supposed to run fifteen to twenty minutes. To maximize patient care and enhance the intervention, mechanical percussions were combined with 15 minutes of nebulization and 10 minutes of positioning methods. Many postures, including lateral, supine, and prone positions, were used to increase the potency of the intervention [18]. Thirty-four teenagers in Group B Control had manual percussion chest physiotherapy while comfortably positioned to maximize treatment of all lung regions. The

bed levels were changed to maximize body mechanics and increase the effectiveness of the percussion approach in line with Group A. The session on percussion was supposed to run fifteen to twenty minutes. The session included 15 minutes of nebulization and positioning techniques, including lateral, supine, and prone postures, in order to maximize the favourable outcomes of the intervention [20]. Designed to meet the particular requirements of every patient, post-percussion suctioning was done at pressures above 20 kPa. This operation sought to give enough airway passage and lower the risk of probable issues. The whole study was represented (Figure 1).

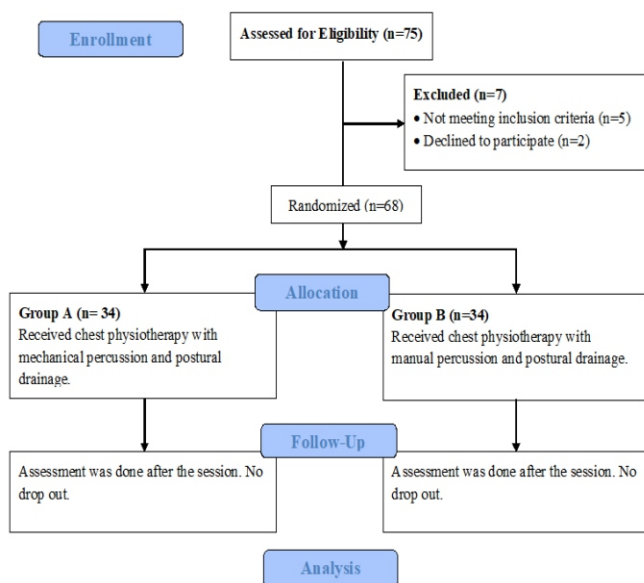


Figure 1: CONSORT flow Representation of Study

RESULTS

Enrolled in the study were 68 children total, ranging in age from birth to five years old. There were 34 youngsters in Group A and 34 children in Group B. Of all the kids in the research, 26 (61.8%) were girls and 42 (61.8%) were boys. The children's respiratory rate was 44.8 ± 15.0 beats per minute, and their average weight was 7.7 ± 4.8 kg. The pneumonia was the predominant diagnosis for the children, accounting for 56 (82.4%) cases (Table 1).

Table 1: Demographic Data of the Participants

| Variables | Study Population | Group A, Mechanical | Group B, Manual |
|------------------------|--------------------|---------------------|-----------------|
| | (Mean ± SD), n (%) | | |
| Weight (kg) | 7.7 ± 4.8 | 7.38 ± 4.2 | 8.01 ± 5.3 |
| Respiratory Rate (b/m) | 44.8 ± 15.0 | 44.3 ± 4.2 | 45.4 ± 14.9 |
| Gender | Male | 21 (61.8%) | 21 (61.8%) |
| | Female | 26 (38.2%) | 13 (38.2%) |
| Age | 0-3 Months | 21 (30.9%) | 10 (29.4%) |
| | 3-6 Months | 13 (19.1%) | 09 (26.5%) |
| | 6-9 Months | 11 (16.2%) | 05 (14.7%) |
| | 9-12 Months | 03 (4.4%) | 01 (2.9%) |

| | | | | |
|-----------|---------------|------------|------------|------------|
| | 1-2 Years | 13 (19.1%) | 07 (20.6%) | 06 (17.6%) |
| | 2-5 Years | 07 (10.3%) | 05 (14.7%) | 02 (5.9%) |
| Diagnosis | Pneumonia | 56 (82.4%) | 28 (82.4%) | 28 (82.4%) |
| | Bronchiolitis | 10 (14.7%) | 06 (17.6%) | 04 (11.8%) |
| | Rad | 02 (2.9%) | 0 (0%) | 02 (5.9%) |
| Total | | 68 | | |

RAD: Reactive Airways Disease

The differences in heart rate, respiratory rate, and oxygen saturation (O2 saturation) between the pre-treatment and post-treatment follow-up were evaluated for both Group A and Group B. A significant difference ($p < 0.05$) was found in Group A's heart rate, FLACC, and modified respiratory distress assessment instrument (mRDAI) when applying the Mann-Whitney for statistical analysis. However, following the session, there was no discernible change in O2 saturation, Wang clinical severity score (WCSS), or respiratory rate ($p > 0.05$). In Group A, the mean score on the modified Respiratory Distress Assessment Instrument (mRDAI) was 28.5 following the session, compared to 30.3 at baseline. In a similar vein, the Wang clinical severity score's (WCSS) mean rank was 30.4 following the session, compared to 31.9 at baseline. The mean rank of oxygen saturation (SPO2) was 35.0 at baseline and 36.5 after the session, while the FLACC score was 30.8 at baseline and 28.2 after the session. The baseline and post-session respiration rates were, respectively, 30.8 and 30.7 (Table 2).

Table 2: Inter-Group Analysis (Mann-Whitney Test)

| Variables | Assessment | Group A, Mechanical | Group B, Manual | Median (IQR) | p-Value |
|-----------|---------------|---------------------|-----------------|--------------|---------|
| | | Mean Rank | | | |
| mRDAI | At Baseline | 30.3 | 38.7 | 6.5 (5) | 0.08 |
| | After Session | 28.5 | 40.5 | 4 (2) | 0.01* |
| WCSS | At Baseline | 31.9 | 37.1 | 6 (5) | 0.27 |
| | After Session | 30.4 | 38.6 | 2 (2) | 0.08 |
| FLACC | At Baseline | 30.8 | 38.3 | 5 (3) | 0.11 |
| | After Session | 28.2 | 40.8 | 0.0 (0) | 0.04* |
| SPO2 | At Baseline | 35.0 | 34.0 | 97 (3) | 0.84 |
| | After Session | 36.5 | 32.5 | 99 (1) | 0.38 |
| RR | At Baseline | 30.8 | 38.2 | 48 (14) | 0.12 |
| | After Session | 30.7 | 38.3 | 38 (13) | 0.11 |

* $p < 0.005$

A significant difference ($p < 0.05$) was found in Group A's heart rate, FLACC, and mRDAI when applying the independent sample t-tests for statistical analysis. The youngsters in Group A had a mean heart rate of 132.3 ± 24.1 at baseline and 125.5 ± 20.5 after the session, according to the independent sample test. Group B's mean mRDAI rank was 38.7 at baseline and 40.5 post-session, while Group B's mean WCSS rank was 37.1 at baseline and 38.6 post-session. With a mean rank of SPO2 of 34.0 at baseline and 32.5 after the session, the FLACC score was 38.3 at baseline and 40.8 after the session. After the session, the

respiratory rate score was 38.3, compared to 38.2 at baseline. The youngsters in Group B had a mean heart rate of 115.5 ± 26.4 at baseline and 112.9 ± 18.8 after the session, according to the independent sample test. When comparing the experimental group (Group A) to the control group (Group B), the results showed a clinically meaningful improvement in Group A ($p < 0.05$) (Table 3).

Table 3: Inter Group Analysis (Independent Sample t-Test)

| Variables | Assessment | Group A, Mechanical | Group B, Manual | Mean Difference | p-Value |
|------------|---------------|---------------------|------------------|-----------------|---------|
| Heart Rate | At Baseline | 132.3 ± 24.1 | 115.5 ± 26.4 | -16.79 | 0.08 |
| | After Session | 125.5 ± 20.5 | 112.9 ± 18.8 | -12.59 | 0.01* |

* $p < 0.005$

DISCUSSION

Newborns and toddlers with impaired lung function are often at risk for pulmonary infections. These people frequently have symptoms like fever, dyspnea, coughing, and inward chest movement. This investigation aimed to assess the effectiveness of mechanical and manual percussion methods in conjunction with appropriate drainage placement for pediatric patients admitted to hospitals with pneumonia diagnoses [2]. The study found substantial improvements ($p < 0.05$) in the modified Respiratory Distress Assessment Instrument (mRDAI), Face Legs Activity Cry CONSOL ability scale (FLACC), and heart rate through the use of a randomized controlled trial (RCT) design. Notably, children's symptoms improved more when they had mechanical percussion as part of their chest physiotherapy. Importantly, there were no problems or side effects noted over the trial. Hue et al. conducted a study to ascertain if mechanical chest physiotherapy (CPT) utilizing the LEGA-Kid® mechanical percussion device is more effective than manual CPT in pediatric patients with lower respiratory tract infections. The amalgamation of percussion and vibrations produced by the LEGA-Kid apparatus can replicate manual chest physiotherapy, facilitating the dislodgment of loosened secretions. Optimal intrathoracic pressure was attained when the precursor was applied firmly and perpendicularly to the chest wall to induce vocal tremor. The precursor demonstrated the ability to sustain a higher and more stable intrathoracic pressure, akin to the efficacy of three physiotherapists [11]. This helps them to clear their airways. In this study, a good number of the subjects had a pneumonia diagnosis. Whereas the experimental group underwent mechanical chest physiotherapy, the control group received manual chest physiotherapy. Heart rate ($p < 0.05$), the Face Legs Activity Cry CONSOL ability scale (FLACC), and the modified Respiratory Distress Assessment Instrument (mRDAI) showed a clear improvement in the outcomes. Conversely, there is negligible enhancement in respiration rate. In group B,

some patients experienced irritation and discomfort, resulting in the FLACC scale values increasing. Therefore, it might be said that mechanical percussion combined with chest physical therapy improves these outcomes more than the control group. Children experiencing a combination of mechanical and manual chest physiotherapy showed alleviation from respiratory discomfort without any recorded side effects, according to earlier research. The study showed how well a combination approach using nebulized hypertonic saline and chest physical therapy removes airway secretions. The therapies in the trial helped to improve rather severe respiratory discomfort. Moreover, it was found that mechanical chest physical treatment reduced respiration rate more effectively than manual chest physical therapy. The findings show that hand and mechanical approaches, together with nebulized hypertonic saline, could be a good way to control children's respiratory discomfort and encourage airway clearing. Mohamed et al. investigated how combining nebulization with chest physical therapy affected pediatric respiratory conditions. The combined strategy proved more effective than nebulization alone, shown by the significant mean variations in heart rate, respiratory rate, and oxygen saturation between the groups seen in the study ($p = 0.000$). The study concludes that nebulization and chest physical therapy may be effective in treating pediatric respiratory disorders. These therapies can alleviate airway obstruction and improve respiratory health [18]. The aforementioned research, along with the current study, provides additional evidence of the efficacy of postural drainage and mechanical percussion in chest physical therapy for airway clearance and secretion removal. Various populations, including children, have exhibited enhanced respiratory outcomes due to these interventions. Notably, none of the research has found any negative impacts of mechanical percussion on kids. Because mechanical percussion did not cause any harm, this study implies that it can be regarded as a safe and effective therapy for clearing children's airways when combined with appropriate treatments like postural drainage. AbdelBasset and Elnegamy looked into the effects of chest physical therapy on hospitalized newborns and children who had pneumonia. The control group received standard pneumonia treatment, whereas the research group received both standard treatment and chest physiotherapy. The study's findings indicate that the group receiving chest physical therapy exhibited significantly elevated oxygen saturation and respiratory rate compared to the control group [4]. In an experimental study assessing the advantages of respiratory physiotherapy for patients with pneumological illnesses, children constituted 50% of the cases, with boys exhibiting the highest prevalence at 60%. The study's findings

indicate that postural and autogenic draining techniques enhanced ventilation, regulated the respiratory cycle, facilitated secretion clearance, and restored the patient's state to normalcy. The study found that employing Huffing and Puffing methods was most effective in treating juvenile patients with respiratory difficulties. The study suggests that individuals with pulmonary issues, regardless of age, may benefit from respiratory physiotherapy interventions [9]. With 61.8% of the instances, most of the impacted young people in the current study were men, suggesting a higher prevalence in this cohort. For all groups, chest physiotherapy lasted 15 to 20 minutes; following that, each person had an extra inspection. The study found that a more effective therapy approach was mechanical percussion mixed with chest physical therapy. It is crucial to remember, though, that the phrase "($p > 0.05$)" usually denotes the absence of a statistically significant difference between the treatment groups. To effectively communicate the results, the sentence should be changed to "($p < 0.05$)" if the goal is to show statistical significance.

This study was limited by its single-center design, relatively small sample size, and short-term outcome assessment immediately after intervention, which may restrict generalizability and long-term interpretation. Additionally, non-probability sampling and the absence of extended follow-up may introduce selection bias and limit causal inference. Future studies should incorporate larger, multi-center populations, probability sampling techniques, and long-term follow-up to assess sustained clinical benefits and safety of mechanical percussion in pediatric respiratory management.

CONCLUSIONS

This study concluded that chest issues in pediatric patients can be effectively managed utilizing both techniques: chest physiotherapy with and without mechanical percussion. Nonetheless, the outcomes of chest physiotherapy utilizing mechanical percussions indicated a greater clinical efficacy in enhancing heart rate, the Face Legs Activity Cry CONSOL ability Scale (FLACC), and the modified Respiratory Distress Assessment Instrument (mRDAI).

Authors' Contribution

Conceptualization: MA, NR

Methodology: SA, AR, NA

Formal analysis: AK, IK

Writing and Drafting: MA, NR, AR

Review and Editing: SA, AR, NA, AK, IK, MA, NR

All authors approved the final manuscript and take responsibilities for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article



Comparative Analysis of Serum Trace and Heavy Metals in Hepatitis B, C, and D Patients from Shaheed Benazirabad, Pakistan

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ARTICLE INFO

Keywords:

Viral Hepatitis, Hepatitis B, Hepatitis C, Hepatitis D, Trace Metals, Heavy Metals, Serum Analysis

How to Cite:Dahri, Z. A., Shah, A., Khuhawar, T. M. J., Shaheen, S., & Buriro, F. (2025). Comparative Analysis of Serum Trace and Heavy Metals in Hepatitis B, C, and D Patients from Shaheed Benazirabad, Pakistan: Analysis of Serum Trace and Heavy Metals in Hepatitis B, C, and D Patients. *Pakistan BioMedical Journal*, 8(8), 09-15. <https://doi.org/10.54393/pbmj.v8i8.1279>***Corresponding Author:**Afsheen Shah
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ABSTRACT

Trace and heavy metals play essential roles in liver metabolism and immune regulation, but their imbalance may exacerbate complications in viral hepatitis. **Objectives:** To compare serum levels of key trace elements (zinc [Zn], iron [Fe], copper [Cu]) and heavy metals (lead [Pb], cadmium [Cd], chromium [Cr], aluminum [Al], arsenic [As], manganese [Mn], nickel [Ni], cobalt [Co]) among patients with Hepatitis B (HBV), Hepatitis C (HCV), and Hepatitis D (HDV). **Methods:** A cross-sectional study was conducted on 130 patients (aged 15-70 years) in Shaheed Benazirabad, Pakistan (2022-2024). Serum metals were quantified using Flame and Graphite Furnace Atomic Absorption Spectrometry (FAAS/GFAAS). Statistical analyses included ANOVA/t-test for normally distributed data and Kruskal-Wallis/Mann-Whitney U for non-normal data. For metals with concentrations reported below the method's limit of detection (LOD), a value of half the LOD (LOD/2) was assigned for statistical analysis. This common strategy minimizes bias and allows for the inclusion of all data points in the analysis, preventing the loss of information that would occur from exclusion. **Results:** Cr was significantly elevated in HCV compared to HBV ($p=0.0035$). Manganese (Mn) was significantly reduced in HDV compared to HBV ($p=0.011$). Other metals (Zn, Fe, Cu, Pb, As, Al, Cd, Ni, Co) showed no statistically significant differences, although trends of accumulation were observed. **Conclusions:** Elevated chromium in HCV and reduced manganese in HDV suggest that hepatitis type influences metal homeostasis, potentially contributing to disease progression.

INTRODUCTION

Hepatitis is a transmissible inflammation of the liver, most often caused by viruses, which alters the functioning of the liver and causes accumulation of toxins. Symptoms are fever, nausea, joint pain, dark urine and abdominal pain [1, 2]. Essential micronutrients play a critical role in the liver metabolic activities including enzymatic activity, protein synthesis, immune system, and antioxidant protection [3, 4]. Reactive oxygen species (ROS) exacerbate liver damage, contributing to hepatocellular carcinoma, particularly in viral hepatitis [5]. Hepatitis A (HAV) does not progress to a chronic stage, whereas Hepatitis B (HBV) can become chronic in about 10% of cases, often driven by

immune-mediated mechanisms [6, 7]. Hepatitis C (HCV) tends to persist due to inadequate antiviral immune responses. HDV and Hepatitis E (HEV) involve varying immune-pathogenic mechanisms, with HAV and HEV typically not leading to chronic disease [8]. Cytokines such as interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6), released during stress or infection, influence trace element levels as part of the immune response [9, 10]. Among these, zinc (Zn) is essential for immune integrity and liver function, impacting the activity of over 300 enzymes [11]. Zn deficiency contributes to complications in chronic liver disease, including hepatic



encephalopathy. Studies have reported reduced plasma Zn levels in patients with HBV-related cirrhosis. Iron (Fe) and copper (Cu) levels are also altered during infection. Cu accumulation, particularly in chronic HCV, can induce oxidative damage and liver injury [12, 13]. Similarly, hepatic iron overload is associated with fibrosis, cirrhosis, and hepatocellular carcinoma, although direct causality remains uncertain [14, 15]. Notably, iron reduction has been shown to improve response to antiviral therapy [16]. Trace elements are crucial for protein synthesis, immune function, and pregnancy health [17]. Accurate measurement of these elements requires sensitive methods such as atomic absorption spectrometry (AAS) [18, 19], often aided by microwave-assisted digestion [20], which reduces acid consumption and vapor production [21, 22].

Imbalance of trace and heavy metals may aggravate oxidative stress and immune dysregulation in viral hepatitis, yet comparative data among HBV, HCV, and HDV patients in local populations remain limited. The lack of region-specific biochemical profiling restricts understanding of how different hepatitis types influence metal homeostasis and disease progression. There is a lack of region-specific comparative data from Pakistan evaluating serum trace and heavy metal imbalances across Hepatitis B, C, and D patients using sensitive analytical techniques. Additionally, limited evidence exists linking hepatitis type-specific metal dysregulation with disease progression, highlighting the need for targeted biochemical profiling in local populations. Therefore, this study aimed to compare serum trace and heavy metal concentrations among HBV, HCV, and HDV patients in Shaheed Benazirabad, Pakistan.

METHODS

This analytical cross-sectional study included 130 viral hepatitis patients (aged 15–70 years, both sexes) recruited from Peoples Medical College Hospital (PMCH), Nawabshah, Rural Health Center (RHC) Shahpur Chakar, and nearby villages such as Qazi Muhammad Jumandahri and Ahmed Abad, covering adjoining areas of Shaheed Benazirabad (SBA). The Ethical Committee of the Institute of Biochemistry approved the study (Ref. IOB/294m/2022). All the participants signed informed consent and the study was performed according to the principles of the Declaration of Helsinki. The study period was from August 2022 to July 2024. The sample size of 130 patients was based on feasibility and availability of hepatitis B, C, and D cases within the study period at the selected hospitals. Although no formal power analysis was performed before data collection, this number exceeds the minimum recommended sample ($n \approx 90$) calculated post hoc using

G*Power software (effect size $f = 0.25$, $\alpha = 0.05$, power = 0.80, three groups, one-way ANOVA). Thus, the study was sufficiently powered to detect medium differences in serum trace and heavy metal concentrations across the hepatitis groups. Patients were recruited through a consecutive sampling technique, enrolling all eligible hepatitis B, C, and D cases presenting at the study sites during the research period. Inclusion criteria were age 15–70 years, confirmed viral hepatitis diagnosis, and informed consent. Patients with co-infections, chronic kidney disease, or on metal supplements were excluded. From each participant, 7 mL of venous blood was drawn using sterile disposable syringes. Out of this, 3 mL was placed in EDTA tubes and stored at 4°C for lead (Pb) and cadmium (Cd) analysis. The remaining sample was transferred to gel tubes, allowed to clot, centrifuged at 3000 rpm for 10 minutes, and the serum was stored at -20°C in Eppendorf tubes for up to two months for the analysis of Zn, Cu, and Fe. Zinc, copper, and lead concentrations were measured using Flame Atomic Absorption Spectrometry (FAAS); cadmium was analyzed using Graphite Furnace Atomic Absorption Spectrometry (GFAAS); and iron levels were determined using a CECIL CE 1011 spectrophotometer with a human manual kit [23, 24]. Descriptive statistics were used to summarize demographic and biochemical data. Data distributions were assessed using the Shapiro–Wilk test [25, 26]. Normally distributed variables were summarized as mean \pm SD, while skewed variables were expressed as median with interquartile range (IQR). Based on data distribution, independent t-tests and one-way ANOVA were applied to normally distributed variables [27], while Mann–Whitney U and Kruskal–Wallis tests were used for non-normally distributed data [28]. Appropriate statistical methods were applied to evaluate associations between trace metal levels and hepatitis across demographic groups. Boxplots were used for data visualization.

RESULTS

This study analyzed trace and heavy metal concentrations in HBV, HCV, and HDV patients, using the Shapiro–Wilk test to classify distributions with descriptive Statistics. Non-normally distributed metals—Al, Cd, Fe, Mn, Ni, Zn exhibited marked skewness and variability. Aluminium was elevated across all types, especially in HCV, indicating possible bioaccumulation. Cadmium had negative means, suggesting levels near detection limits, slightly higher in HDV. Iron showed extreme variability, particularly in HCV, pointing to potential overload. Manganese declined from HBV to HDV with decreasing variability, indicating tighter regulation. Nickel increased modestly from HBV to HDV, with detection issues implied by negative minima. Zinc remained stable, slightly higher in HCV. Normally

distributed metals, As, Co, Cr, Cu, and Pb, showed consistent trends. Arsenic rose with disease progression. Cobalt remained negligible, likely due to low bioavailability. Chromium was highest among these metals, elevated and variable in HCV and HDV, suggesting altered metabolism. Copper remained stable. Lead showed high variability and right-skew in HCV and HDV, hinting at environmental or occupational exposure. Overall, chromium and lead showed the highest variability and concentration, while arsenic accumulated progressively, and cobalt appeared insignificant (Table 1).

Table 1: Descriptive Statistics and Shapiro-Wilk Normality Test for Metal Variables

| Non-Normal Variables: Variables that Follow A Non-Normal Distribution | | | | | | | | | |
|---|-------|-------|-------|-------|-------|-------|--------|--------|-------|
| Variables | Al-B | Al-C | Al-D | cd-B | cd-C | cd-D | fe-B | fe-C | fe-D |
| Mean | 2.26 | 2.54 | 2.16 | -0.19 | -0.19 | -0.10 | 26.87 | 29.17 | 17.24 |
| Median | 1.54 | 1.89 | 1.50 | -0.05 | -0.03 | -0.03 | 8.23 | 5.49 | 7.00 |
| Std Dev | 1.78 | 2.06 | 1.84 | 0.25 | 0.33 | 0.14 | 35.16 | 47.60 | 21.28 |
| Min | 0.91 | 0.82 | 0.82 | -1.02 | -1.44 | -0.47 | 0.95 | 0.87 | 0.87 |
| Max | 10.10 | 10.10 | 10.10 | -0.01 | 0.01 | 0.01 | 148.82 | 209.80 | 79.97 |
| Shapiro Stat | 0.64 | 0.73 | 0.62 | 0.75 | 0.64 | 0.75 | 0.75 | 0.63 | 0.74 |
| Shapiro p-value | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Variables | Mn-B | Mn-C | Mn-D | Ni-B | Ni-C | Ni-D | Zn-B | Zn-C | Zn-D |
| Mean | 0.05 | 0.04 | 0.04 | 0.03 | 0.04 | 0.05 | 0.91 | 1.01 | 0.91 |
| Median | 0.04 | 0.03 | 0.03 | 0.04 | 0.05 | 0.05 | 0.91 | 0.95 | 0.93 |
| Std Dev | 0.03 | 0.03 | 0.01 | 0.03 | 0.05 | 0.03 | 0.44 | 0.60 | 0.42 |
| Min | 0.02 | 0.02 | 0.02 | -0.07 | -0.14 | 0.00 | 0.33 | 0.26 | 0.26 |
| Max | 0.15 | 0.17 | 0.07 | 0.09 | 0.11 | 0.18 | 2.49 | 2.66 | 2.35 |
| Shapiro Stat | 0.81 | 0.65 | 0.87 | 0.93 | 0.83 | 0.87 | 0.83 | 0.85 | 0.91 |
| Shapiro p-value | 0.00 | 0.00 | 0.00 | 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 |
| Normal Variables: Variables That Follow A Normal Distribution | | | | | | | | | |
| Variable | As-B | As-C | As-D | Co-B | Co-C | Co-D | Cr-B | Cr-C | Cr-D |
| Mean | 0.03 | 0.04 | 0.04 | -0.03 | -0.04 | -0.03 | 0.10 | 0.12 | 0.12 |
| Median | 0.03 | 0.04 | 0.04 | -0.03 | -0.03 | -0.03 | 0.09 | 0.12 | 0.11 |
| Std Dev | 0.02 | 0.02 | 0.02 | 0.01 | 0.02 | 0.01 | 0.02 | 0.03 | 0.04 |
| Min | -0.01 | -0.01 | 0.02 | -0.06 | -0.06 | -0.06 | 0.03 | 0.07 | 0.01 |
| Max | 0.07 | 0.07 | 0.07 | -0.01 | 0.00 | 0.00 | 0.16 | 0.17 | 0.19 |
| Shapiro Stat | 0.98 | 0.99 | 0.97 | 0.98 | 0.97 | 0.97 | 0.94 | 0.94 | 0.95 |
| Shapiro p-value | 0.68 | 0.94 | 0.44 | 0.80 | 0.53 | 0.54 | 0.11 | 0.08 | 0.19 |
| Variables | Cu-B | Cu-C | Cu-D | Pb-B | Pb-C | Pb-D | - | - | - |
| Mean | 0.07 | 0.07 | 0.08 | 0.11 | 0.11 | 0.11 | - | - | - |
| Median | 0.07 | 0.07 | 0.08 | 0.13 | 0.13 | 0.12 | - | - | - |
| Std Dev | 0.04 | 0.05 | 0.04 | 0.11 | 0.11 | 0.10 | - | - | - |
| Min | 0.01 | 0.00 | 0.00 | -0.07 | -0.06 | -0.06 | - | - | - |
| Max | 0.18 | 0.19 | 0.19 | 0.34 | 0.36 | 0.36 | - | - | - |
| Shapiro Stat | 0.94 | 0.97 | 0.98 | 0.96 | 0.95 | 0.97 | - | - | - |
| Shapiro p-value | 0.09 | 0.41 | 0.91 | 0.24 | 0.15 | 0.42 | - | - | - |

Boxplot visualizations were used to assess differences in the concentrations of As, Co, Cr, Cu, and Pb among patients with Hepatitis B, C, and D. The boxplot for Chromium (Cr) showed a clearly elevated median and wider spread in Hepatitis C patients compared to groups B and D, indicating a notable difference in distribution. This visual

trend was supported by the ANOVA result ($p = 0.0092$), confirming a statistically significant variation. In contrast, the boxplots for Arsenic, Cobalt, Copper, and Lead displayed overlapping medians, similar interquartile ranges, and consistent overall shapes across all three groups. These visual patterns suggest no meaningful differences in the distributions of these four metals. Thus, boxplot analysis highlights Chromium as the only metal with a distinct concentration pattern, particularly elevated in Hepatitis C patients, while the other metals remained visually consistent regardless of hepatitis type (Figure 1).

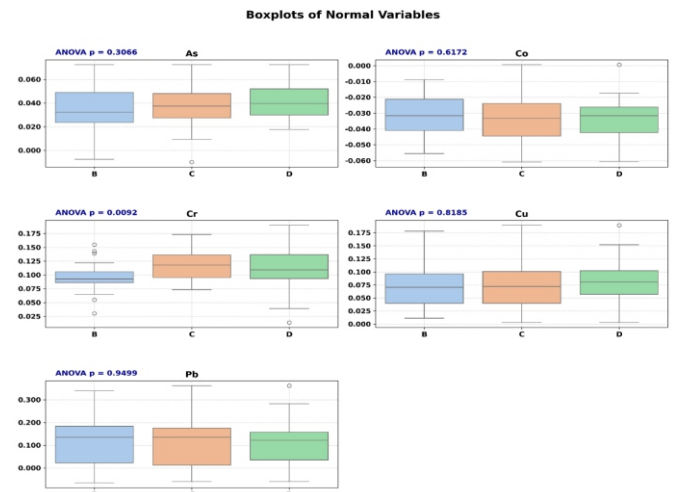


Figure 1: Boxplot of Normally Distributed Metal Variables

Boxplot visualizations were used to assess differences in the concentrations of Al, Cd, Fe, Mn, Ni, and Zn among groups B, C, and D. The boxplot for Manganese (Mn) revealed a noticeably higher median and broader spread in Group B compared to Groups C and D, suggesting a distinct variation in its distribution. This visual pattern was supported by the Kruskal-Wallis test ($p = 0.0352$), indicating a statistically significant difference. In contrast, the boxplots for Aluminum (Al), Cadmium (Cd), Iron (Fe), Nickel (Ni), and Zinc (Zn) showed overlapping medians, comparable interquartile ranges, and similar distribution shapes across all three groups. These consistent visual patterns imply no substantial differences in the distributions of these five metals. Therefore, the boxplot analysis highlights Manganese as the only element with a distinct concentration pattern, particularly elevated in Group B, while the others remained visually uniform across the groups (Figure 2).

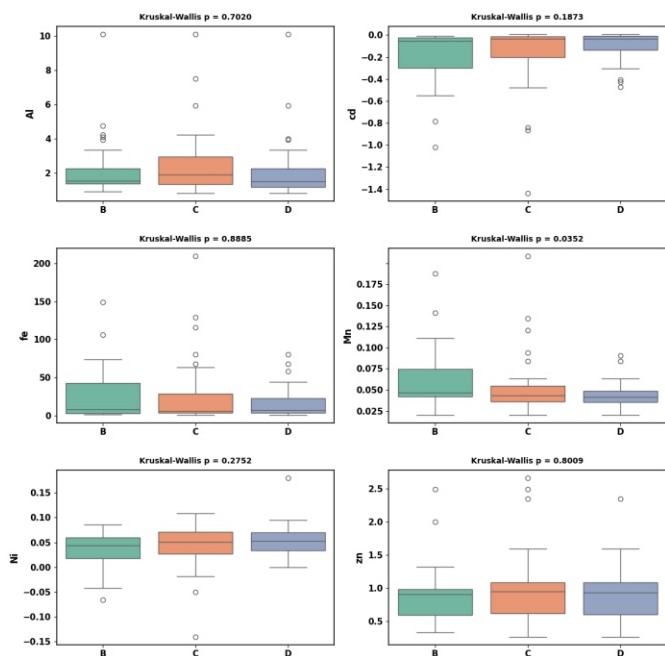


Figure 2: Boxplot of Non-Normally Distributed Metal Variables

To investigate potential differences in heavy metal concentrations among patients with Hepatitis B (HBV), Hepatitis C (HCV), and Hepatitis D (HDV), statistical analyses including One-Way ANOVA and independent sample t-tests were conducted for each metal. The analysis revealed that chromium (Cr) was the only metal showing a statistically significant difference among the three groups. The ANOVA result for chromium was $F = 4.9386$ with a p-value of 0.0092, indicating a significant variation. Specifically, the pairwise comparison between HBV and HCV showed a significant difference ($t = -3.4058$, $p=0.0035$), suggesting elevated chromium concentrations in HCV patients. No significant differences were observed between HBV and HDV or between HCV and HDV. In contrast, arsenic (As), cobalt (Co), copper (Cu), and lead (Pb) showed no statistically significant differences across the hepatitis types. Both ANOVA and pairwise t-tests confirmed uniform concentrations of these metals among all patient groups, indicating that their levels are not influenced by hepatitis type (Table 2).

Table 2: Hypothesis Testing of Normally Distributed Metal Variables Using Parametric Tests

| Test | One-Way ANOVA | T-test | | | One-Way ANOVA | T-test | | |
|-----------|---------------|-----------|---------|--------|---------------|---------|--------|---------|
| | | B vs C | B vs D | C vs D | | B vs C | B vs D | C vs D |
| | | As | | | Co | | | |
| Statistic | 1.198 | -0.506 | -1.522 | -1.069 | 0.4851 | 0.9429 | 0.7783 | -0.2032 |
| p-value | 0.307 | 1 | 0.4 | 0.869 | 0.6172 | 1 | 1 | 1 |
| | | Cr | | | Pb | | | |
| Statistic | 4.9386 | -3.4058 | -2.3491 | 0.4457 | 0.0515 | -0.2238 | 0.0745 | 0.3165 |
| p-value | 0.0092 | 0.0035 | 0.0664 | 1 | 0.9499 | 1 | 1 | 1 |

| | | Cu | | | | - |
|-----------|--------|-----------|---------|--------|--|---|
| Statistic | 0.2007 | -0.0296 | -0.5737 | -0.536 | | |
| p-value | 0.8185 | 1 | 1 | 1 | | |

To investigate potential differences in heavy metal concentrations among patients with Hepatitis B (HBV), Hepatitis C (HCV), and Hepatitis D (HDV), non-parametric statistical tests were used due to non-normal data distribution. The Kruskal-Wallis test assessed overall group differences, followed by Mann-Whitney U tests for pairwise comparisons. Among the metals analyzed, only manganese (Mn) showed a statistically significant difference across the hepatitis types. The Kruskal-Wallis test yielded a statistic of 6.6921 with a p-value of 0.0352, indicating significant variation. Pairwise comparisons revealed a significant difference between HBV and HDV ($U=661.5$, $p=0.011$), while differences between HBV and HCV and between HCV and HDV were not significant. In contrast, aluminium (Al), cadmium (Cd), iron (Fe), nickel (Ni), and zinc (Zn) showed no statistically significant differences across the groups. Both Kruskal-Wallis and Mann-Whitney U tests confirmed that concentrations of these metals remained consistent among all hepatitis types, suggesting they are not influenced by hepatitis infection status (Table 3).

Table 3: Hypothesis Testing of Non-Normally Distributed Metal Variables Using Non-Parametric Tests

| Test | Kruskal-Wallis | Mann-Whitney U | | | Kruskal-Wallis | Mann-Whitney U | | |
|-----------|----------------|----------------|--------|--------|----------------|----------------|--------|--------|
| | | B vs C | B vs D | C vs D | | B vs C | B vs D | C vs D |
| | | Al | | | Cd | | | |
| Statistic | 0.7075 | 460.5 | 520 | 538.5 | 3.3496 | 397.5 | 351.5 | 439.5 |
| p-value | 0.702 | 0.7836 | 0.5829 | 0.4181 | 0.1873 | 0.2454 | 0.0704 | 0.5684 |
| | | Fe | | | Mn | | | |
| Statistic | 0.2365 | 487.5 | 516 | 502.5 | 6.6921 | 605 | 661.5 | 526.5 |
| p-value | 0.8885 | 0.9271 | 0.6221 | 0.7621 | 0.0352 | 0.0808 | 0.011 | 0.5216 |
| | | Ni | | | Zn | | | |
| Statistic | 2.5808 | 400 | 370 | 453.5 | 0.4439 | 435.5 | 456 | 507.5 |
| p-value | 0.2752 | 0.26 | 0.1214 | 0.709 | 0.8009 | 0.5309 | 0.7354 | 0.709 |

DISCUSSION

This study revealed disease-specific alterations in trace and heavy metal concentrations among patients with hepatitis B, C, and D, highlighting the importance of metal metabolism in viral hepatopathology. The significantly elevated chromium (Cr) levels observed in HCV patients compared to HBV are of particular interest. Chromium has been implicated in oxidative stress-mediated hepatocellular damage and fibrosis [29]. The association between Cr dysregulation and chronic HCV infection may reflect virus-induced disruption of redox balance and warrants consideration as a potential biomarker of disease severity. Manganese (Mn) levels showed a progressive decline from HBV to HDV, which may indicate differences in

hepatic processing or biliary excretion. Previous studies have emphasized the dual role of Mn in health and disease, with excess linked to neurotoxicity and deficiency contributing to impaired enzymatic activity [30]. This trend suggests that Mn metabolism could be differentially affected by the viral etiology of hepatitis, although mechanistic clarification requires further research. Although aluminium (Al), cadmium (Cd), and nickel (Ni) levels were elevated but non-significant, their presence underscores the possible contribution of environmental exposures to liver injury in chronic viral hepatitis. Similar findings in experimental hepatology indicate that such metals exacerbate oxidative stress and mitochondrial dysfunction [29]. These results suggest that subclinical metal accumulation may contribute cumulatively to hepatocellular injury without clear disease-specific patterns. Iron (Fe) variability, especially in HCV patients, is notable. While our findings were not statistically significant, iron overload is a recognized cofactor for hepatic fibrosis and carcinogenesis in chronic liver disease [31]. The absence of a strong association in this study may reflect the need for larger cohorts or stratification by fibrosis stage. Similarly, copper (Cu) and lead (Pb) showed wide inter-individual variability, likely influenced by heterogeneous environmental or occupational exposures rather than direct viral effects [32]. Interestingly, zinc (Zn) levels remained stable across all groups despite its essential role in hepato-protection and immune modulation. Prior studies have reported zinc depletion in advanced liver disease, particularly cirrhosis [30]. Our results may suggest either compensatory mechanisms at earlier disease stages or uniform depletion across hepatitis types that obscures group-specific differences. Taken together, these findings support the hypothesis that viral hepatitis not only causes direct hepatocellular damage but also perturbs systemic metal homeostasis. This dysregulation may contribute to oxidative injury, altered immune responses, and progression to fibrosis or cirrhosis. The novelty of this study lies in comparing multiple viral hepatitis types, revealing both shared and unique patterns of metal imbalance. Clinically, trace metal profiling could aid in risk stratification and guide supportive interventions such as nutritional correction or chelation in selected cases.

However, this study has limitations, including a modest sample size, a lack of dietary and occupational exposure data, and a cross-sectional design, which precludes causal inference. This study was limited by its cross-sectional design, modest sample size, and absence of detailed dietary, environmental, and occupational exposure assessments, which may influence serum metal levels. Additionally, the lack of fibrosis staging and viral load correlation restricted deeper clinical interpretation.

CONCLUSIONS

This study demonstrates that specific trace and heavy metals, particularly chromium and manganese, exhibit significant variations among patients with different types of viral hepatitis. Chromium levels were notably elevated in hepatitis C, while manganese showed a progressive decline from hepatitis B to hepatitis D. Although other metals did not display strong statistical associations, their potential contribution to liver dysfunction cannot be excluded and warrants continued monitoring. Overall, these findings provide deeper biochemical insights into viral hepatitis and underscore the importance of incorporating trace and heavy metal profiling into future diagnostic, prognostic, and therapeutic strategies.

Authors' Contribution

Conceptualization: ZAD

Methodology: ZAD, AS

Formal analysis: ZAD, AS, TMJK, SS, FB

Writing and Drafting: ZAD, SS

Review and Editing: ZAD, AS, TMJK, SS, FB

All authors approved the final manuscript and take responsibilities for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article

Association of *HSD17B1* Gene Polymorphisms with Male Infertility in the Khyber Pakhtunkhwa Population, Pakistan

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ARTICLE INFO

Keywords:

Male Infertility, Single Nucleotide Polymorphisms (SNPs), Spermatogenesis, *HSD17B1* Gene

How to Cite:

Khan, M. F., Muhammad, H., Irfan, M., Shah, S. S., Rehman, F. U., Alamgeer, M., Din, K. U., Ilyas, M., & Khan, S. (2025). Association of *HSD17B1* Gene Polymorphisms with Male Infertility in the Khyber Pakhtunkhwa Population, Pakistan: Association of *HSD17B1* Gene Polymorphisms with Male. *Pakistan BioMedical Journal*, 8(8), 16-22. <https://doi.org/10.54393/pbmj.v8i8.1272>

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Received Date: 18th June, 2025

Revised Date: 8th August, 2025

Acceptance Date: 13th August, 2025

Published Date: 31st August, 2025

ABSTRACT

Male infertility is a complex disease recognized by the World Health Organization as a global health concern that affects men's reproductive health. This study investigated the association of the *HSD17B1* gene, a key regulator of the hormone testosterone, with male infertility.

Objectives: To find out the genetic variation in the *HSD17B1* gene and the association of *HSD17B1* gene polymorphisms with male infertility. **Methods:** The study involved 106 male patients with infertility issues and 80 healthy controls. Hormonal profiles were evaluated using *ELISA*, and semen parameters such as sperm count, morphology, and motility were examined to identify any abnormalities. Target genomic sequencing was performed to identify three SNPs, rs605059, rs992310724, and rs2676530, in the *HSD17B1* gene that are associated with male infertility. **Results:** The findings indicated a significant association between rs992310724 variations and testosterone levels (p -value=0.041). However, rs605059 (p -value=0.783) and rs2676530 (p -value=0.381) were not significantly associated with male infertility. **Conclusions:** The findings suggest the potential for personalized diagnostic and therapeutic strategies, as well as the need for a multidisciplinary approach in male infertility research. Male reproductive health is influenced by genetic variations, with different SNPs emerging as potential contributors.

INTRODUCTION

Infertility is a reproductive system disease in which a woman fails to achieve pregnancy after regular unprotected sexual contact for one year or more [1]. It affects 8-12% of the world's population, with secondary infertility being more prevalent. The prevalence of infertility varies globally, with males experiencing it at a greater incidence than women [2, 3]. Factors such as low sperm counts, poor morphology, and other health issues, such as heart disease, type 2 diabetes, prostate tumors,

and testicular cancer, can affect fertility [4, 5]. Pakistan has one of the highest rates globally, with 21.9%, 3.5% and 18.4% of married individuals having primary infertility and secondary infertility, respectively [6, 7]. Spermatogenesis and Steroidogenesis are both essential testicular functions. Spermatogenesis is a 74-day process occurring in the testes and involves mitotic cell division, meiotic cell division, and spermiogenesis [8, 9]. Sertoli cells provide structural and nutritional support to germ cells and

maintain the blood testis barrier [10, 11]. Spermatogenesis is regulated by two major hormones, FSH and LH. LH stimulates testosterone production in testicular Leydig cells (Lcs), releasing androgens that maintain physical characteristics, support sexual organ development, and regulate androgen-dependent activities. Both gonadotropins have distinct functions and feedback loops. The hypothalamic-pituitary-gonadal (HPG) axis regulates this process, and maintaining hormonal balance is vital for male fertility [12, 13]. Figure 1 illustrates this normal physiological pathway of spermatogenesis, highlighting the interactions between Sertoli cells, Leydig cells, gonadotropins, and testosterone production, providing context for understanding the *HSD17B1* role (Figure 1).

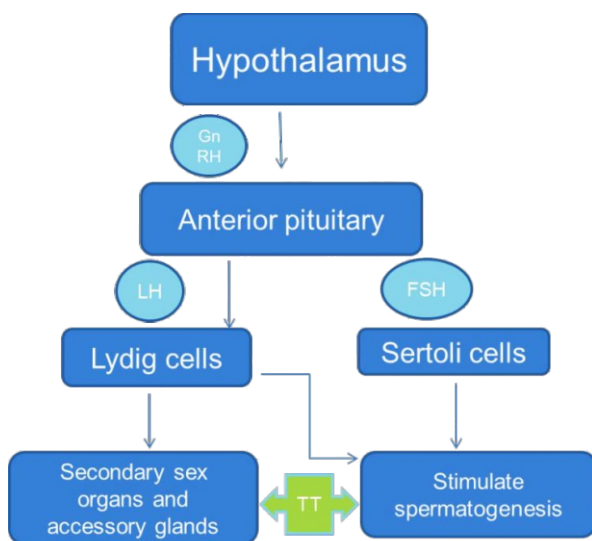


Figure 1: Normal Physiological Pathway of Spermatogenesis

Steroidogenesis is the process of converting cholesterol, primarily in Leydig cells, into androgens such as testosterone. Androgens support various developmental processes, including secondary sexual traits and spermatogenesis [14, 15]. In lateral lobes (LCs), male gonads produce steroid hormones, which are important for reproductive health. [16]. Testosterone is the primary hormone produced by LCs and affects fetal growth and secondary sexual function [17]. The enzyme *HSD17B* is essential for the conversion of hormones to more potent forms. Fourteen distinct *HSD17B* enzymes are present in various tissues and organs, regulating hormone activities and potentially causing hormone-related disorders such as breast cancer and endometriosis. Therefore, targeting *HSD17Bs* could be a promising therapeutic approach. *HSD17B1* is responsible for testosterone production in the gonads [18]. Hydroxysteroid (17b) dehydrogenase type 1 "*HSD17B1*" is an enzyme that plays a role in the synthesis of steroids in humans and other animals. This enzyme belongs to the enzyme family known as hydroxysteroid (17b) dehydrogenases (*HSD17Bs*), which convert low-potency 17-

ketosteroids to high-potency 17b-hydroxysteroids. *HSD17B1* is highly expressed in tissues known for their ability to produce estradiol, such as rat and human ovaries, as well as in the human placenta [19]. On chromosome 17q21, near BRCA1, the *HSD17B1* gene encodes 17b-hydroxysteroid dehydrogenase 1 (17b-HSD-1). 17b-HSD-1 is required for the production of oestrogens and testosterone. The principal site of testosterone production, the testis, is where 17b-HSD-1 is mostly expressed [20, 21]. Three SNPs in the *HSD17B1* gene (rs605059, rs992310724, and rs2676530) were selected based on their reported functional consequences and potential impact on steroid metabolism and testosterone production, making them strong candidates for evaluating genetic susceptibility to male infertility. Three SNPs in the *HSD17B1* gene were selected based on their functional consequences.

Male infertility is a multifactorial condition in which genetic determinants, particularly polymorphisms in steroidogenic genes such as *HSD17B1*, remain insufficiently characterized in the Pakistani population. Limited regional data regarding the association of specific SNPs (rs605059, rs992310724, and rs2676530) with hormonal profiles and infertility risk creates uncertainty in understanding their clinical relevance. There is limited population-specific evidence from Pakistan examining the association between *HSD17B1* gene polymorphisms (rs605059, rs992310724, rs2676530) and male infertility, particularly in relation to hormonal profiles such as serum testosterone. Moreover, the functional and clinical relevance of these SNPs in the local ethnic context remains insufficiently characterized, necessitating targeted genetic studies. Therefore, this study aimed to evaluate the association between selected *HSD17B1* gene polymorphisms and male infertility, as well as their relationship with serum testosterone levels.

METHODS

This case-control study was conducted between January 2023 to January 2024 at the Imperial Poly Clinic in Dabgari Garden, Peshawar, Pakistan, and involved 186 participants. The study was approved by the Khyber Medical University Ethical Research Committee (ASRB Reference No. KMU/IBMS/IRBE/7th meeting/2023/1209-3). Cases and controls were matched by age and weight to minimize confounding effects. The mean age and weight of both groups were compared using independent samples t-tests to confirm successful matching. Participants were included if, according to WHO criteria, they failed to achieve pregnancy with their partners after at least one year of regular unprotected sexual intercourse. The control group included men who were currently having children with their partners. Demographic data were carefully recorded, and

biochemical information, including blood components, hormone levels, and semen analysis, was collected. Semen samples were obtained by masturbation into a sterile plastic cup, and sperm morphology was manually assessed. The overall motile sperm count was calculated as (Concentration \times ejaculate volume \times % overall motility). The total normal count was calculated as (Concentration \times ejaculate volume \times % morphologically normal). Sperm parameters were classified according to the WHO lower reference limits: total sperm count 39 million per ejaculate, sperm concentration 15 million per mL, total sperm motility 40%, progressive motility 32%, morphologically normal sperm 4%, and ejaculate volume 1.5 mL. Serum hormone assays were performed to measure testosterone, LH, and FSH levels. Testosterone was measured using a chemiluminescence kit (Siemens, Germany, Lot No. CIA37K3K2) with a detection threshold of 0.2 ng/mL. Serum levels of FSH and LH were assessed using ELISA kits (Lot No. 4K11x 13 and CIA-6K1B2, respectively). Genomic DNA was extracted from blood samples using the phenol-chloroform method. SNPs(rs605059, rs992310724, and rs2676530) were genotyped using PCR according to standard protocols. PCR amplification was performed in a Conversion TCY48 thermocycler under standard cycling conditions, and selected PCR products were confirmed by Sanger sequencing. Sequencing outputs were obtained in ABI and SEQ formats and analyzed using FinchTV and BioEdit software for accuracy and precision. Age and weight distributions were analyzed using Microsoft Excel. SPSS version 25 was used to evaluate associations between SNPs and clinical parameters, including testosterone levels, sperm count, hormone profiles, and sperm morphology/motility. The normality of testosterone level distribution and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests, respectively. When assumptions were met, t-tests and Pearson correlation were applied; otherwise, non-parametric alternatives such as the Mann-Whitney U test or Spearman's correlation were employed. Chi-square tests were used for categorical comparisons (e.g., genotype frequencies), independent samples t-tests for continuous variables (e.g., testosterone levels between groups), and Pearson correlation to assess relationships between genetic variants and hormone levels. Logistic regression was applied for association analyses under additive genetic models, and the Bonferroni method was used to correct for multiple comparisons. Allelic distributions were tested for Hardy-Weinberg equilibrium in the control group. A p-value <0.05 was considered statistically significant.

RESULTS

The study involved 186 male individuals, including 106 infertile patients (cases) and 80 healthy controls. The mean age of the patients was 31.87 ± 6.07 years, while the mean age of the controls was 30.16 ± 4.75 years. Our research data categorized the cases into two groups: primary infertility (77 patients; 72.64%) and secondary infertility (29 patients; 27.35%), each presenting unique issues within the context of reproductive health. The mean age of the participants was 31.87 ± 6.07 years. The minimum age of the participants was 21 years, and the maximum age was 50 years (Figure 2).

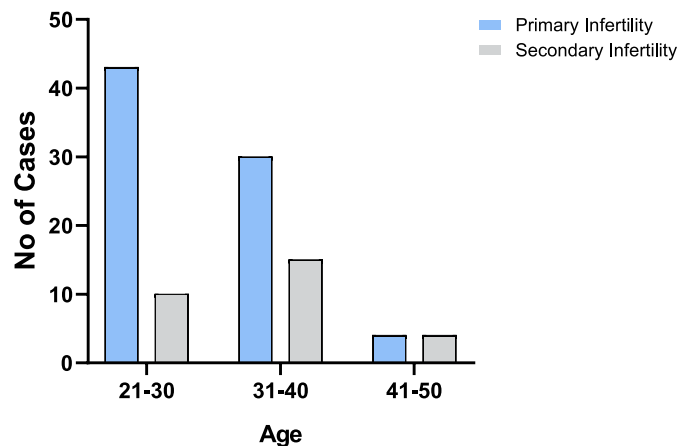


Figure 2: Age Distribution of Participants with Primary and Secondary Infertility

The mean weight of the participants was 72.4 ± 8.74 kg. The minimum weight of the participants was 60 kg, and the maximum weight was 118 kg (Figure 3).

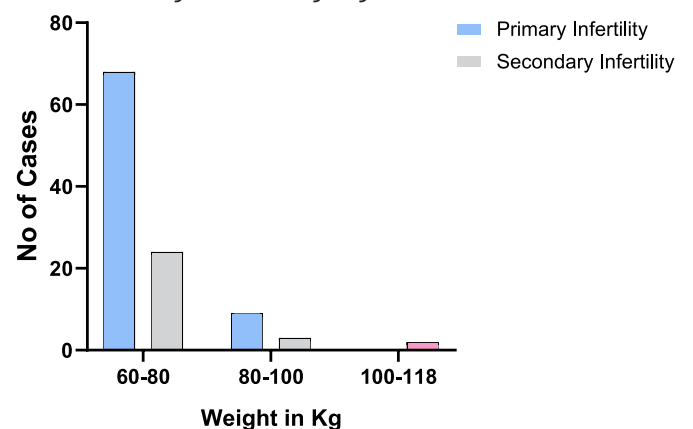


Figure 3: Weight Distributions of Participants with Primary and Secondary Infertility

Semen samples were collected from participants following WHO guidelines; their ejaculation time was recorded, and ejaculation was avoided for at least three days. The sperm morphology was manually examined using high-resolution oil-immersion microscope optics. The samples were categorized into five groups: azoospermic (29), oligospermic (3), asthenospermic (41), teratospermic (14),

and normal (19) individuals. The data presented in Figure IV are based on the sperm count. Numbers indicate the sample size for each group: azoospermic (n=29), oligospermic (n=3), asthenospermic (n=41), teratospermic (n=14), and normal (n=19)

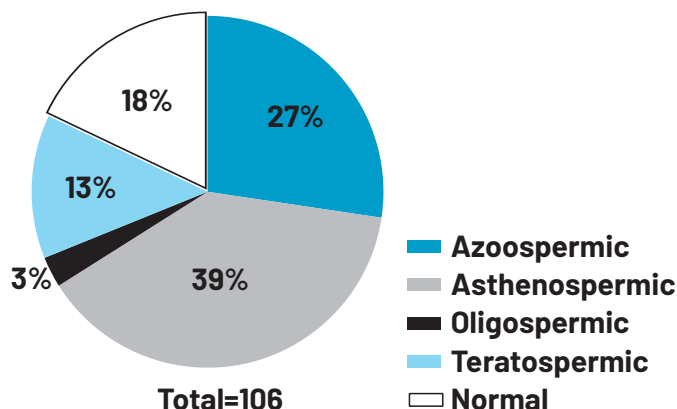


Figure 4: Distribution of Sperm Count Categories Among Infertile Participants

The genetic variations in selected SNPs were analysed using Sanger sequencing, ensuring quality and mutation detection. Using the specialized software Finch TV and BioEdit, the sequencing data were analysed for reliability and precision. Each chromatogram displays nucleotide composition and quality for the indicated SNP in infertile and control samples (sample sizes: n=106 cases, n=80 controls)(Figure 5).

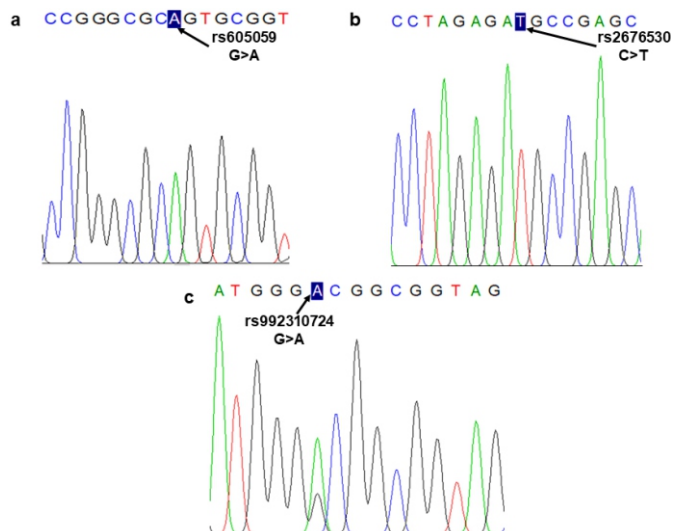


Figure 5: Representative Sanger Sequencing Chromatograms for Selected SNPs: (a)rs605059, (b)rs2676530, and (c)rs992310724

The study investigated the allelic distribution of selected SNPs in male infertility patients. Allelic distributions for the three SNPs are summarized in Table I. Briefly, rs605059 showed a higher frequency of allele A (69.8% in cases) compared to allele G (30.8%). For rs2676530, allele C was most frequent (89.6% in cases), whereas allele T was rare (5.7%). In rs992310724, allele G predominated (96.2%), with allele A being uncommon (3.77%)(Table 1).

Table 1: Genotype and Allelic Distribution for Selected SNPs in Male Infertility Patients

| SNP | Genotype / Allele | Cases (N, %) | Controls (N, %) | OR (95% CI) | p-Value | Reference | |
|-------------|-------------------|--------------|-------------------|---------------------|---------|-----------|--|
| rs605059 | GG | 73 (69.8%) | 75 (93.75%) | 1 | - | G | |
| | GA | 32 (30.8%) | 4 (5.0%) | 8.22 (2.77-24.40) | 0.001 | - | |
| | AA | 1 (0.94%) | 1 (1.25%) | 1.03 (0.06-16.74) | 0.984 | - | |
| | GA + AA | 74 (69.81%) | 5 (6.25%) | 15.21 (5.81-39.76) | <0.001 | - | |
| | Alleles | | | | | | |
| | G | 147 (69.3%) | 154 (96.3%) | 1 | - | G | |
| A | 34 (16.03%) | 6 (3.7%) | 5.94 (2.42-14.56) | 0.001 | - | | |
| rs2676530 | CC | 95 (89.6%) | 40 (50.0%) | 1 | - | C | |
| | CT | 10 (9.43%) | 39 (48.75%) | 0.11 (0.05-0.24) | <0.001 | - | |
| | TT | 1 (0.94%) | 1 (2.27%) | 0.42 (0.03-6.90) | 0.544 | - | |
| | CT + TT | 11 (10.37%) | 40 (50.0%) | 0.12 (0.05-0.25) | <0.001 | - | |
| | Alleles | | | | | | |
| | C | 191 (90.0%) | 81 (50.62%) | 1 | - | C | |
| T | 12 (5.7%) | 41 (25.62%) | 0.12 (0.06-0.25) | <0.001 | - | | |
| rs992310724 | GG | 99 (93.39%) | 79 (98.8%) | 1 | - | G | |
| | GA | 6 (5.6%) | 0 (0%) | 10.39 (0.58-187.19) | 0.112 | - | |
| | AA | 1 (0.94%) | 1 (1.25%) | 0.80 (0.05-12.96) | 0.873 | - | |
| | GA + AA | 7 (6.67%) | 1 (1.25%) | 5.59 (0.67-46.35) | 0.111 | - | |
| | Alleles | | | | | | |
| | G | 204 (96.2%) | 159 (99.4%) | 1 | - | G | |
| A | 8 (3.77%) | 2 (1.25%) | 3.12 (0.65-14.89) | 0.154 | - | | |

In addition, the potential associations between particular single-nucleotide polymorphisms (SNPs)(rs605059, rs992310724,

and 2676530) and testosterone levels in men were investigated. Analysis of the Independent sample t-test showed no significant association between the SNP rs605059 and testosterone levels in male participants, with a *p*-value of 0.783. There was a weak positive correlation with the SNP rs992310724, with a *p*-value of 0.041. Further analysis by using Pearson correlation showed a correlation coefficient of 0.1, indicating a very weak positive correlation. SNP 2676530 had no significant association with testosterone levels, with a *p*-value of 0.318. These findings suggest that polymorphisms in these SNPs do not affect testosterone function (Table 2).

Table 2: Association of Selected SNPs with Serum Testosterone Levels

| SNP | Allele (n) | Mean Testosterone (ng/mL) | <i>p</i> -Value | Pearson Correlation (r) |
|-------------|----------------|---------------------------|-----------------|-------------------------|
| rs605059 | G 147 (69.3%) | 1.140–7.850 | 0.783 | Not applicable* |
| | A 34 (16.03%) | | | |
| rs992310724 | G 204 (96.02%) | 1.140–7.850 | 0.041 | 0.1 |
| | A 8 (3.77%) | | | |
| rs2676530 | C 191 (90.0%) | 1.140–7.850 | 0.318 | Not applicable* |
| | T 12 (5.7%) | | | |

DISCUSSION

Frequency analysis of SNPs in the *HSD17B1* gene in male infertility is essential for understanding genetic factors influencing reproductive health. Variants in this gene are linked with changes in sex steroid hormone metabolism and potentially impair male fertility. Previous study reported that the *HSD17B1* gene is expressed in the testis, contributes to the synthesis of steroids, and is essential for male fertility. This study demonstrates that the *HSD17B1* gene may cause disturbances in the metabolism of sex steroid hormones, thereby affecting the health of male reproduction [19]. The ultimate goal of this study is to find new diagnostic and treatment strategies by expanding our understanding of these genetic factors. We analyzed data from participants with a mean age of 31.87±6.07 years (range 21–50 years) and a mean weight of 72.4±8.74 kg (range 60–118 kg). At rs605059, allele A was dominant (69.8%) and allele G was less common (30.8%), aligning with previous studies on *HSD17B1* in hormone metabolism and male reproductive health [20, 22]. Prior research has also investigated the rs605059 polymorphism in estrogen-dependent diseases (e.g., endometriosis, breast, prostate, endometrial, and uterine cancers), suggesting a potential link with increased enzyme activity. Previous studies indicated that this variant may not affect enzyme function in infertility or other contexts [23–29]. Following the rs605059 analysis, we examined rs2676530. Allele C predominated, found in 95 participants (89.6%), while allele T was observed in only 10 participants (9.43%), revealing notable allelic distribution changes in male infertility.

Previous studies found no association of this SNP with endometriosis across different ethnic groups and no effect on Alzheimer's disease [30, 31]. These inconsistent results highlight the disease-specific nature of genetic variants and the need for further investigations to clarify the role of rs2676530 in male infertility [32]. Additionally, we identified rs992310724 as a novel SNP in the context of male infertility. Analysis revealed that 99 participants (93.39%) carried the dominant allele G, while only 6 participants (5.6%) carried allele A. These results demonstrate the unique and significant allelic distribution of rs992310724 and provide a new direction for exploring the genetic causes of male infertility. We examined the correlation between SNPs and testosterone levels. Rs605059 (*p*=0.783) and rs2676530 (*p*=0.318) showed no statistically significant association, whereas rs992310724 (*p*=0.041) showed a weak positive correlation. Similar findings were reported by previous studies, concluding that *HSD17B1* polymorphisms do not significantly impact testosterone levels [20, 33, 34]. These data, along with our findings, illustrate the intricate nature of genetic contributions to male infertility and suggest that sample size and environmental factors may influence outcomes. The study had a modest sample size and an ethnically homogeneous population, limiting generalizability. Environmental and lifestyle factors were not fully controlled, and the borderline significance of rs992310724 requires cautious interpretation and further validation.

This study was limited by its modest sample size, single-center design, and ethnically homogeneous population, which may restrict generalizability of the findings. Additionally, environmental, lifestyle, and epigenetic factors influencing infertility were not comprehensively assessed, and the borderline significance observed for rs992310724 warrants cautious interpretation. Future studies should incorporate larger, multi-center populations, detailed exposure assessments, and functional molecular investigations to better clarify the biological mechanisms linking *HSD17B1* polymorphisms with male infertility.

CONCLUSIONS

In conclusion, the SNP rs992310724 showed a suggestive association with male infertility and testosterone levels; further research studies are needed due to its borderline *p*-value, and this SNP may be a potent target for precision or targeted medicine. However, neither SNP rs605059 nor SNP rs2676530 showed any significant relationship with testosterone levels. These findings emphasize the complexities of male infertility, highlighting the importance of ongoing research to fully comprehend the genetic and hormonal components that contribute to this condition.

Authors' Contribution

Conceptualization: MFK, HM, MI

Methodology: MFK, HM, MI, SSS, MA, SK

Formal analysis: SSS, MA, KUD, MI

Writing and Drafting: MFK, HM, KUD, MI

Review and Editing: MFK, HM, MI, SSS, MA, SK, KUD

All authors approved the final manuscript and take responsibilities for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article



Acceptance of Telemedicine in Healthcare Customers of District Karachi, Pakistan: A Cross-Sectional Analysis

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ARTICLE INFO

Keywords:

Telemedicine, Internet Browsing, Immunosuppression, Hospital-Acquired Infections, Healthcare Technology Adoption

How to Cite:

Ajaz, A., & Memon, S. I. (2025). Acceptance of Telemedicine in Healthcare Customers of District Karachi, Pakistan: A Cross-Sectional Analysis: Acceptance of Telemedicine in Healthcare Customers. *Pakistan BioMedical Journal*, 8(8), 23-28. <https://doi.org/10.54393/pbmj.v8i8.1210>

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Received Date: 21st January, 2025Revised Date: 8th August, 2025Acceptance Date: 19th August, 2025Published Date: 31st August, 2025

ABSTRACT

The global healthcare industry faced significant revenue and volume losses following the emergence of COVID-19 in December 2019. Telemedicine emerged as a potential solution to mitigate these challenges. **Objectives:** To determine the influence of internet browsing and immunosuppression on telemedicine acceptance and to evaluate the mediating role of fear of acquiring hospital-induced infections (HAI) in this relationship. **Methods:** A quantitative, cross-sectional study was conducted from April to September 2021. Data were collected using a validated online questionnaire distributed to patients, physicians, and health insurance providers in Karachi. The data were analyzed using SPSS version 24, employing correlation, regression, and mediation analysis. A p-value < 0.05 was considered statistically significant. **Results:** The study found that internet browsing significantly influenced telemedicine acceptance ($p < 0.01$), while immunosuppression did not ($p = 0.39$). The mediating role of fear of HAI was partially supported. The model's explanatory power was weak, with an R value of 0.29, indicating limited predictive capability. **Conclusions:** The findings suggested limited acceptance of telemedicine among Karachi's population, highlighting the need for targeted awareness campaigns and policy adjustments. While internet browsing positively influenced telemedicine acceptance, immunosuppression did not. The study underscored the importance of addressing technological and health-related barriers to improve telemedicine adoption.

INTRODUCTION

The emergence of Covid-19 On December 1, 2019 cases resulted in a decrease in healthcare revenue and volume globally. Numerous systems have profited from the increased use of "telehealth," synonymous with "telemedicine." Telemedicine is the quick electronic communication of medical information between clinical practice locations for treatment and education [1]. The pandemic encouraged a shift from in-person consultations to telehealth services in Karachi, Pakistan, and other regions to reduce virus transmission risks. This shift has shown the potential of telemedicine to improve healthcare access but also raised questions about customer acceptance [2]. In addition, studies reveal that the perception of telemedicine has predominantly been

favorable, with several patients reporting satisfaction with their telehealth experiences during the pandemic [3, 4]. Factors affecting its acceptability encompass perceived advantages, usability, and the immediacy of healthcare need throughout the crisis [5]. Comprehending the dynamics of telemedicine adoption in Karachi is essential for guiding future healthcare policies and practices, especially as the healthcare environment evolves in the post-pandemic era [6]. The biggest problems in the health care system are creating telemedicine policies, licensing and accrediting doctors who practice telehealth, strengthening the information system, ensuring patients can access and understand technology, being at ease with it, and having a good relationship with their provider and



the doctors that practice telemedicine struggle with time management, team building, professional health, and medical education [7]. The majority of studies examine social influence, effort expectancy, and enabling situations, with perceived usefulness (PU) and PEU mediating [8]. The study is grounded in the Technology. Internet browsing was chosen as a determinant due to its role in facilitating access to telemedicine services, while immunosuppression was selected to explore the impact of health vulnerabilities on telemedicine acceptance. Thereby, this study aims to fill this gap by examining the impact of these variables on telemedicine acceptance among healthcare customers in Karachi, Pakistan. These findings can assist both public and private healthcare organizations in Pakistan in making informed decisions about the implementation of telemedicine projects. This, in turn, can inform health policymakers about the role of telemedicine as an initiative to enhance the country's healthcare sector [9-12].

Telemedicine adoption in Karachi remains inadequately understood, particularly regarding how technological exposure and health vulnerability influence acceptance in a post-COVID healthcare landscape. Despite growing digital transformation, limited empirical evidence exists on the predictors of telemedicine acceptance and the psychological mechanisms underlying its use. The present study evaluated the dependent variable, acceptance of telemedicine, against the independent variables: internet browsing and immunosuppression. It was also identified the impact of the mediating variable, fear of acquiring HAI, on the relationship between immunosuppression and acceptance of telemedicine [13].

METHODS

This study employed a quantitative, cross-sectional design with a deductive approach (April-September, 2021). The target population included patients, physicians, and health insurance providers in Karachi, with a sample size of 384 respondents. The sample size was determined using a 95% confidence level and a 5% margin of error, based on the population of Karachi (16,459,000). Convenience sampling was used due to time constraints and accessibility. Data were collected using an online questionnaire adapted from validated instruments, including the COVID-19 Fear Scale, the Prevalence of Immunosuppression Scale, and the Telemedicine Acceptance Scale. The questionnaire was distributed via Google Forms, and responses were recorded in Excel before being imported into SPSS for analysis. The data were analyzed using SPSS version 24.0. Descriptive statistics, correlation, regression, and mediation analysis were performed. Cronbach's alpha was used to assess the reliability of the scales, with values

above 0.7 considered acceptable. The mediation analysis was conducted using Hayes' Process macro.

RESULTS

Table 1 highlighted the demographic characteristics of respondents. The sample consists of 384 respondents, with a nearly equal gender distribution (47.4% male, 52.6% female). The majority fall within the 21-35 age group (57%), followed by 36-50 years (21.9%) and 51-65 years (14.6%). Most respondents have a graduate degree (41.1%) and belong to the middle class (44.3%) or upper-middle class (43.2%). These demographic variables help contextualize the study's findings regarding telemedicine acceptance.

Table 1: Demographic Characteristics of Respondents (N=384)

| Characteristics | Frequency (%) |
|-----------------------------|---------------|
| Age Group | |
| 21-35 Years | 219 (57.0%) |
| 36-50 Years | 84 (21.9%) |
| 51-65 Years | 56 (14.6%) |
| Gender | |
| Male | 182 (47.4%) |
| Female | 201 (52.6%) |
| Education Level | |
| Graduate Degree | 158 (41.1%) |
| College Degree | 82 (21.4%) |
| High School | 59 (15.4%) |
| Socioeconomic Status | |
| Middle Class | 170 (44.3%) |
| Upper Middle Class | 165 (43.2%) |

Table 2 revealed the descriptive statistics of constructs in which the mean scores and standard deviations indicate moderate levels of Internet Browsing (Mean = 2.90, SD = 0.78), Immunosuppression (Mean = 2.03, SD = 1.14), and Telemedicine Acceptance (Mean = 2.81, SD = 0.79). Fear of Healthcare-Associated Infections (HAI) shows a relatively higher mean score (3.64, SD = 1.10), suggesting a notable concern among respondents. These values provide an overview of the distribution of key variables used in the regression and mediation analyses.

Table 2: Descriptive Statistics of Constructs

| Variables | Mean ± SD | Range |
|------------------------------|-------------|-------------|
| Internet Browsing (IV1) | 2.90 ± 0.78 | 1.00 - 4.70 |
| Immunosuppression (IV2) | 2.03 ± 1.14 | 1.00 - 5.00 |
| Fear of HAI (Mediator) | 3.64 ± 1.10 | 1.00 - 5.00 |
| Telemedicine Acceptance (DV) | 2.81 ± 0.79 | 0.86 - 5.00 |

Table 3 the regression model showed a weak relationship ($R = 0.293$) and a low explanatory power ($R^2 = 0.086$, Adjusted $R^2 = 0.081$). This suggests that only 8.6% of the variance in telemedicine acceptance is explained by Internet Browsing and Immunosuppression. The small R^2 value indicates that other unmeasured factors may influence telemedicine

acceptance, which is acknowledged as a limitation in the discussion.

Table 3: Model Summary(Regression Analysis)

| Model | R | R ² | Adjusted R ² | Standard Error |
|-------|-------|----------------|-------------------------|----------------|
| 1 | 0.293 | 0.086 | 0.081 | 0.754 |

Table 4 indicated the ANOVA results confirm that the overall regression model is statistically significant (F = 17.740, p < 0.001), indicating that at least one of the predictor variables significantly contributes to explaining variations in Telemedicine Acceptance. However, despite statistical significance, the low R² suggests the need for additional predictors to improve the model's explanatory power.

Table 4: ANOVA Results

| Model | Sum of Squares | df | Mean Square | F | p-Value |
|------------|----------------|-----|-------------|--------|---------|
| Regression | 20.151 | 2 | 10.075 | 17.740 | <0.001 |
| Residual | 215.246 | 379 | 0.568 | - | |
| Total | 235.397 | 381 | - | - | |

Table 5 showed Internet Browsing has a significant positive effect on Telemedicine Acceptance (B = 0.285, p < 0.001), suggesting that increased browsing is associated with higher acceptance. In contrast, Immunosuppression has a non-significant effect (B = -0.029, p = 0.390), implying that it does not significantly influence telemedicine acceptance. The confidence intervals confirm the significance of Internet Browsing while showing that Immunosuppression's effect crosses zero, reinforcing its non-significance.

Table 5: Regression Coefficients with 95% Confidence Intervals

| Variables | B | Standard Error | β | t | p-Value | 95% CI (Lower-Upper) |
|--------------------------|--------|----------------|--------|--------|---------|----------------------|
| Constant | 2.045 | 0.173 | - | 11.849 | <0.001 | 1.706 - 2.384 |
| Internet Browsing (IV1) | 0.285 | 0.050 | 0.284 | 5.726 | <0.001 | 0.187 - 0.383 |
| Immuno-suppression (IV2) | -0.029 | 0.034 | -0.043 | -0.861 | 0.390 | -0.096 - 0.038 |

V1: Internet Browsing; IV2: Immunosuppression; DV: Telemedicine Acceptance. Confidence intervals(CI) derived from 5,000 bootstrap samples. Bolded values indicate statistical significance(p<0.05).

Table 6 revealed the mediation analysis and it suggests that Immunosuppression does not significantly predict Fear of HAI (B = 0.060, p = 0.224). However, Fear of HAI significantly influences Telemedicine Acceptance (B = 0.116, p = 0.001). The indirect effect of Immunosuppression on Telemedicine Acceptance via Fear of HAI is not significant (B = 0.007, 95% CI = -0.005 to 0.023), indicating that Fear of HAI does not mediate the relationship between Immunosuppression and Telemedicine Acceptance.

Table 6: Mediation Analysis Results(Fear of HAI)

| Path | Effect | Boot SE | 95% CI (Lower - Upper) | p-Value |
|------------------------------|--------|---------|------------------------|---------|
| IV2 → Mediator (Fear of HAI) | 0.060 | 0.050 | -0.037 - 0.158 | 0.224 |
| Mediator → DV (Telemedicine) | 0.116 | 0.036 | 0.045 - 0.186 | 0.001 |
| Indirect Effect | 0.007 | 0.007 | -0.005 - 0.023 | - |

Confidence intervals(CI) derived from 5,000 bootstrap samples

DISCUSSION

The current study findings highlight the significant role of internet browsing in telemedicine acceptance, consistent with prior research on technology adoption frameworks such as the Technology Acceptance Model(TAM) and Social Cognitive Theory. These theories emphasize that familiarity with technology, as facilitated by frequent internet use, enhances perceived ease of use and usefulness, thereby driving acceptance [14, 15]. For instance, younger populations (e.g., Generation Y and Z), who are more tech-savvy, demonstrated higher telemedicine adoption rates, aligning with global trends where digital literacy correlates with telehealth utilization [16, 17]. Contrary to expectations, immunosuppression did not significantly influence telemedicine acceptance (p = 0.39). This suggests that health vulnerabilities alone may not drive adoption, potentially due to cultural preferences for in-person consultations or distrust in remote diagnostics among immunocompromised patients [5]. However, the partial mediation effect of fear of hospital-acquired infections (HAI) indicates that while immunosuppression itself is not a direct predictor, the psychological fear of infection in clinical settings may indirectly encourage telemedicine use. This aligns with studies showing that perceived health risks during pandemics amplify reliance on telehealth [6, 7]. The present study is critically necessary at that time when COVID-19 lockdown restrictions began to relax, prompting healthcare regulators and professionals to seek a healthcare plan to mitigate the impact of future pandemics. This study found a low R value attributable to the negligible correlation between immunosuppression and the absence of a mediating effect of fear of getting HAI on the adoption of telemedicine [9]. This has facilitated opportunities for future researchers to include new variables to enhance the R value. The acceptability of telemedicine among healthcare consumers in Karachi during the COVID-19 pandemic can be ascribed to various interconnected reasons. The pandemic's urgency facilitated the swift development of telemedicine services. Research indicates that patients were predominantly content with telemedicine, highlighting advantages such as convenience, time efficiency, and less risk of infection [10, 11]. A recent survey has revealed that a substantial majority of participants deemed telemedicine beneficial

during the epidemic with numerous individuals indicating a preference to persist with these services moving forward [2]. This corresponds with data from other regions, where patient satisfaction with telemedicine has been reported as high, especially when patients could connect successfully with their healthcare practitioners [12, 13]. Nonetheless, despite the favorable welcome, some challenges persist that may impede the long-term adoption of telemedicine in Karachi with inadequate technology infrastructure, insufficient training of healthcare personnel, and apprehensions about privacy and data security have been recognized as substantial obstacles [18]. A thorough assessment indicated that although telemedicine services were broadly accepted, their implementation encountered challenges associated with technical issues and regulatory frameworks [19]. The present study has tackled critical obstacles to facilitate the efficient integration of telemedicine into the healthcare system in Karachi and abroad. Furthermore, the significance of healthcare practitioners in promoting telemedicine acceptability is paramount. Their thoughts and attitudes around telemedicine substantially affect patient acceptability. Research indicates that healthcare providers that are adequately trained and supportive of telemedicine are more inclined to promote its utilization among patients [20, 21]. Thereby, enhancing provider education and addressing their concerns regarding telemedicine can lead to improved patient outcomes and satisfaction. The present study also bridges the gap that existed in prior researches from the perspective of the independent variables and mediating variables. Another advantage of this study is that it critically evaluates the customer's preferences and inclination towards telemedicine and telehealth in order to align independent organizational efforts in quality care delivery and growth of healthcare industry. The research is helpful for health legislative bodies in making policies for telemedicine and telehealth keeping in view the customer behavior and priorities. This study facilitates the Federal and Provincial Government of Pakistan to make discretion regarding allocation of budget for restructuring telehealth in Pakistan. The most prominent limitation of this study is it is quantitative study and it introduces biasness in responses since respondents have to choose any option from the fixed set of answers. In addition, it is a cross sectional research that studies a population at one point of time. The scope of this study is limited to Karachi City, only leaving behind a huge chunk on population of Pakistan and the other countries. The usage of simple random sampling technique has narrowed the scope of the study. The effect of predictors, outcome and mediator is checked but the effect of moderator is not studied in this research. Lastly, this research is conducted at the student level with in

duration of four months only. First of all, it is imperative for the future studies to incorporate new independent variables to increase the R value of the research. Since, the role of mediation proved partial in this study, and future researchers could study the mediator as predictor to check its complete effect on acceptance of telemedicine, as well as introduce a moderator in the study. Further, this research could be done in countries where the scope of telemedicine is still emerging. Lastly, the worthy researchers may conduct this research as longitudinal design and could use systematic- random method of sampling.

The study was limited by its cross-sectional design, convenience sampling, and focus on Karachi, which restrict generalizability and causal inference. The low explanatory power ($R^2 = 0.086$) suggests that other influential variables were not captured, and self-reported responses may introduce bias. Further, this research could be done in countries where the scope of telemedicine is still emerging. Lastly, the worthy researchers may conduct this research as longitudinal design and could use systematic- random method of sampling.

CONCLUSIONS

This study highlighted that internet browsing significantly influences telemedicine acceptance, while immunosuppression does not. Fear of healthcare-associated infections (HAI) did not mediate this relationship, suggesting that telemedicine adoption is more technology-driven than health-risk motivated. Despite the model's statistical significance, its low explanatory power ($R^2 = 0.086$) indicates that additional factors influence telemedicine acceptance.

Authors' Contribution

Conceptualization: AA, SIM

Methodology: AA, SIM

Formal analysis: AA, SIM

Writing and Drafting: AA, SIM

Review and Editing: AA, SIM

All authors approved the final manuscript and take responsibilities for the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article



Resurgent Measles in Pakistan: A 5-Year Analysis of Vaccination Gaps, Surveillance Challenges, and Urban-Rural Disparities in Khyber Pakhtunkhwa

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ARTICLE INFO

Keywords:

Measles Surveillance, Zero-Dose Children, Vaccination Coverage, Disease Elimination, Health Systems Strengthening

How to Cite:Nasir, A., Ullah, W., Izaz, M., & Islam, Z. U. (2025). Resurgent Measles in Pakistan: A 5-Year Analysis of Vaccination Gaps, Surveillance Challenges, and Urban-Rural Disparities in Khyber Pakhtunkhwa: A 5-Year Analysis of Vaccination Gaps, Surveillance Challenges, and Urban-Rural. *Pakistan BioMedical Journal*, 8(8), 29-35. <https://doi.org/10.54393/pbmj.v8i8.1238>***Corresponding Author:**

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ABSTRACT

Measles remains a substantial public health concern in low and middle-income countries.

Objectives: This study aimed to provide a comprehensive analysis of measles surveillance data from Khyber Pakhtunkhwa (KP) province in Pakistan from 2020 to 2024, examining epidemiological trends, vaccination coverage, and the effectiveness of the surveillance system.**Methods:** This retrospective descriptive study conducted a thorough surveillance analysis using several data sources, including weekly vaccine-preventable disease (VPD) reports, zero reports, and standardized case investigation forms provided to the EPI monitoring information system (EPI-MIS). For statistical analysis, Stata 17.0 and R 4.2.1 software programs were used.**Results:** 82% of cases comprised unvaccinated children (zero-dose) aged 6-59 months, suggesting severe gaps in regular vaccination. Laboratory testing verified 5,550 (39%) measles cases, whereas 6,398 (44%) were rejected, with a non-measles/non-rubella discard rate of 19.4/100,000, indicating a better differential diagnosis. The surveillance system achieved high performance indicators, with 97% district reporting completeness and 87% timeliness. However, case investigation adequacy decreased to 65% in 2024 ($p < 0.001$), especially in low-compliance districts like North Waziristan and Peshawar. Urban centers administered fewer booster doses (Peshawar: 0) than rural areas (Charsadda: 21,155 doses; $p < 0.001$). While specimen collection matched WHO standards (88%), South Waziristan's low rates indicated regional diagnostic shortcomings. **Conclusions:** The study concluded the critical need for tailored vaccination programs in high-risk areas, improved real-time surveillance, and health system improvement in KP to achieve measles control.

INTRODUCTION

Measles remains a substantial public health concern in low and middle-income countries (LMICs), despite the availability of an effective vaccine and global attempts to eradicate it [1]. Measles is a highly infectious viral illness that can cause serious consequences such as pneumonia, encephalitis, and death, especially in unvaccinated children under the age of five [2]. The World Health Organization (WHO) recommends at least 95% coverage with two doses of the measles-containing vaccine (MCV1 and MCV2) to achieve herd immunity and avoid outbreaks

[3]. However, several places, particularly Pakistan's Khyber Pakhtunkhwa (KP) province, continue to experience recurring outbreaks due to low vaccination rates, gaps in surveillance, and logistical obstacles in vaccine distribution [4]. Measles is still common in Pakistan, with sporadic outbreaks linked to inadequate immunization rates, relocation brought on by conflict, and inadequate healthcare facilities [5]. The province of Khyber Pakhtunkhwa, which borders Afghanistan, has particular obstacles, such as vaccination reluctance, poor cold chain



management, and trouble reaching isolated communities [6]. In Pakistan, the Expanded Program on Immunization (EPI) uses a surveillance system that uses laboratory confirmation and case-based reporting to monitor vaccine-preventable illnesses (VPDs), including measles [7]. Effective epidemic response is hampered, therefore, by disparities in vaccination coverage among districts, delays in case notification, and inconsistent data quality [8]. Recent epidemiological statistics from KP show a disturbing increase in measles infections, with incidence rates rising from 28 per million people in 2020 to 135 per million in 2024, considerably above the WHO's eradication objective of less than 5 cases per million [9]. Alarming, 82% of these cases are reported in zero-dose children (those who have not received measles immunization), with the majority (82%) occurring between the ages of 6 and 59 months [10]. This pattern indicates major deficiencies in routine vaccination programs, emphasizing the need for specialized interventions in high-risk regions. Furthermore, discrepancies in urban and rural vaccination coverage hinder epidemic containment, with urban centers like Peshawar reporting shockingly low booster dose delivery when compared to rural locations like Charsadda and Bajaur [11]. The performance of surveillance systems is also important in the control of measles. While KP has excellent reporting completeness (95%) and timeliness (80%) in weekly VPD monitoring, the adequacy of case investigations has decreased to 65% in 2024, indicating possible gaps in data accuracy and epidemiological follow-up [12]. Furthermore, laboratory confirmation rates vary by area, with South Waziristan reporting particularly poor specimen collection rates, which may result in underreporting or misclassification of cases [13]. The non-measles, non-rubella (NMNR) discard rate of 19.4 per 100,000 population, while indicating improved differential diagnosis, might potentially represent errors in case criteria or insufficient physician training in measles detection [14, 15].

The persistent resurgence of measles in Khyber Pakhtunkhwa reflects substantial gaps in routine immunization coverage, particularly among zero-dose children, alongside declining case investigation adequacy despite high reporting completeness. Urban-rural disparities, weak booster dose uptake in metropolitan districts, and inconsistent surveillance quality further hinder progress toward elimination targets. Strengthening targeted outreach for zero-dose populations, enhancing real-time digital surveillance integration within the EPI-MIS framework, and improving cold chain and district-level accountability mechanisms are urgently recommended. Focused interventions in high-risk districts such as Peshawar and border regions should be prioritized to achieve sustainable measles control. This study aims to provide evidence-based suggestions for boosting the

region's measles control efforts by identifying high-risk districts, reviewing vaccine gaps, and investigating monitoring deficiencies. The findings may help to inform continuing conversations about improving EPI methods in LMICs, particularly in conflict-affected and hard-to-reach areas where measles poses a chronic danger to child health.

METHODS

A retrospective surveillance data analysis was carried out using all available records from the EPI Monitoring Information System (EPI-MIS). The study used a descriptive and analytical epidemiological approach to evaluate trends in measles incidence, vaccination coverage, and surveillance effectiveness. Data on suspected, clinically verified, and laboratory-tested measles cases, as well as vaccination records, were collected from 1,851 health institutions throughout KP. The study was carried out in Khyber Pakhtunkhwa (KP), a region in Northwest Pakistan from July 2024 to December 2024, with 37 districts and a mixed urban and rural population. Disparities in healthcare access, relocation brought on by conflict, and low vaccination rates have all contributed to the region's repeated measles epidemics. With an emphasis on measles case reporting, vaccination records, and laboratory-confirmed diagnoses from 2020 to 2024, data were gathered from all districts taking part in the Expanded Program on Immunization (EPI) surveillance system. The ethical board does not need to conduct a thorough review of the study because the data did not include any patient-identifiable information. The data holders provided all necessary consents and permissions before the commencement of the study. The study conducted a thorough surveillance analysis using several data sources, including weekly vaccine-preventable disease (VPD) reports, zero reports, and standardized case investigation forms provided to the EPI Monitoring Information System (EPI-MIS). The collected data included key variables such as demographic characteristics (age, gender, and district of residence), vaccination status (categorized as zero-dose, MCV1, or MCV2 recipients), laboratory confirmation results (IgM-positive cases and discarded negative cases), and critical surveillance performance indicators (including reporting timeliness, data completeness, and specimen collection rates). For statistical analysis, Stata 17.0 and R 4.2.1 software programs were used. The analytical technique used descriptive statistics to calculate frequencies, proportions, and population-based incidence rates, resulting in a basic understanding of measles distribution patterns. Temporal trends were analyzed using linear regression modeling and Cochran-Armitage tests, while district-level differences in vaccination coverage were examined using Chi-square and Fisher's exact tests to uncover statistically significant discrepancies.

Throughout all studies, a preset alpha level of 0.05 was used to evaluate statistical significance. Advanced visualization tools, such as heat maps and epidemic curves, were used to highlight spatiotemporal patterns of disease transmission. These were supplemented with logistic regression models that identified important risk variables linked with zero-dose measles cases in the community (Figure 1).

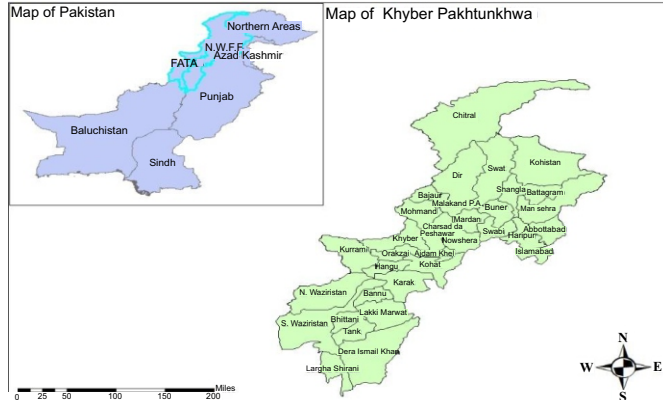


Figure 1: Study Area Map Showing Sampling Districts of Khyber Pakhtunkhwa

RESULTS

Measles surveillance statistics from Khyber Pakhtunkhwa (KP) for the reporting period showed key trends in case incidence, vaccination coverage, and system efficiency. Out of 37 districts, 36 (97%) reported surveillance data to the EPI-Monitoring Information System (EPI-MIS), indicating excellent reporting compliance. Furthermore, 95% of health institutions (1,763 out of 1,851) provided zero reports, demonstrating strong engagement in measles monitoring. A total of 18,075 probable measles cases were recorded, with 14,366 (82%) line-listed, indicating extensive case recording. In EPID Week 47 alone, 220 suspected cases were recorded, indicating that transmission is ongoing (Figure 3.1). A concerning rise in vaccination coverage was noted, with 82% of measles-infected cases being zero-dose children (unvaccinated) over the age of nine months, with the majority (82%) falling into the 6-59-month age category. Laboratory tests confirmed 5,550 (39%) cases as measles positive, while 6,398 (44%) were ruled out as negative. The surveillance system achieved 95% completeness and 80% timeliness in weekly VPD Zero reporting, with 35 out of 37 districts (95%) meeting the 80% completeness threshold. However, Kurram Lower and Central, North Waziristan, Palas Kohat, Peshawar, Dir-Upper, Hangu, and Kolai were designated as low-compliance areas. Cumulative data from Weeks 1-47 showed 97% completeness and 87% timeliness in VPD reporting, whereas AEFI surveillance reached 95% completion and 82% timeliness (Figure 2).

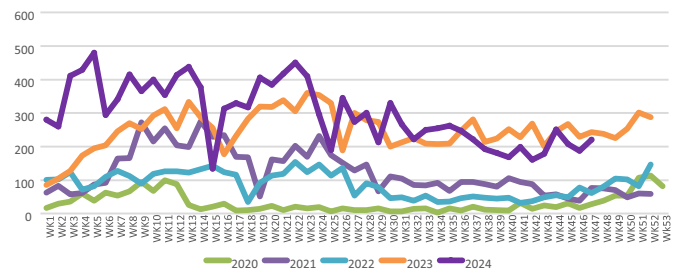


Figure 2: Weekly Trend of Measles Cases from 2020 to 2024

Measles incidence fluctuated, with 17,717 suspected cases reported between 2020 and 2024. The highest occurrence was in 2023 (12,675 cases), with a slight fall in 2024 (14,366 cases by Week 47) (Table 1).

Table 1: Measles Surveillance Performance Indicators (2020–2024)

| Indicators | Target | 2020 | 2021 | 2022 | 2023 | 2024 (Wk 1-47) | p-value (Trend Analysis) |
|------------------------------|--------|-------|-------|-------|--------|----------------|--------------------------|
| Suspected Measles Cases | - | 1,717 | 6,403 | 4,492 | 12,885 | 14,366 | <0.001 (↑) |
| Lab-Confirmed Measles Cases | - | 909 | 2,996 | 1,997 | 5,482 | 5,550 | <0.001 (↑) |
| Measles Incidence Rate (/M) | <5 | 28 | 101 | 56 | 118 | 135 | <0.001 (↑) |
| Rubella Incidence Rate (/M) | <5 | 1 | 1 | 2 | 1.7 | 0.6 | 0.112 (NS) |
| NMNR Discard Rate (/100K) | 2 | 3 | 9 | 6 | 15.2 | 19.4 | <0.001 (↑) |
| Specimen Collection Rate (%) | >80% | 89% | 97% | 95% | 99% | 88% | 0.003 (↓ in 2024) |
| Adequate Investigation (%) | >80% | 82% | 92% | 93% | 94% | 65% | <0.001 (↓) |

The measles incidence rate per million population increased from 28 in 2020 to 135 in 2024, well above the WHO objective of <5 cases per million. In contrast, rubella incidence remained low (0.6 per million), while the non-measles, non-rubella (NMNR) discard rate increased to 19.4 per 100,000 population, showing improvement (Figure 3).

District Wise Measles Suspected Cases
Khyber Pakhtunkhwa

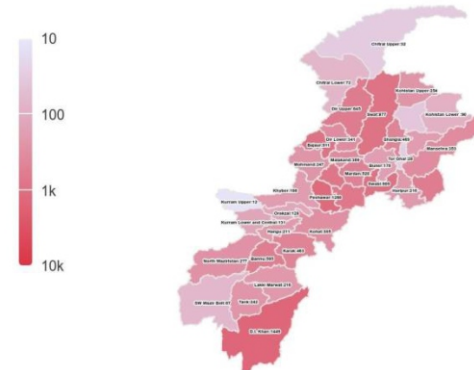


Figure 3: A Choropleth Map of Measles Cases in Khyber Pakhtunkhwa

Vaccination efforts varied by district, with a total of 67,643 measles booster doses delivered. Charsadda had the highest MSL booster coverage (21,155 doses), followed by Bajaur (9,708) and Kohistan Upper (7,682) (Table 2).

Table 2: District-Wise Vaccination Coverage of Measles in Khyber Pakhtunkhwa

| Districts | BCG | Penta -3 | MSL -1 | MSL -2 | MSL Booster | Coverage Gap (%) | p-value (vs.Target) |
|------------------|-----|----------|--------|--------|-------------|------------------|---------------------|
| Charsadda | 118 | 503 | 831 | 774 | 21,155 | 12% | |
| Bajaur | 0 | 0 | 203 | 0 | 9,708 | 28% | |
| Kohistan Upper | 0 | 140 | 288 | 196 | 7,682 | 19% | |
| Peshawar | 3 | 4 | 42 | 108 | 0 | 45% | |
| North Waziristan | 170 | 205 | 1,870 | 1,601 | 318 | 23% | |

However, variations were observed, with Peshawar, Nowshera, and Malakand reporting low doses, indicating poor immunization drives in urban areas (Figure 4).

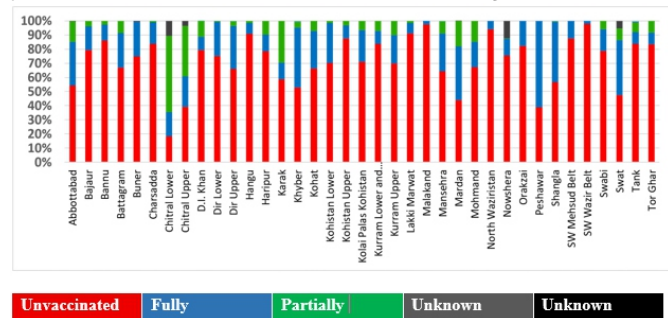


Figure 4: District-wise Vaccination Status of Suspected Cases. An epidemiological examination of measles surveillance data from Khyber Pakhtunkhwa (KP) suggests a disturbing rising trend in disease incidence, with 135 confirmed cases per million population in 2024, greatly exceeding the WHO-recommended threshold of <5 cases per million. This increase is mostly due to a high proportion of zero-dose children (82%) remaining unvaccinated against measles, as well as inadequate coverage of MCV1 and MCV2 doses across numerous districts. The majority of cases in the 6-59-month age group (82%) highlight a critical gap in routine immunization efforts, particularly among groups with poor healthcare access. Furthermore, the continually high incidence rate—a fourfold increase since 2020 (28 to 135 per million) indicates systemic inadequacies in achieving herd immunity, prompting an immediate review of vaccination policies and outreach initiatives. Despite high reporting completeness (95% in district-level monitoring), the adequacy of case investigations has dropped to 65% by 2024, showing significant gaps in data quality and epidemiological follow-up. This weakness is especially visible in low-compliance districts (such as Kurram Lower and Central, North Waziristan, and Peshawar), where delayed case verification and poor documentation impede effective outbreak response.

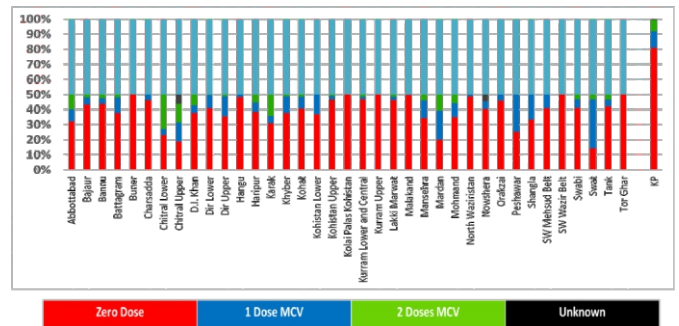


Figure 5: District-Wise Vaccination Status of Lab-Confirmed Measles Cases

A severe urban-rural difference in vaccination coverage exacerbates transmission dynamics, with urban areas (Peshawar, Nowshera) reporting dangerously low booster dose administration, whilst rural districts (Charsadda, Bajaur, Kohistan Upper) show higher but inconsistent uptake. Charsadda delivered 21,155 measles booster doses, whereas Peshawar received nil booster vaccines, highlighting inequitable resource allocation and logistical issues in metropolitan areas. Despite higher vaccination rates in rural areas, prolonged outbreaks suggest either cold chain issues, inadequate vaccine efficacy, or substantial population mobility, all of which contribute to long-term transmission (Figure 5).

DISCUSSION

The results of this extensive analysis of measles surveillance data in Khyber Pakhtunkhwa (KP) demonstrate serious gaps in vaccination coverage, surveillance performance, and outbreak response, necessitating immediate public health interventions. The rising measles incidence (135 cases per million in 2024) exceeds the WHO criterion of <5 cases per million, aligning with global trends of diminishing immunization rates leading to measles resurgence in previously controlled regions [16,17]. 82% of cases in zero-dose children highlight a fundamental failure in routine immunization regimens, which is consistent with research associating measles outbreaks with unvaccinated populations [18]. This tendency is especially concerning given that 82% of cases were among children aged 6-59 months, a group that is very susceptible to serious sequelae such as pneumonia and encephalitis [19]. The high reporting completeness (95%) and timeliness of 80% in weekly VPD Zero reporting imply a solid surveillance infrastructure, comparable to systems in other low- and middle-income countries (LMICs) with strong EPI frameworks [20]. However, the fall in case investigation adequacy (65% in 2024) indicates declining data quality, which could be related to personnel shortages or insufficient training, as shown in similar contexts [21]. The awaiting cross-notification of 34 diphtheria cases reveals systemic inefficiencies in inter-district cooperation, which mirrors issues seen during Pakistan's polio eradication

efforts [22]. Disparities in healthcare access are reflected in the glaring differences in vaccination coverage between urban and rural districts. Peshawar recorded nil booster vaccinations, whereas Charsadda and Bajaur reported substantial booster doses (21,155 and 9,708, respectively). This is probably because of vaccine hesitancy, logistical challenges, or conflicting urban health goals [23]. This is consistent with research demonstrating that mobile populations, disinformation, and inadequate communication initiatives are frequently the cause of urban vaccination gaps [24]. Ironically, ongoing outbreaks despite greater vaccination rates in remote areas point to either increased population mobility, poor vaccine efficacy, or cold chain breakdowns, as observed in Afghanistan's border regions [25]. Although South Waziristan's low rates suggest regional diagnostic problems and may result in underreporting, the 88% specimen collection rate satisfies WHO criteria [26]. Due to vague case definitions, which are also a problem in Nigeria's measles monitoring, the high NMNR discard rate (19.4/100,000) may indicate either improved differential diagnosis or over-reporting [27]. Refining case confirmation requires strengthening laboratory networks and training clinicians in measles detection [28].

The persistent resurgence of measles in Khyber Pakhtunkhwa reflects substantial gaps in routine immunization coverage, particularly among zero-dose children, alongside declining case investigation adequacy despite high reporting completeness. Urban-rural disparities, weak booster dose uptake in metropolitan districts, and inconsistent surveillance quality further hinder progress toward elimination targets. Strengthening targeted outreach for zero-dose populations, enhancing real-time digital surveillance integration within the EPI-MIS framework, and improving cold chain and district-level accountability mechanisms are urgently recommended. Focused interventions in high-risk districts such as Peshawar and border regions should be prioritized to achieve sustainable measles control. This study was limited by its retrospective design and reliance on secondary surveillance data, which may be subject to underreporting, misclassification, and variability in district-level data quality. The absence of individual-level socioeconomic, mobility, and vaccine hesitancy data restricted deeper causal interpretation of zero-dose prevalence. Future research should incorporate prospective, mixed-method approaches, geospatial risk modeling, and community-based assessments to better understand barriers to vaccination and strengthen outbreak preparedness strategies across KP.

CONCLUSIONS

The findings of this study show a critical resurgence of measles in Khyber Pakhtunkhwa, which is primarily caused by inadequate vaccination coverage among zero-dose children, as well as systemic inadequacies in surveillance and outbreak response. Despite high reporting completeness, decreased case investigation adequacy, and urban-rural vaccination disparities, particularly in regions like Peshawar without booster doses, there are continuing gaps in measles management. The fourfold increase in incidence (2020-2024) highlights the critical need for targeted interventions. Addressing cold chain issues and increasing diagnostic capacity in underperforming locations (such as South Waziristan) is also critical. A multi-sectoral approach that includes digital surveillance and equitable resource allocation is required to meet measles elimination targets and safeguard vulnerable populations in KP and similar settings.

Authors' Contribution

Conceptualization: AN, WU

Methodology: AN, WU, MI

Formal analysis: MI

Writing and Drafting: AN, WU, ZUI

Review and Editing: AN, WU, MI, ZUI

All authors approved the final manuscript and take responsibilities of the integrity of the work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article



Assessment of Post-COVID-19 Complications in the Pakistani Population: A Detailed Survey into Late-Onset Adverse Events through Cross-Sectional Analysis

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ARTICLE INFO

Keywords:

Covid-19 Pandemic, Vaccination, Adverse Effects, Immunity

How to Cite:

Madni, A. U. H., Hassan, N., Hameed, S., Satti, M. N., Ullah, R. H., Adnan, H., Mushtaq, M. F., & Faridi, T. A. (2025). Assessment of Post-COVID-19 Complications in the Pakistani Population: A Detailed Survey into Late-Onset Adverse Events through Cross-Sectional Analysis: Post-COVID-19 Complications in the Pakistani Population: Late-Onset Adverse Events. *Pakistan BioMedical Journal*, 8(8), 36-44. <https://doi.org/10.54393/pbmj.v8i8.1299>

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Received Date: 18th June, 2025

Revised Date: 19th August, 2025

Acceptance Date: 25th August, 2025

Published Date: 31st August, 2025

ABSTRACT

COVID-19 was a health crisis that severely obstructed the healthcare system and public well-being. Although vaccination has been very instrumental in reducing mortality and morbidity due to COVID-19, concerns are still prevalent regarding its short- and long-term side effects.

Objectives: To investigate the incidence and severity of the pandemic, which vary considerably across different demographic groups, especially in older age. **Methods:** The current descriptive exploratory study examines post-vaccination effects among various age and gender groups in Pakistan through an online survey of 220 individuals. **Results:** Nearly 24% of respondents have suffered from COVID-19 during the pandemic, with a high prevalence in older age. A vast majority, i.e., 92% were vaccinated for COVID-19. Results show that 47 percent of respondents have been affected by one or more kinds of post-vaccination effects. Segregating results, 28 percent faced neurological effects, 16% cardiovascular effects, 11% bleeding complications, 11% gastrointestinal effects, and 18% allergic reactions. Among female, 22% reported menstrual changes after receiving the vaccination for COVID-19. Two deaths were also reported among the respondents temporally following vaccination; however, the small number and the study design preclude any causal inference with multiple post-vaccination effects.

Conclusions: Pre-existing health conditions, experiencing surgery, and growing age were significantly associated with a higher likelihood of reporting post-vaccination complications. This study plays an important part in tailoring future vaccination approaches and plans, eventually assisting Pakistan's long-term public health preparedness.

INTRODUCTION

COVID-19 became a health crisis in late 2019 in Wuhan, China, due to the spread of the SARS-CoV-2 virus. It has caused more than 770 million confirmed cases and above 7 million deaths globally [1]. In Pakistan, over 1.58 million cases were confirmed, and more than 30,000 deaths were reported due to the numerous waves of the pandemic. It

severely obstructed the healthcare system and public well-being. The morbidity rates were high, especially amongst persons who had pre-existing conditions. The epidemic likewise uncovered weaknesses in the healthcare setup, predominantly in rural and neglected populations. As a countermeasure, the Government of Pakistan executed

mitigation plans including lockdowns, measures for social distancing, and campaigns for mass vaccination to contain the virus's spread and lessen mortality rates. In this regard, Pakistan started its national vaccination program in early 2021, arranging a variety of vaccines that were WHO-approved. They included Sinopharm, Sinovac, AstraZeneca, Pfizer, and Moderna [2]. The execution of the vaccination campaign greatly helped in controlling the spread and severity. The existing literature highlights that the incidence and severity of the pandemic vary considerably across different demographic groups. The studies also reflect that older persons with diabetes, cardiovascular diseases, and hypertension were at high risk of morbidity and mortality [3]. Furthermore, men experienced higher mortality rates compared to women. Although vaccination has been very instrumental in reducing mortality and morbidity due to COVID-19, concerns are still prevalent regarding its short-term and long-term side effects. These effects have been reported differently for vaccine type, age, gender, and underlying health conditions. Examining these effects is critical for numerous reasons, including addressing public concerns, improving healthcare response, informing public health policies, and contributing to global research. The reported side effects' symptoms are fever, fatigue, and muscle pain, whereas in some situations, severe health effects have been reported [4, 5]. Against this backdrop, some studies highlight that females and young adults report stronger immunity compared with older persons [6, 7]. Nevertheless, vaccination has had successes in reducing morbidity and mortality, yet a research gap still exists regarding post-vaccination effects in the country. In this regard, understanding these effects is necessary for improving post-vaccination care as well as informing public policies. The current study examines post-vaccination effects among various age and gender groups in Pakistan. Although COVID-19 vaccination has substantially reduced morbidity and mortality, limited empirical evidence exists in Pakistan regarding late-onset and system-specific post-vaccination adverse events across different demographic and clinical groups. Most available data are derived from international surveillance systems, leaving a contextual gap in understanding age-, gender-, and comorbidity-based variations within the Pakistani population. The absence of structured national-level post-vaccination monitoring has created uncertainty in risk communication and public confidence. Therefore, there is a critical need to systematically evaluate reported adverse events and their associated predictors to inform evidence-based vaccination strategies and public health preparedness. This study aims to investigate post-vaccination effects,

future vaccination approaches, and plans, eventually assisting Pakistan's long-term public health preparedness.

METHODS

This descriptive exploratory cross-sectional study was conducted between December 2024 and February 2025. An online survey was conducted on the general population with a minimum age of 18 years and a maximum age of 65 years. The sample size was calculated using Raosoft software, with a 95% confidence level and a 50% response distribution due to the unavailability of prior data on post-vaccination effect prevalence in Pakistan. A margin of error of 6.6% was selected to achieve a feasible sample size given the exploratory nature of the study and the challenges in participant recruitment for an online survey on this topic. This resulted in a sample size of 220, which was deemed adequate for the primary descriptive and exploratory objectives of this research. The vaccination effect was assumed to be 50% which was a standard practice in calculating sample size for an adequate sample. This makes the sample size 220 individuals. Inclusion criteria were individuals aged 18 and above and those who were willing to participate in the survey, while exclusion criteria were children lower the age of 18 and those not willing to participate. A structured questionnaire was developed through an extensive literature review and converted into Google Forms. The questionnaire was shared through various social media platforms, including WhatsApp, Facebook, Instagram, etc., and also through sharing with universities and hospitals. Snowball technique was also applied to reach out to the deaths after vaccination, and respondents' immediate family members filled out the forms on their behalf. This approach introduces the potential for proxy or recall bias, as the family members' reporting of symptoms and medical history may not be fully accurate. Although convenient sampling and an online survey may lead to selection bias and limited access to certain individuals, various platforms have been used, as mentioned above, to reach the maximum number of individuals. Moreover, the purpose of the study was to capture the response of willing individuals who were also easily accessible. However, acknowledging the limitations, future studies should be done to incorporate probability sampling for a more representative sample. Furthermore, the sample size of 220, while calculated for feasibility, may not have been large enough to reliably capture rare adverse events or to allow for robust subgroup analyses (e.g., among the elderly or individuals with specific pre-existing conditions). In particular, the regression analysis for predictors with very small subgroups (e.g., smoking, n=12) may have produced unstable estimates, as indicated by wide confidence intervals, and should be interpreted with caution. Consent

was embedded in the start of the Performa, and respondents were asked to give consent before filling in the forms. The questionnaire carries important social, demographic and health-related information including gender, age, education, occupation, marital status, number of children, smoking, exposure to air pollution or road traffic, sleeping pattern, physical activity, pre-existing health conditions, undergone any surgery, previously had Covid-19, and vaccination status (though data on the specific vaccine type received, e.g., mRNA vs. adenoviral, was not collected). pre-existing health conditions, undergone any surgery, previously had Covid-19, and received a vaccination or not. To explore post-vaccination effects in the respondents' different groups, the different symptoms faced by the respondents were examined. These broad groups were neurological effects, cardiology effects, platelet count and bleeding complications, gastrointestinal effects, allergic reactions, and menstrual changes (applicable for female only). A pilot test was conducted on a sample of 20 participants to ensure the validity of the questionnaire. The validity of the questions was assessed through expert review by three public health specialists for clarity, relevance, and comprehensiveness. The internal consistency reliability of the multi-item sections of the questionnaire (e.g., symptom checklists) was assessed using Cronbach's alpha on the main study data, which yielded a value of 0.78, indicating good reliability. Data were analyzed in SPSS version 27.0 in the form of univariate, bivariate, and multivariate analyses. Bivariate analysis was performed for the purpose of examining the differentials in COVID-19 infection rate and adverse effects of vaccination by age and gender. For bivariate analysis, both the Chi-square Test and Fisher's Exact Test were applied as per requirements. For the variable of age, certain cells contain a sample size less than 5, which makes the results of the Chi-square test less reliable, so Fisher's Exact test was applied there; however, for the variable of gender, the Chi-square test is applied. For multivariate analysis, *Binary Logistic Regression* is applied due to the dichotomous nature of dependent variables (in Yes/No options). Seven separate regressions are run firstly by taking the combined variable of post-vaccination effects, and then all six other categories of post-vaccination effects separately. All social, demographic, and health variables collected through the questionnaire were taken as independent variables in the models. A p-value of <0.05 was considered significant. Odds ratios were reported alongside their 95% confidence intervals (CIs) to indicate the precision of the estimates.

RESULTS

The demographic profile of the respondents reveals that more than 2/3rd of the respondents belonged to the 20-35 age group and were female. Regarding education and occupation, more than half of the respondents were undergraduate students. Respondents who were working in any sector are 31% and those who were not working and neither studying are 11%. The majority, i.e., 75% of the respondents, are not married, and 24% are married with an average of 2 children. Behavioral factors of the respondents were also explored, according to which very few, i.e., 6 percent of the respondents, have a habit of smoking. While smoking is very low among respondents, exposure to traffic and air pollution is very prevalent, i.e., 84%. A very high percent, i.e., 59% take less than eight hours, 36 percent take eight hours or more, and only 6 percent take more than 8 hours. Regarding staying physically active, 73 percent of the respondents are physically active. If the study explores the type of most prevalent physical activity among those who stay active, walking is most common, with 60 percent of responses.

Table 1: Demographic and Behavioral Factors of the Respondents

| Variables | Categories | Frequency (%) |
|---|-------------------|---------------|
| Demographic Factors | | |
| Age | Below 20 | 32 (14.5%) |
| | 20-35 | 148 (67.3%) |
| | 36-50 | 26 (11.8%) |
| | 51-65 | 14 (6.4%) |
| Gender | Male | 77 (35.0%) |
| | Female | 143 (65.0%) |
| Education | Higher Secondary | 25 (11.4%) |
| | Undergraduate | 127 (57.7%) |
| | Postgraduate | 68 (30.9%) |
| Occupation | Not working | 23 (10.5%) |
| | Students | 116 (52.7%) |
| | Working | 81 (36.8%) |
| Marital Status | Single | 165 (75.0%) |
| | Married | 53 (24.1%) |
| | Divorced | 2 (0.9%) |
| Total | — | 220 (100.0%) |
| Mean Number of Children | — | 2.2 Children |
| Behavioral Factors | | |
| Smoking | Yes | 12 (5.5%) |
| | No | 208 (94.5%) |
| Exposure to Air Pollution or Road Traffic | Yes | 184 (83.6%) |
| | No | 36 (16.4%) |
| Sleeping Pattern | Less Than 8 Hours | 129 (58.6%) |
| | 8 Hours | 78 (35.5%) |
| | More Than 8 Hours | 13 (5.9%) |
| Physical Activity | Yes | 160 (72.7%) |
| | No | 60 (27.3%) |
| Total | — | 220 (100.0%) |

| | | |
|---|----------|-------------|
| Type of Physical Activity (Multiple Response) | Walking | 119 (60.4%) |
| | Exercise | 44 (22.3%) |
| | Cycling | 3 (1.5%) |
| | Sports | 21 (10.7%) |
| | Others | 10 (5.1%) |

¹Those questions that were mentioned as multiple responses throughout the paper, for which more than one response was selected by the respondents. Therefore, the percentage was calculated among the responses, not respondents, and their total was not 220.

Respondents were asked whether they had suffered from COVID-19 during the pandemic, and 24 percent had previously had COVID-19. Male and female were both equally affected by COVID; however, these results were significant. With age, the risk of getting infected with COVID-19 increases significantly (Figure 1).

Value of Significance: 0.543

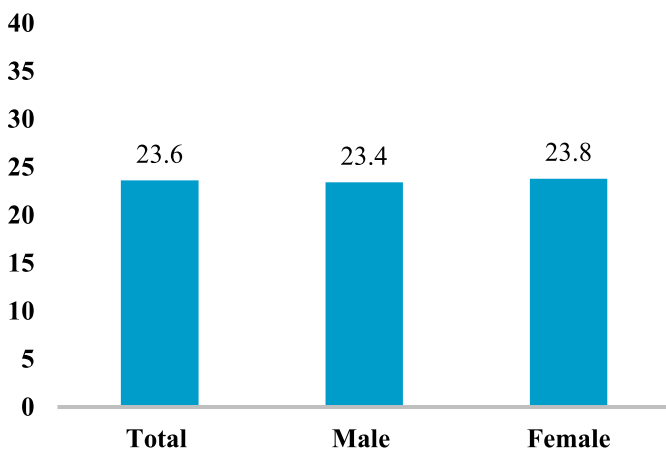


Figure 1: Percentage of Respondents Suffering from COVID-19 by Gender

Among respondents who are below 20 years old, only 13 percent suffered from COVID-19. However, among those who are between 51 and 65 years of age, 50 percent of the respondents had previously gotten infected by the pandemic (Figure 2).

Value of significance: 0.035

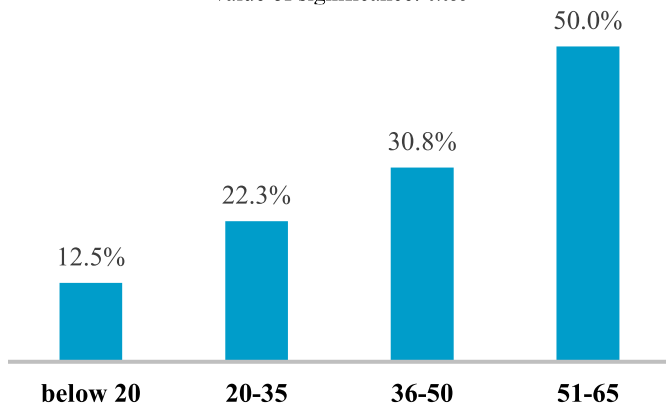


Figure 2: Percentage of Respondents Suffered from Covid-19 by Age

A large number of respondents i.e., 92 percent, have received the vaccination for Covid-19 (Figure 3).



Figure 3: Percentage of Respondents Who Received Covid-19 Vaccination

Information regarding the presence of any comorbidity and surgeries undergone was also explored from the respondents. Only 7 percent of the respondents had pre-existing conditions before Covid-19, among which the majority reported hypertension (8 responses), closely followed by Diabetes (6 responses). The majority of them are undoing medical treatment for their disease. Similarly, only 13 percent of the respondents had undergone any kind of surgery, including C-section, appendix removal, Craniotomy, femto-LASIK surgery, toe surgery, removal of an iron rod from the leg, root canal, stenting, and ENT-related surgeries. Within different types of post-vaccination effects, the symptoms faced by the respondents were also probed. According to the results, among neurological effects, most respondents face difficulty in concentrating or focusing on tasks (12 percent), followed by memory difficulties (8 percent). It is important to interpret this data with caution due to the potential for proxy bias. Cardiology effects highlight chest pain and difficulty breathing (8 percent) as the common symptoms. Abdominal pain (7 percent) was most prevalent among gastrointestinal effects. Similarly, skin rashes or itching (9 percent) and having trouble breathing or wheezing (7 percent) were common symptoms reported in allergic reactions. In females who reported menstrual changes post vaccination, they reported changes in timings and duration of periods (15 percent), mood swings and bloating (12 percent), and more pain or irregular periods than before (8 percent) (Table 2).

Table 2: Pre-existing Diseases and Surgeries Undergone by Respondents and Type of Effects with Symptoms on the Respondents

| Variables | Categories | Frequency (%) |
|---|------------------------------------|---------------|
| Demographic Factors | | |
| Pre-existing Conditions Before Covid-19 Vaccination | Yes | 15 (6.8%) |
| | No | 205 (93.2%) |
| | Total | 220 (100.0%) |
| Name of Disease (Pre-Existing Health Condition) (Multiple Response) | Diabetes | 6 (30.0%) |
| | High Blood Pressure (Hypertension) | 8 (40.0%) |
| | Cardiovascular Conditions | 2 (10.0%) |
| | Genetic Disease | 1 (5.0%) |
| | Others | 3 (15.0%) |
| Total | 20 (100.0%) | |
| Undergoing Any Medication/ Treatment | Yes | 13 (86.7%) |
| | No | 2 (13.3%) |
| | Total | 15 (100.0%) |

| | | |
|--|---|--------------|
| Any Surgeries/ Operations | Yes | 29 (13.2%) |
| | No | 191 (86.8%) |
| | Total | 220 (100.0%) |
| Neurological Effects | Frequent Migraines/Severe Headaches | 15 (6.6%) |
| | Difficulty Concentrating or Focusing on Tasks | 28 (12.3%) |
| | Memory Difficulty | 19 (8.3%) |
| | Strange Body Sensations | 17 (7.5%) |
| | Others | 3 (1.3%) |
| | None | 146 (64.0%) |
| | Total | 228 (100.0%) |
| Cardiology Effects | Heart Problems Like A Heart Attack | 6 (2.7%) |
| | Chest Pain or Difficulty Breathing | 18 (8.2%) |
| | Feeling Weak or Paralyzed | 13 (5.9%) |
| | Changes in Your Blood Pressure | 11 (5.0%) |
| | None | 171 (78.1%) |
| | Total | 219 (100.0%) |
| PC and Bleeding Complications | Nose Bleeding | 5 (2.4%) |
| | Platelet Levels Checked | 8 (3.9%) |
| | Wound Healing Delays | 4 (1.9%) |
| | More Bruises | 6 (2.9%) |
| | Any Other Unusual Bleeding | 3 (1.4%) |
| | None | 181 (87.4%) |
| | Total | 207 (100.0%) |
| Gastrointestinal Effects | Persistent Nausea or Vomiting | 7 (3.4%) |
| | Abdominal Pain Related to GERD | 14 (6.8%) |
| | Others | 6 (2.9%) |
| | None | 180 (87.0%) |
| | Total | 207 (100.0%) |
| Allergic Reactions | Skin Rashes or Skin Itching | 19 (9.0%) |
| | Lips, Tongue, or Throat Swell | 4 (1.9%) |
| | Trouble Breathing or Wheezing | 14 (6.6%) |
| | Allergies to Meds, Food, or Vaccines | 3 (1.4%) |
| | Others | 4 (1.9%) |
| | None | 167 (79.1%) |
| | Total | 211 (100%) |
| Menstrual Changes (Only Applicable for Female) | More Pain or Irregular | 11 (7.9%) |
| | Mood Swings or Bloating | 17 (12.2%) |
| | Bleeding Much Different | 3 (2.2%) |
| | Period Timing or Duration Changed | 21 (15.1%) |
| | Others | 2 (1.4%) |
| | None | 85 (61.2%) |
| Total | 139 (100.0%) | |

Note: All of these are Multiple Response Questions, so the total varies as per responses in each category and can be greater than 220. The percentages calculated are among the responses, not the respondents.

Respondents were asked what type of effects they experienced after receiving the vaccination. Nearly 47 percent of respondents have been affected by one or more kinds of effects after vaccination. As represented in Graph 3, 28 percent have neurological effects, 16 percent have cardiovascular effects, 11 percent have platelet count and

bleeding complications, 11 percent have gastrointestinal effects, and 18 percent have allergic reactions. Among female, 22 percent reported menstrual changings after receiving vaccination of Covid-19 (Figure 4).

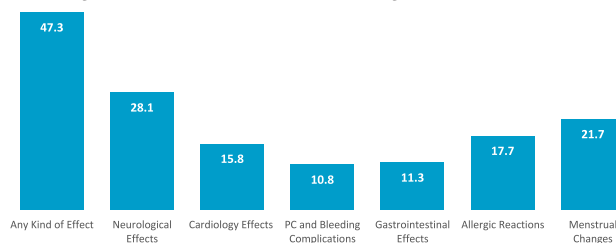


Figure 4: Percentage of Respondents Reported Post-Vaccination Effects

There were two male death reports among the respondents, and their immediate family members have filled out questionnaires on their behalf. This makes the percent mortality rate among the respondents who have been administered vaccination 2 percent among those who have reported vaccination. One of the two previously suffered from Covid-19. Neither has any pre-existing disease nor undergone any surgery. One reported all types of post-vaccination effects, and the other reported neurological, cardiology, and allergic effects (Table 3).

Table 3: Post-Vaccination Deaths Reported among Respondents

| Variables | Respondent 1 | Respondent 2 |
|--------------------------------|--------------|--------------|
| Gender | Male | Male |
| Age | 51-65 | 51-65 |
| Had Covid-19 | Yes | No |
| Vaccination | Yes | Yes |
| Pre-Existing Health Conditions | No | No |
| Undergone Surgery | No | No |
| Neurological Effects | Yes | Yes |
| Cardiology Effects | Yes | Yes |
| Bleeding Complications | Yes | No |
| Gastrointestinal Effects | Yes | No |
| Allergic Reactions | Yes | Yes |

When a difference in post-vaccination effects was seen by gender, female reported a slightly higher number of effects due to a higher number reporting menstrual changes. While for all other types, male reported higher post-vaccination effects than female, but the results were insignificant (Table 4).

Table 4: Post-Vaccination Effects by Gender

| Effects | Male | | Female | | Significance |
|---------------------------------|------|------|--------|------|--------------|
| | Yes | No | Yes | No | |
| Any Kind of Effect | 41.9 | 58.1 | 50.4 | 49.6 | 0.154 |
| Specific Kind of Effects | | | | | |
| Neurological Effects | 29.7 | 70.3 | 27.1 | 72.9 | 0.405 |
| Cardiology Effects | 18.9 | 81.1 | 14.0 | 86.0 | 0.230 |
| PC and Bleeding Complications | 12.2 | 87.8 | 10.1 | 89.9 | 0.405 |
| Gastrointestinal Effects | 12.2 | 87.8 | 10.9 | 89.1 | 0.472 |

| | | | | | |
|--------------------|------|------|------|------|-------|
| Allergic Reactions | 21.6 | 78.4 | 15.5 | 84.5 | 0.182 |
| Menstrual Changes | NA | NA | 34.1 | 65.9 | NA |

Results show the difference in post-vaccination effects by age. Data show that older ages, that was above 36 years,

Table 5: Post-Vaccination Effects by Age

| Effects | Below Age 20 | | 20-35 Years | | 36-50 Years | | 51-65 Years | | Significance |
|---------------------------------|--------------|------|-------------|------|-------------|------|-------------|------|--------------|
| | Yes | No | Yes | No | Yes | No | Yes | No | |
| Any Kind of Effect | 39.3 | 60.7 | 43.4 | 56.6 | 68.0 | 32.0 | 64.3 | 35.7 | 0.059 |
| Specific Kind of Effects | | | | | | | | | |
| Neurological Effects | 17.9 | 82.1 | 23.5 | 76.5 | 48.0 | 52.0 | 57.1 | 42.9 | 0.004 |
| Cardiology Effects | 10.7 | 89.3 | 12.5 | 87.5 | 20.0 | 80.0 | 50.0 | 50.0 | 0.007 |
| PC and Bleeding Complications | 7.1 | 92.9 | 10.3 | 89.7 | 12.0 | 88.0 | 21.4 | 78.6 | 0.499 |
| Gastrointestinal Effects | 3.6 | 96.4 | 9.6 | 90.4 | 12.0 | 88.0 | 42.9 | 57.1 | 0.005 |
| Allergic Reactions | 10.7 | 89.3 | 15.4 | 84.6 | 24.0 | 76.0 | 42.9 | 57.1 | 0.052 |
| Menstrual Changes | 21.4 | 78.5 | 18.4 | 81.6 | 44.0 | 56.0 | NA | NA | 0.148 |

Binary logistic regression is performed firstly with the variable of the combined effect of post-vaccination and then separately with all types of reported effects. Results show that for overall effects, pre-existing conditions, surgery, and marital status are significant variables. The results, presented as odds ratios (OR) with 95% confidence intervals (CI), show that for overall effects, pre-existing conditions, surgery, and marital status are significant variables. It is important to note that variables with very wide confidence intervals indicate less stable estimates, often due to small numbers in specific subgroups. Those who have experienced a surgery have 3 times more chances of post-vaccination effects than those who have not undergone any surgery. Similarly, those who have any comorbidity have 8 times more chances of suffering from post-vaccination effects as compared to those who do not have any comorbidity. This suggests an association where individuals with a pre-existing health condition or a history

were profoundly affected compared to younger age groups. Results are significant for neurological, cardiology, and gastrointestinal effects (Table 5).

of surgery were more likely to report post-vaccination effects. Marital status was also significant in overall and neurological effects, with married people less likely to report these effects than single individuals. It may be due to different lifestyles, health behaviors, and stress in unmarried individuals, leading to weaker immunity. Age came out to be a significant variable in neurological, cardiology, gastrointestinal, and allergic effects. Education is a significant variable in gastrointestinal effect. Those who have previously suffered from Covid-19 are highly and significantly affected by allergic reactions, while less likely and significantly affected by menstrual changes. For menstrual effects among female, physical activity and sleeping patterns are also significant variables. Those who are involved in physical activity and those with more than 8 hours are more likely to report menstrual changes (Table 6).

Table 6: Results of Regression Analysis

| Independent Variables | Any Effect | Neurological Effects | Cardiology Effects | PC and Bleeding Complications | Gastrointestinal Effects | Allergic Reactions | Menstrual Changes |
|-------------------------------|------------|----------------------|--------------------|-------------------------------|--------------------------|--------------------|-------------------|
| Gender (Male) | 0.846 | 1.244 | 1.793 | 1.536 | 1.449 | 2.101 | — |
| Previously Had Covid (Yes) | 1.038 | 1.068 | 1.241 | 1.085 | 0.970 | 3.205* | 0.293* |
| Surgery (Yes) | 3.160* | 0.688 | 0.519 | 2.139 | 1.200 | 1.962 | 1.781 |
| Smoking (Yes) | 3.497 | 3.716 | 1.564 | 2.855 | 1.833 | 0.756 | 0.000 |
| Exposure to Pollution (Yes) | 1.257 | 1.427 | 1.012 | 10.172 | 4.036 | 1.291 | 1.864 |
| Physical Activity (Yes) | 1.229 | 0.773 | 0.751 | 0.941 | 1.367 | 0.736 | 4.099* |
| Pre-Existing Conditions (Yes) | 8.501* | 5.738* | 7.049* | 0.987 | 3.507 | 0.753 | 5.763 |
| Age (20-35) | 1.291 | 1.774 | 1.425 | 2.060 | 11.319 | 2.235 | 0.478 |
| Age (36-50) | 5.363 | 42.621* | 5.364 | 26.938 | 16.645 | 4.360 | 1.121 |
| Age (51-65) | 2.365 | 31.701* | 14.020* | 54.726 | 45.76* | 12.047* | 0.065 |
| Occupation (Students) | 0.292 | 0.651 | 0.764 | 0.435 | 1.065 | 0.258 | 0.324 |
| Occupation (Working) | 0.628 | 0.900 | 1.149 | 0.301 | 0.544 | 0.646 | 0.845 |
| Education (Undergraduate) | 0.816 | 0.901 | 0.708 | 1.418 | 0.116* | 0.339 | 0.716 |
| Education (Postgraduate) | 0.700 | 0.668 | 0.592 | 1.115 | 0.257 | 0.318 | 0.842 |
| Sleep (8 hours) | 0.961 | 0.669 | 0.877 | 1.527 | 1.238 | 0.998 | 0.881 |
| Sleep (More than 8 hours) | 2.085 | 1.190 | 1.836 | 3.968 | 5.103 | 0.509 | 8.404* |

| | | | | | | | |
|----------------------------|--------|--------|-------|-------|-------|-------|-------|
| Marital Status (Married) | 0.252* | 0.084* | 0.256 | 0.079 | 0.868 | 0.231 | 1.439 |
| Marital Status (Divorced) | 0.231 | 0.121 | 1.927 | 0.000 | 0.000 | 2.166 | 2.509 |
| Model Predicted Percentage | 64.0% | 75.0% | 86.0% | 89.0% | 90.0% | 83.0% | 74.0% |

Reference Categories: Gender (Female), Previously Had Covid (No), Surgery (No), Smoking (No), Exposure to Pollution (No), Physical Activity (No), Pre-Existing Conditions (No), Age (Below 20 years), Occupation (Not working), Education (Higher secondary), Sleep (Less than 8 hours), Marital Status (Single). *Indicates significant values

DISCUSSION

The respondents of the study were largely young adults, constituting around 1/3rd the population aged 20–35 years, with 65% being female and highly educated, with 58% undergraduate and 31% being postgraduate. More than half are students (53%), while 37% respondents are employed, and 11% are not working at all. Around 3/4th of respondents are single. The findings highlight a strong representation of young, educated female, mainly students. This demographic insight is critical for understanding vaccine effects and improving future efforts. Existing studies highlight that age significantly increases vulnerability to post-COVID-19 vaccination effects. The CDC reports that the death rate was 360 times higher for those aged 85 years and above when compared with young age groups [8, 9]. These findings highlight that older persons face far greater effects of COVID-19 vaccination. Similarly, the findings reflect that around one out of ten respondents had pre-existing conditions before COVID-19 vaccination. The pre-existing conditions included hypertension (40%) and diabetes (30%), which were the most common. Additionally, 87% were on medication, and 13% had a history of surgeries. The literature shows that individuals with hypertension, diabetes, and cardiovascular diseases face a higher risk of severe Covid-19 vaccination outcomes [10]. The findings regarding post-vaccination effects comply with existing studies, which show neurological effects have been observed but remain rare [11]. Similarly, cardiac effects cases have been reported post-mRNA vaccination, though the CDC confirms these cases are infrequent [8, 9]. The studies also show that allergic reactions following COVID-19 vaccination are rare [12]. In accordance with key findings, existing literature shows that gastrointestinal effects, including nausea, diarrhea, and abdominal discomfort, are generally reported [13]. Regarding menstrual and bleeding issues, the existing studies report menstrual irregularities and bleeding post-vaccination. While observational studies suggest a likely link, no conclusive underlying connection has been recognized [14]. Two deaths were reported in the survey among males aged 51–65 years. It is critical to emphasize that this study cannot establish causality, and these reports represent temporal associations only. This complies with existing research that highlights that older persons with chronic illnesses are more at risk of adverse health outcomes post-vaccination, mainly because of a

vulnerable immune system [4]. The research also reflects that prior Covid-19 infections, particularly recurrent cases, can result in immunity dysregulation, thus contributing to the possibility of inflammatory or cardiovascular complications post-vaccination [15]. Some studies suggest that persons with previous infections can also experience stronger immunity reactions, possibly adding to neurological and cardiac effects [16]. Both respondents reporting on behalf of the deceased patients highlighted neurological and cardiovascular effects, which adequately align with documented cases of post-vaccine complications [17, 18]. Moreover, one respondent suffered from mild bleeding and gastrointestinal problems, whereas the other had additional severe complications. These signs have been associated with rare vaccine-induced immune thrombotic thrombocytopenia (VITT) and inflammatory responses in persons with pre-existing conditions [19, 20]. Although rare, post-vaccination mortality has been experienced by persons with multiple comorbidities, as established by studies conducted across the world [21]. The symptoms reported by respondents, including difficulty concentrating, memory issues, and cognitive impairment, resemble post-COVID-19 vaccine-associated neuro-inflammatory responses, which have been documented in studies linking mRNA vaccines to mild cognitive disturbances [17, 18]. Moreover, research highlights that systemic inflammation caused by vaccination can lead to brief neurological effects, mostly in persons with pre-existing problems [17]. The study also reported chest pain and difficulty in breathing, which are consistent with research on post-vaccination. Studies indicate that mRNA-based COVID-19 vaccines can result in mild myocarditis, though cases are rare [18]. The study found that 7% of respondents experienced abdominal pain, which aligns with reports of post-vaccine gastrointestinal disturbances. Covid-19 vaccines, particularly adenoviral vector vaccines, have been associated with nausea, diarrhea, and abdominal discomfort due to mild systemic inflammation [18]. These signs are generally short-lived and improve without any medication. This is particularly relevant for variables like smoking and pollution exposure, where the small size of the exposed group limits the reliability of the point estimates. The findings regarding skin rashes or itching, as well as trouble breathing or wheezing, highlight results from prior research on vaccine-

related allergic responses. Some people get mild dermatological reactions, possibly because of vaccine adjuvants or immune activation [22]. Similarly, the study respondents reflected changes in period timing and duration, mood swings, bloating, and increased menstrual pain or irregularity, which is in compliance with existing research. It highlights that menstrual cycle disturbances post-vaccination are associated with transient immune activation and inflammatory responses affecting ovarian function [14]. However, a comprehensive study established that though some females undergo minor menstrual changes, this impact is provisional and does not influence fertility [22]. To alleviate these hazards, improved pre-vaccination screening and post-vaccine monitoring are critical, especially for high-risk persons. Effective communication on the benefits and hazards of vaccination is essential to safeguard learnt decision-making while upholding community assurance in vaccination programs. A follow-up mechanism should be established for guiding individuals reporting adverse post-vaccination effects to reduce their risk and severity. Secondly, the inclusion of data from family members for deceased individuals, while necessary to capture these rare events, introduces the potential for proxy and recall bias, which may affect the accuracy of the reported symptoms and medical history. Thirdly, the regression analysis for some specific outcomes, particularly those with low prevalence, yielded estimates with wide confidence intervals, indicating instability and a lack of precision.

The study was limited by its small sample size, online convenience sampling, and cross-sectional design, which restrict generalizability and prevent causal inference. Self-reported data and proxy responses for deceased individuals introduce recall and reporting bias, while wide confidence intervals in regression models indicate estimate instability. Future research should adopt probability sampling, larger multi-center cohorts, and longitudinal follow-up designs to validate findings. Incorporating vaccine-type data and clinical verification of adverse events would further enhance the robustness of evidence for public health decision-making.

CONCLUSIONS

The present study has assessed that a significant proportion of individuals had faced any kind of adverse effects from vaccination against COVID-19. Individuals who are in their later age groups not only have a higher chance of contracting the disease but also report higher post-vaccination effects. Moreover, pre-existing health conditions and a history of surgery were associated with a higher reported susceptibility to post-vaccination effects, which may be related to underlying health status.

Authors' Contribution

Conceptualization: AUHM
 Methodology: AUHM, NH
 Formal analysis: AUHM, SH, RHU, MFM, TAF
 Writing and Drafting: MNS, HA, TAF
 Review and Editing: AUHM, NH, SH, RHU, MFM, TAF, MNS, HA

All authors approved the final manuscript and take responsibilities for integrity of work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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Original Article



Double Burden of Malnutrition among Children Under Five in Pakistan: Evidence from Pakistan Demographic and Health Survey 2017–18

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ARTICLE INFO

Keywords:

Malnutrition, Stunting, Overweight, Double Burden, Pakistan Demographic and Health Survey

How to Cite:Farooq, I., Imran, M., & Ahmad, N. (2025). Double Burden of Malnutrition among Children Under Five in Pakistan: Evidence from Pakistan Demographic and Health Survey 2017–18: Double Burden of Malnutrition in Pakistan: Evidence from PDHS 2017–18. *Pakistan BioMedical Journal*, 8(8), 45–51. <https://doi.org/10.54393/pbmj.v8i8.1303>***Corresponding Author:**Irzah Farooq
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ABSTRACT

Malnutrition in Pakistan presents a dual challenge, with high levels of undernutrition persisting alongside a rising prevalence of childhood overweight. The coexistence of these conditions in the same population, often referred to as the DBM, is an emerging but underexplored concern. **Objective:** To examine the prevalence and determinants of stunting, underweight, wasting, and overweight among children under five years in Pakistan. **Methods:** It was a cross-sectional secondary analysis of the data on the Pakistan Demographic and Health Survey (PDHS) 2017. A total of 150 children aged 0–59 months and whose anthropometric data were complete, were analyzed. The WHO child growth standards were used to determine the nutritional status. Descriptive statistics, bivariate tests, and multivariate logistic regression were used. **Results:** Overall, 53.3% of children were stunted and 22.7% were overweight, while 13.3% exhibited individual-level DBM. Stunting was uniformly high across wealth quintiles, whereas overweight rose from 10.0% in the poorest to 36.7% in the richest quintile. Regression analysis showed that the age variable was found to contribute to stunting (adjusted odds ratio (aOR): 1.06, 95 per cent confidence interval (CI): 1.0325.29, $p=0.023$), and children in the wealthiest households were over five times more likely to be overweight (aOR: 5.14, 95 per cent CI: 1.3725.29, $p=0.023$). **Conclusions:** These findings reveal a pronounced DBM among Pakistani children. Integrated strategies are urgently needed to address chronic undernutrition while preventing the rise of childhood overweight.

INTRODUCTION

Malnutrition has been among the most urgent international issues in public health, and children in low- and middle-income countries (LMICs) are disproportionately affected. Conventionally the focus has been on undernutrition, especially stunting, wasting, and underweight, which is highly linked to high child morbidity, mortality, poor cognitive development, and low productivity in later life [1, 2]. In 2022, it is estimated that there are 148 million stunted children under five, 45 million wasted children, and 37 million overweight children globally, as both persistently under-informed and gaining weight/obesity [3]. Over the past few decades, LMICs have undergone dramatic socioeconomic and nutritional shifts that are

characterized by urbanization, nutrition, and decreased physical activity. Such developments have added to the increased childhood overweight and obesity, despite undernutrition being rife [4]. Generally, this is known to occur as the so-called double burden of malnutrition (DBM), which is a combination of undernourishment and over nutrition at either the population and household levels or even at the individual level [5]. DBM poses unique challenges because it requires public health systems to simultaneously tackle two seemingly opposite problems, nutrient deficiency and energy excess, within resource-limited settings [6, 7]. At the regional level, South Asia continues to exhibit some of the highest stunting rates



worldwide, with Pakistan, India, Bangladesh, and Nepal collectively contributing a significant share of the global stunted child population [8]. However, alongside these high rates, evidence of overweight and obesity in preschool children is increasing, particularly in urban and wealthier households [9]. This transition reflects broader nutrition and epidemiological shifts, as populations move from traditional diets to energy-dense, processed foods with lower micronutrient quality [10]. In Pakistan, the nutritional landscape is complex and evolving. With the high rates of chronic undernutrition, the country still faces a high level of maternal and child health programs despite having heavily invested in them. PDHS 2017/18 indicated that almost 38 percent of children below the age of five years were stunted, 7 percent were wasted, and 23 percent were underweight, and that overweight tendencies in children were emerging in urban areas [11]. The increasing sedentary lifestyles, changing eating habits, and urbanization have increased the rate at which children in the upper socioeconomic classes are becoming overweight and obese [12]. Stunting and overweight co-occurring in the same child or household increase the risk of health-related effects in the long-term, such as growth retardation, decreased education, and heightened susceptibility to non-communicable diseases (NCDs) like diabetes and cardiovascular conditions [13, 14]. Although several studies have largely described the undernutrition in Pakistan, there has been little research on the dual burden of malnutrition in children under five years. Most existing analyses focus on either stunting or overweight in isolation, without accounting for their coexistence or shared determinants [15]. A deeper understanding of this phenomenon is critical for designing integrated interventions that can address both undernutrition and emerging overweight in early childhood.

Despite extensive evidence on undernutrition in Pakistan, limited research has examined the coexistence of undernutrition and overweight (double burden of malnutrition) at the individual and household levels among children under five. Most prior studies have analyzed stunting or overweight in isolation, overlooking their shared determinants within rapidly transitioning socioeconomic contexts. This study aims to estimate the prevalence and determinants of stunting, underweight, wasting, and overweight in children under five in Pakistan based on the data of PDHS 2017/18, and to shed light on the co-occurrence of stunting and overweight as malnutrition indicators.

METHODS

The analysis was founded on a secondary analysis of cross-sectional data on the Pakistan Demographic and Health Survey (PDHS) 2017–18, a nationally representative survey that used a stratified two-stage cluster design, collecting data about demographic, socioeconomic, and health indicators in the country. The study duration was from December 2017 to February 2018. For the present analysis, a subsample of 150 children aged 0–59 months was randomly selected from the full PDHS dataset. This sample size was determined to be adequate for a preliminary analysis of the double burden of malnutrition, providing a 5% margin of error and 80% power to detect significant associations, and was consistent with sample sizes used in similar methodological studies focusing on specific anthropometric outcomes. Written informed consent was taken. The children with implausible anthropometric values were eliminated according to World Health Organization (WHO) growth standards cutoffs, including a height-for-age Z-score (HAZ) of less than -6 or more than +6, a weight-for-age Z-score (WAZ) of less than -6 or more than +5, and a weight-for-height Z-score (WHZ) of less than -5 or more than +5. Children who lacked data regarding these variables were also left out. WHO child growth standards transformed anthropometric measurements to Z-scores. Stunting was determined as those below the -2 SD of HAZ, wasting as those below the -2 SD of WHZ, and underweight as those below the -2 SD of WAZ, and overweight/obesity as those above the +2 SD of WHZ. The composite DBM was determined at an individual and a household level, and this aspect presupposes the existence of a combination of stunting and overweight. The independent variables were: child-level (age, sex), and household-level (place of residence (urban/rural), wealth quintile, which was obtained through household asset data that PDHS reported). The statistical tests were conducted in several steps. Sample characteristics and the prevalence of various types of malnutrition were summarized by means of descriptive statistics. The chi-square tests were used for categorical variables, and independent-sample t-tests of continuous variables to test bivariate associations between nutritional outcomes and independent variables. It was also conducted using multivariate logistic regression analysis as a way to ascertain the determinants of malnutrition, where the results gave adjusted odds ratios (aORs) that had a 95% confidence limit (CI). The entire inferential analyses were considered to be statistically significant at a p-value of less than 0.05.

RESULTS

A total of 150 children aged 0–59 months were included in the analysis, of whom half were male (50.0%) and half

female (50.0%). The mean age was 31.7 ± 13.1 months. A majority of children resided in urban areas (60.0%), and household wealth quintiles were equally distributed across the sample (20.0% each).

Table 1: Sample Characteristics and Overall Malnutrition Prevalence (n=150)

| Characteristic | n (%) / mean ± SD |
|-------------------------------|-------------------|
| Sex | |
| Male | 75 (50.0%) |
| Female | 75 (50.0%) |
| Residence | |
| Urban | 90 (60.0%) |
| Rural | 60 (40.0%) |
| Wealth quintile | |
| Poorest | 30 (20.0%) |
| Poorer | 30 (20.0%) |
| Middle | 30 (20.0%) |
| Richer | 30 (20.0%) |
| Richest | 30 (20.0%) |
| Malnutrition Indicator | |
| Stunting (HAZ < -2) | 80 (53.3%) |
| Overweight (WHZ > +2) | 34 (22.7%) |
| Individual DBM | 20 (13.3%) |
| Household DBM | 31 (20.7%) |
| HAZ Score | -1.81 ± 0.95 |
| WHZ Score | 0.77 ± 1.33 |
| Age (Months) | 31.7 ± 13.1 |

All variables are complete with no missing data.

The analysis reveals divergent socioeconomic patterning: stunting persists at high levels across all wealth strata, indicating its resilience, while overweight exhibits a clear positive gradient, increasing from 10.0% in the poorest to 36.7% in the richest quintile. Furthermore, the equal stunting prevalence in urban and rural areas (53.3%), coupled with a significantly higher overweight prevalence in urban settings (26.7% vs. 16.7%), underscores that the double burden is a universal challenge, albeit manifested with greater intensity in more affluent and urban contexts (Table 2).

Table 2: Prevalence of Stunting and Overweight by Socioeconomic and Geographic Factors

| Stratified | Category | Stunting, n (%) | Overweight, n (%) |
|-----------------|----------|-----------------|-------------------|
| Wealth quintile | Poorest | 17 (56.7%) | 3 (10.0%) |
| | Poorer | 15 (50.0%) | 7 (23.3%) |
| | Middle | 15 (50.0%) | 5 (16.7%) |
| | Richer | 17 (56.7%) | 8 (26.7%) |
| | Richest | 16 (53.3%) | 11 (36.7%) |
| Residence | Urban | 48 (53.3%) | 24 (26.7%) |
| | Rural | 32 (53.3%) | 10 (16.7%) |

Note: Percentages represent row percentages within each category.

Multivariable regression models provided further insights into determinants of malnutrition. Older age was significantly associated with stunting, with each additional month increasing the odds by 6% (aOR: 1.06, 95% CI: 1.03-1.10, p<0.001). In contrast, no significant associations were found for sex or wealth quintile. For being overweight, children from the richest households had five times higher odds compared to those from the poorest (aOR: 5.14, 95% CI: 1.37-25.29, p = 0.023). Female children showed a non-significant trend toward overweight (aOR: 2.13, p = 0.079). Individual-level DBM appeared more common among children in higher wealth quintiles (aOR range: 4.5-6.1 compared to the poorest), although none reached statistical significance. Household-level DBM analysis produced unstable odds ratios due to small sample sizes, leading to unreliable estimates. The logistic regression model for household-level DBM showed signs of complete separation, resulting in extreme odds ratios and implausibly wide confidence intervals. This indicates a statistical limitation due to sparse data in certain categories, and these estimates should therefore be interpreted with caution.

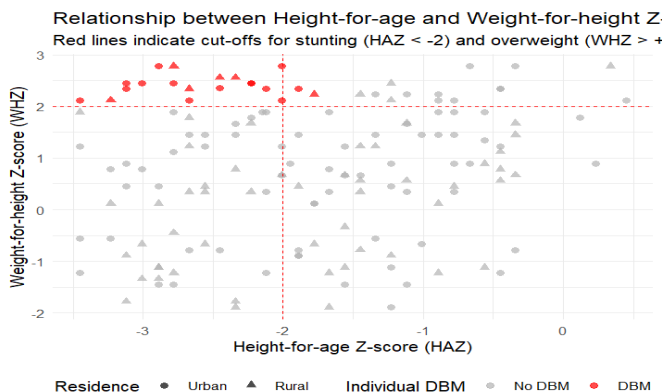


Figure 1: Relationship Between Height for Age and Weight for Z

The analysis reveals concerning trends for individual-level DBM, with wealthier quintiles showing 4.5-6.1 times higher odds compared to the poorest, though these associations lack statistical significance (all p>0.05). The household-level DBM model exhibits complete separation with extreme odds ratios, indicating model instability likely due to small cell sizes or perfect prediction, rendering these results unreliable. These findings suggest potential socioeconomic patterning of individual DBM, but methodological limitations preclude definitive conclusions about household-level determinants (Table 3).

Table 3: Multivariable Logistic Regression Analysis of Double Burden of Malnutrition Determinants

| Determinant | Category | Individual DBM aOR (95% CI) | p-Value | Household DBM aOR (95% CI) | p-Value |
|-----------------|--------------------|-----------------------------|---------|-----------------------------------|---------|
| Age | Per month | 1.01(0.98-1.05) | 0.514 | 0.90(0.66-1.17) | 0.420 |
| Sex | Female vs Male | 1.47(0.54-4.15) | 0.451 | 494,220,842.21(0-NA) | 0.998 |
| Wealth Quintile | Poorer vs Poorest | 5.95(0.88-118.42) | 0.115 | 0.00(NA-Inf) | 0.999 |
| | Middle vs Poorest | 4.53(0.62-91.96) | 0.190 | 0.00(NA-Inf) | 0.999 |
| | Richer vs Poorest | 6.07(0.89-120.90) | 0.111 | 0.00(NA-Inf) | 0.999 |
| | Richest vs Poorest | 5.88(0.87-117.00) | 0.118 | 46,206,933,838,458,290,176(0-Inf) | 0.997 |

Statistical Technique: Multivariable Logistic Regression

Age has a strong positive correlation with stunting, with each month adding odds by 6%. The overweight gradient is also clear among the wealthy, with the children in the wealthiest quintile being found to be 5.14 times more likely to be overweight than their counterparts in the poorest quintile (95% CI: 1.37-25.29, p=0.023). Female sex shows a non-significant trend toward higher overweight odds(aOR: 2.13, p=0.079), while no wealth-based patterns emerge for stunting (Table 4).

Table 4: Determinants of Stunting and Overweight

| Determinant | Category | Stunting aOR (95% CI) | p-Value | Overweight aOR (95% CI) | p-Value |
|-----------------|--------------------|-----------------------|---------|-------------------------|---------|
| Age | Per Month | 1.06(1.03-1.10) | <0.001 | 0.98(0.95-1.01) | 0.254 |
| Sex | Female vs Male | 0.71(0.34-1.46) | 0.365 | 2.13(0.93-5.06) | 0.079 |
| Wealth Quintile | Poorer vs Poorest | 0.80(0.27-2.35) | 0.682 | 2.70(0.66-13.81) | 0.187 |
| | Middle vs Poorest | 0.71(0.24-2.11) | 0.543 | 1.86(0.41-9.93) | 0.432 |
| | Richer vs Poorest | 1.21(0.41-3.67) | 0.729 | 3.14(0.79-15.88) | 0.124 |
| | Richest vs Poorest | 0.94(0.32-2.76) | 0.912 | 5.14(1.37-25.29) | 0.023 |

Based on the provided forest plots, the determinants exhibit varying associations with the different malnutrition outcomes. Socioeconomic factors like wealth quintiles and residence generally show significant gradients, while demographic variables like sex display more outcome-specific effects (Figure 2).

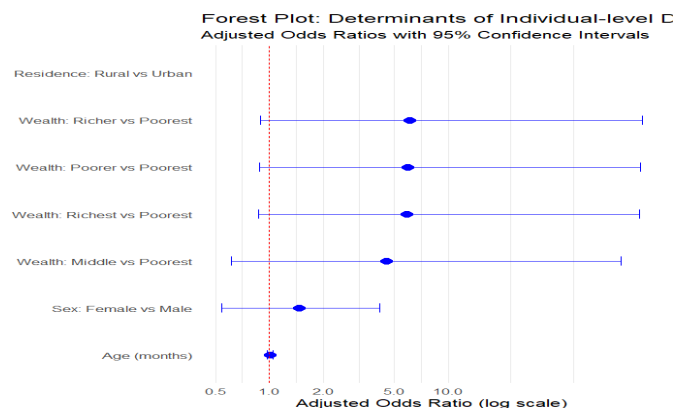


Figure 2: Forest Plot: Determinants of Individual Level

The magnitude and direction of these associations differ across the household/individual Double Burden of Malnutrition (DBM), overweight, and stunting, indicating distinct underlying causal pathways (Figure 3).

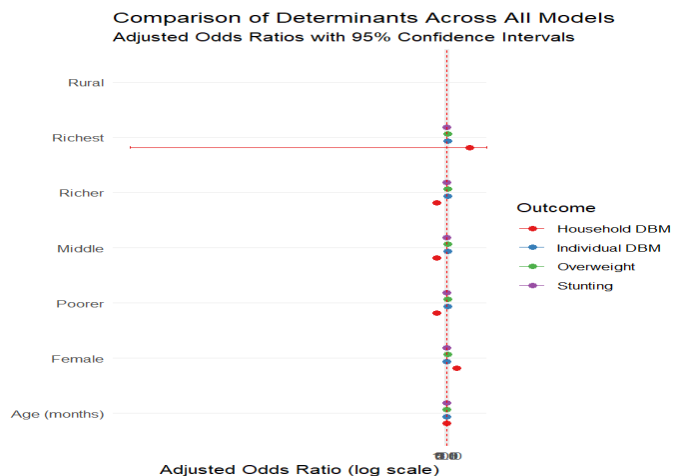


Figure 3: Comparison of Determinants Across all Models

The analysis indicates stunting is a generalized issue, showing no significant association with wealth, residence, or sex, but a significant association with older child age. Conversely, overweight and the individual double burden of malnutrition, while not statistically significant, demonstrate clear socio-demographic gradients, with higher prevalence in wealthier quintiles, urban areas, and among female. This suggests an emerging nutrition transition where undernutrition persists alongside rising overnutrition in more affluent segments (Table 5).

Table 5: Bivariate Analysis of Stunting, Overweight, and Individual Double Burden of Malnutrition (DBM) Determinants

| Variable | Category | Stunting Prevalence (%) | Overweight Prevalence (%) | Individual DBM Prevalence (%) | Statistical Test (Stunting) | p-Value | Statistical Test (Overweight) | p-Value | Statistical Test (DBM) | p-Value |
|-----------------|----------------------|-------------------------|---------------------------|-------------------------------|-----------------------------|---------|-------------------------------|---------|------------------------|---------|
| Wealth Quintile | Poorest | 56.7 | 10.0 | 3.3 | $\chi^2 = 0.54$ | 0.970 | $\chi^2 = 7.00$ | 0.136 | $\chi^2 = 3.46$ | 0.484 |
| | Poorer | 50.0 | 23.3 | 16.7 | | | | | | |
| | Middle | 50.0 | 16.7 | 13.3 | | | | | | |
| | Richer | 56.7 | 26.7 | 16.7 | | | | | | |
| | Richest | 53.3 | 36.7 | 16.7 | | | | | | |
| Residence | Urban | 53.3 | 26.7 | 15.6 | $\chi^2 = 0.00$ | 1.000 | $\chi^2 = 1.52$ | 0.217 | $\chi^2 = 0.54$ | 0.462 |
| | Rural | 53.3 | 16.7 | 10.0 | | | | | | |
| Sex | Male | 52.0 | 17.3 | 10.7 | $\chi^2 = 0.03$ | 0.870 | $\chi^2 = 1.86$ | 0.172 | $\chi^2 = 0.52$ | 0.471 |
| | Female | 54.7 | 28.0 | 16.0 | | | | | | |
| Age (Months) | Not Affected/ No DBM | 27.1* | 32.2* | 31.4* | t = -4.29 | <0.001 | t = 0.80 | 0.428 | t = -0.72 | 0.480 |
| | Affected/DBM | 35.8* | 30.1* | 33.8* | | | | | | |

Statistical Techniques: Chi-square tests for categorical variables, independent samples t-tests for continuous variables. Note: * indicates mean age in months for the respective groups

DISCUSSION

This study provides critical, nationally representative evidence of the evolving DBM among Pakistani children, revealing a complex landscape where high stunting (53.3%) coexists with a concerning rise in overweight (22.7%). This transition, driven by rapid urbanization and shifting dietary patterns, signals a pressing public health crisis that challenges traditional, single-focus nutrition interventions, necessitating the "double-duty actions" recommended by global health authorities [16, 17]. The stark socioeconomic gradient for overweight increasing from 10.0% in the poorest to 36.7% in the richest quintile underscores the influence of growing household purchasing power on access to processed, energy-dense foods, a pattern increasingly documented in South Asia and similar developing contexts [18, 19]. Conversely, the pervasive nature of stunting across all wealth strata highlights persistent systemic failures in addressing underlying determinants like food insecurity, poor sanitation, and suboptimal infant feeding practices, consistent with findings from other low-income settings where stunting remains intractable across economic classes [20]. Our analysis confirms that the risk of stunting accumulates with age, a testament to the long-term consequences of nutritional deficits and environmental enteropathy. Recent research on gut microbiota and linear growth faltering provides a biological basis for this observed cumulative deficit, where prolonged exposure to suboptimal conditions manifests in worsening stunting with age [21]. The trend toward higher overweight in urban areas and among females, while not statistically significant in our sample, aligns with global data on obesogenic environments and gendered feeding practices, warranting targeted investigation as seen in studies of the triple burden of malnutrition [22]. These findings collectively

necessitate a paradigm shift in Pakistan's public health policy. Moving forward, integrated, multi-sectoral strategies are urgently needed to break the intergenerational cycle of stunting while simultaneously curbing the rise of childhood overweight through regulatory measures and public awareness, echoing the comprehensive approach advocated by UNICEF and other international bodies [1]. Furthermore, the statistical power of the bivariate and regression analyses was limited by the sample size and its distribution, such as having only 30 children per wealth quintile, which constrained our ability to detect significant associations for some nutritional outcomes.

The study is limited by its cross-sectional design, which precludes causal inference, and by the relatively small analytical subsample, resulting in limited statistical power and unstable estimates for household-level DBM. Additionally, reliance on secondary data restricted the inclusion of dietary intake and physical activity variables. Future research should employ larger nationally representative samples, longitudinal designs, and incorporate behavioral and environmental factors to better understand pathways driving the double burden and to guide targeted, double-duty nutrition interventions in Pakistan.

CONCLUSIONS

Based on the study findings, the double burden of malnutrition is evident among Pakistani children under five, characterized by high stunting (53.3%) and emerging overweight (22.7%). Stunting persists uniformly across all wealth quintiles, while overweight demonstrates a clear socioeconomic gradient, rising from 10.0% in the poorest to 36.7% in the richest households. Older child age

significantly increased stunting odds, and wealth was a strong determinant of overweight. These findings highlight the urgent necessity of concerted nutrition policies that will at once solve problems of chronic under-nutrition and the escalating problem of overweight in Pakistan.

Authors' Contribution

Conceptualization: IF
Methodology: MI
Formal analysis: MI
Writing and Drafting: IF, NA
Review and Editing: IF, MI, NA

All authors approved the final manuscript and take responsibilities for integrity of work.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The author received no financial support for the research, authorship and/or publication of this article.

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