

Epidemiology, Prevalence and Preventive Guidelines of Hepatitis B Virus Infection

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ABSTRACT

This study aims to determine the incidence of hepatitis B infection in the District Multan region while also focusing on risk factors and epidemiology, which are the primary causes of HBV infection. Individuals whose ages are between 18-50 are included in data sampling. Individuals who have a history of any hepatitis infection or having any chronic illness involved in exclusion criteria. By taking ethical approval and informed consent from individuals summarized data like medical history, behavioral pattern and recent travel to



high frequency rate areas is gathered. To inquire about HBsAg blood sample is collected, synthesized and examined by using serologic methods. Further analysis is done on participants whom are tested positive for HBV using diagnostic techniques. The findings of this research is support in understanding the roots of HBV and linked risk factors and help us in reaching insights to take preventive steps for curing HBV and further which strategies are to be taken in future.

Keywords: HBV, Prevalence, Risk Factors, Hepatitis.

1. INTRODUCTION

Particularly in the last 20 years, Pakistan's high hepatitis prevalence has been a serious public health concern. The term "hepatitis" comes from the Latin and means "liver tissue inflammation." This serious public health issue globally, especially in developing Asia. The disease is mostly viral in nature, often leading to further liver complications and in rare cases, hepatocellular carcinoma. Even though a single virus subtype usually causes infections, co-infections involving many viruses are possible (Lavanchy and Kane, 2016). Hepatitis can cause more serious conditions including cancer or liver cirrhosis, as well as acute or chronic symptoms. Even with the presence of non-viral causes such autoimmune illnesses and drugs, viral infections remain the most common cause (Qureshiet *al.*, 2010). Hepatitis B virus is a serious threat to international health. HBV is a serious worldwide health risk that is more prevalent in underdeveloped nations. Among the approximately 2 billion affected individuals worldwide, 400 million suffer from the chronic form. Nine million people in the developing country of Pakistan are affected by the high frequency of HBV. Newborns with HBV infection rates drop from over 90% at birth to about 25% by the time they turn five years old. Numerous risk factors influence the complex epidemiology of these viruses. Globally, hepatitis B virus infection is a major health concern. Worldwide, there are over 2 billion HBV infections, 400 million of which are chronic HBV infections. Nine million people in Pakistan are afflicted with HBV, and the country is becoming more and more endemic for the virus (Razavi-Shearer et al., 2018).

The absence of adequate medical facilities is the basic state of the economy, and minimal awareness among the public of the spread of serious infectious illnesses like HBV.



Each person has a different chronic hepatitis clinical course and outcome. HBV infection can produce a variety of clinical symptoms, such as HCC, acute self-destructive infection, aggressive hepatic failure, or chronic hepatitis leading to liver disease, as well as a silent carrier condition. The number of studies is too small to present a whole image of the national prevalence of HBV, particularly in otherwise healthy people. Since the majority of earlier research focused on particular small groups of people with specific clinical indications, it is inaccurate to say that these findings fully represent Pakistan's prevalence overall. This research provides a brief overview of the frequency, protective factors, and knowledge level, and common HBV genotypes among Pakistani people (Sheikh *et al.*, 2011). Hepatitis B is a highly drastic disease that is highly fatal and produces a high rate of morbidity worldwide. HBV infections affect about one-third of the global populace. A quarter of the population, or around 5% of them, are chronic carriers, and they have the potential to develop every liver condition such as cirrhosis, hepatic cancer, and chronic hepatitis. Globally, 780000 deaths linked to HBV are reported each year. The age of infection has an inverse relationship with the likelihood of having a chronic illness and its aftereffects. If the infection happens there is a 90% likelihood that childbearing will result in chronic illness and its aftereffects (Basit *et al.*, 2014). In East Asian and sub-Saharan African nations, HBV is typically transmitted by the perinatal or horizontal route. However, high-risk sexual behaviour or injectable drug use account for most illnesses that affect young adults in affluent nations.

World Health Organization (WHO) statistics show that 2 to 5% of the subcontinent's population has hepatitis B, making it is among the most highly infected in the world. Nearly one-third of Pakistan's HBV-infected population is also found to be co-infected with hepatitis D virus, a defective RNA virus that needs HBV to replicate. The prevalence of these blood-borne illnesses has been evaluated in the two groups at high risk and the seemingly wholesome populace. This is most likely because the study venues, sampling strategy, and eligibility requirements differed. Overall frequency of HBV among adult populations and blood donors that seems to be healthy is found to be between 0.84, 6.9%, 0.19, and 22.2%, respectively, in a number of studies (Chen *et al.*, 2000).

In wealthy countries, piercings drug use, needle-related damage, transfusions of blood, and sexual encounters and prenatal exposure are the primary modes of transmission. However, in developing countries such as Pakistan, the use of contaminated injections and other non-sterilized medical equipment often contributes to the spread of the disease, resulting in 8–16 million cases of HBV (Houet *et al.*, 2005). In wealthy nations, HBV infections are falling, while in developing nations, they are rising. These illnesses have spread swiftly throughout Pakistan in the last 20 years 2007–2008; the US conducted the first comprehensive nationwide analysis of HBV prevalence (Kwon and Lee, 2011). Then, in June 2010, Pakistan launched a national hepatitis sentry site surveillance program. However, this system’s scope is limited to Islamabad and regional capitals due to financial constraints. Furthermore, the national survey did not include high-risk populations; instead, it concentrated on seropositivity within the broader populace. In Pakistan, the prevalence of hepatitis B is a major public health concern (Razavi-Shearer *et al.*, 2018).

Multiple strategies are needed for an effective approach to viral hepatitis prevention, as listed below. Education disease awareness-focused education initiatives reduce the spread of disease. In poor nations, a significant percentage of people with chronic hepatitis are unaware that they have the illness (Fanning *et al.*, 2019). The introduction of local health measures and awareness initiatives aimed at educating the entire community are crucial. These include establishing efficient screening procedures for the distribution of donated blood transfusions and teaching the community about safe blood transfusion practices practices (Mast *et al.*, 1999). Administering safe injections in medical settings and among drug users, as well as implementing safer sexual behaviours, should be part of health education. Health workers should also receive occupational safety training. Additionally, it’s critical to discuss clearly and stress the significance of testing for the medication to help eradicate viral hepatitis, including monitoring appointments and follow-up visits (Revillet *et al.*, 2019).

1.1 Problem Statement

Globally, hepatitis B virus affects 240 million people which resulting in 1.45 million death rates annually. Mortality rate is higher in low-middle income countries especially in Pakistan as lack of knowledge of screening, lack of sources, and illiteracy is



common which results in greater number of death cases and some cases are untreated and undetected. World Health Organization set different strategies to prevent from hepatitis virus and reduce mortality rate to 90% by 2030. To gain this objective proper knowledge of risk factors, causes of hepatitis, epidemiology and preventive measures are to be known.

1.2 Objective(S)

The objectives of the study were

- 1) To assess the frequency rate of hepatitis B virus.
- 2) To identify risk factors related to hepatitis B virus.

1.3 Hypothesis

The implementation of a preventive measure program in order to reduce risk factors which cause hepatitis B prevalence, addressing specific precautionary measures, will lead to reduce prevalence among individuals.

2. Literature review

This investigation aimed to identify the many risk variables linked to infections as well as Pakistan's viral hepatitis prevalence rate. There has also been research done on the genotype's distribution. The inflammation of the liver tissue is called hepatitis. People are seriously at danger for health problems from it, especially in Asia's developing countries. The following portals were searched through Google Scholar. The nationwide prevalence rate was determined using the weighted average. For the hepatitis B virus (HBV), the weighted averages for the antigen and antibodies in non-blood donors were 1.98%, 7.44%, and 5.62%, respectively.

For blood donors, the comparable numbers were 2.41% and 3.31%. The degree of relationship between each and the various illnesses varied; for example, the highest correlation (44.45%) was seen between HBV and chronic liver disease. Intravenous drug users (IDUs) had the highest rate of HCV at 68.3%, whereas healthcare workers had the highest rate of HBV among high-risk groups. Genotype 3 was more prevalent than genotypes 1 and 2. In summary, Punjab, the war-torn districts, and the interior of Sindh were the most affected areas of Pakistan. Internally displaced individuals (IDPs) are forced to live in various makeshift camps throughout Pakistan as a result of mass movement



activities, which serve as refuges for the spread of viruses. The most popular channels of transmission include IDUs, blood transfusions, and barbers(Mehmood *et al.*, 2020).

In an attempt to save lives, around 118.5 million units of blood are donated annually. Blood-borne illnesses may potentially be linked to blood transfusions. Pakistan, home to more than 247 million people, is a country where viral hepatitis is endemic. Of healthy donors, a considerable proportion will be asymptomatic. The population-wide incidence of viral hepatitis is 2.5 percent, which has Pakistani health authorities quite worried about the safety of blood screening and donation.

It is recommended by the World Health Organization to screen for asymptomatic viral hepatitis in order to exclude individuals who may be mildly unwell. Comprehending the prevalence of transfusion-transmissible infectious agents in healthy blood donors may enhance the efficacy of treatment, avert severe consequences for impacted persons, and facilitate the evaluation of the disease burden within any given community. In this study, blood donors who attended the Armed Forces Institute of Transfusion (AFIT) in Rawalpindi, Pakistan, were asked to provide information about their visitation patterns and frequency of transfusion-related infections (TTIs). This cross-sectional descriptive study involved the screening of 15,405 blood donors for HIV, syphilis, HBV, HCV, and malaria. Just 0.7% of donors had AB-negative blood types, whereas O-positive blood types made up the majority. 1.06% HBV, 0.54% HCV, 0.19% HIV, and 0.31% asymptomatic blood donors with syphilis were among the study population. Despite this, no blood donor tested positive for malaria. The majority of respondents were young adults (18–27 years old), and the region of Punjab was found to have the greatest rate of TTIs. These findings imply the need for national awareness campaigns regarding TTIs. Stakeholders must reaffirm the requirements for blood collection and closely assess successful performance through internal and external audits in order to obtain non-infectious blood products(Zorobet *et al.*, 2023).

In underdeveloped nations where vertical transmission is still a common mechanism of transmission, the incidence of hepatitis B infections places a significant financial burden on such nations. In this cross-sectional investigation, 375 pregnant women receiving antenatal care at health institutions were asked about the seroepidemiology and



associated risk factors of hepatitis B virus infections. The study was conducted at the DHQ Swabi in Khyber Pakhtunkhwa, Pakistan, and the Bacha Khan Medical Complex (BKMC) in Shahmansoor. Ten millilitres of blood were taken from 375 pregnant patients who were chosen from both institutions using a rigorous random sample method. The 3rd Generation Enzyme-Linked Immunosorbent Assay (ELISA) and the Immuno-Chromatographic Test (ICT) were then used to evaluate the blood serum in order to find markers. To collect data on prospective risk factors and sociodemographic traits, pre-structured questionnaires were used. A statistical program called SPSS 23.0 was used to analyse the data. The association between the variables was ascertained by the application of chi-square analysis. For statistical analysis, a significance criterion of $P < 0.05$ was used. The study's 375 pregnant individuals had an overall frequency of 2.1% HBV infections (Israret *al.*, 2021).

Since most blood transfusion recipients in Pakistan are patients with beta-thalassemia major, they are more susceptible to diseases like hepatitis B that are transmitted by blood transfusions. This study includes a comprehensive examination of the HBV status in individuals with beta-thalassemia major who are Pakistani. In order to do this, we examined primary studies assessing the HBV epidemiology in individuals suffering from transfusion-dependent thalassemia. Between January and February of 2020, we searched ten of the biggest subscription databases: The following resources are available: Google Scholar, Embase, Science Direct, Web of Science, Medline, PakMediNet, CINAHL, Scopus, PubMed, and Directory of Open Access Journals. We also looked through the database of the World Health Organization to find pertinent reports. There were no language constraints and the search criteria included published papers published up until December 31, 2019. The selected papers were loaded into the Endnote version X9 application, where a relevancy and duplication check was performed. The results were shown as the overall research prevalence as well as for regionally-based subgroups. The evaluation included thirty-three papers published between 1995 and 2019. Of the 33 studies, information on HBV prevalence was present in 19, while it was present in every one of them. The sample size for assessing the prevalence of HBV in thalassemia patients totaled 8,554 individuals. The sample of 6,184 individuals from the 19 research that looked at the prevalence of HBV (Waheed *et al.*, 2021).

One of the major occurring cause of hepatocellular carcinoma (HCC) in the globe is hepatitis B virus (HBV). Depending on the part of the world, the prognosis, incidence, and age of occurrence vary significantly. The varying prevalence of HBV, the age at which the virus transmits, the time at which the virus incorporate into the human genetic machinery, the various HBV genotypes, and environmental factors all play a significant role in this geographic diversity. It causes significant variations in the way viruses interact with the immune system, modify the genome, and ultimately lead to the uplifting of HCC in a particular person. In this study, we outline numerous variables linked to the development of HCC, offer information on populations that are in danger, and clarify social norms around monitoring for HCC in people with HBV worldwide (Anugwomet *al.*, 2021).

Hepatitis B virus (HBV) and/or hepatitis C virus (HCV) combination with HIV has become an important issue in global public health due to their similar modes of transmission, especially in Africa where these viruses are widespread. Demographic data on HBV and/or HCV coinfection with HIV in Africa have been by a small number of systematic research, and none of them have supplied data on the case death rate (CFR) of the combination. Studying the rate and fatal accident rate of HBV and/or HCV infections in African HIV-positive patients was the aim of the course of this research. For the meta-analysis, a random-effects model was used. Subgroup analysis was used to look into sources of heterogeneity, and Egger tests and funnel plots were used to evaluate publication bias. 314 studies out of the 4388 publications that were obtained from the databases satisfied all inclusion requirements. The estimate for the total HBV case fatality rate was 4.4% (95% CI: 0.7–10.3). HBV infection, HCV infection, and HBV/HCV coinfection were found to be seroprevalent in PLHIV at 10.5% [95% CI = 9.6–11.3], 5.4% [95% CI = 4.6–6.2], and 0.7% [95% CI = 0.3–1.0], in that order. In people living with HIV, the pooled seroprevalences of acute HBV infection, current HBsAg, and current HBeAg were 3.6% [95% CI = 0.0–11.0], 7.0% [95% CI = 4.7–9.7], and 10.7% [95% CI = 9.8–11.6], respectively. The seroprevalences of HBV and HCV infection are determined by HBV-DNA and HCV-RNA confirmation(Kenfack-Momoet *al.*, 2022).

Persistent viral liver disease is mostly caused by the hepatitis B virus (HBV). Approximately two million kids below the age of 5 contract the virus each year,

primarily as a consequence of untreated horizontal and vertical spread. The high efficacy phenomenon of vertical HBV transmission, which occurs when preventive measures are not performed, can vary from 10% to 40% for mothers who are antigen-negative for hepatitis E to 70% to 90% for mothers individuals test positive for the virus's antigen. One of the main dangers for HBV transmitted vertically is pregnant viraemia.

Maternal screening is the main protection against HBV vertical transmission. The two most crucial approaches to completely eradicate HBV infection worldwide are antiviral therapy for mothers who are extremely viraemic and hepatitis B passive and active immunoprophylaxis at birth. It takes strategies to advance introduction of hepatitis B immunoglobulins and birth-dose immunization in low- and middle-income nations, where the virus is most common (Veronese *et al.*, 2021).

The frequency and clinical implications of occult HBV infection and HBeAg-negative chronic hepatitis B (e-CHB) are two distinct clinical entities that still need to be investigated. The most common modes of transmission vary according to the extent of HBV infection. In locations where HBV endemicity is low, sexual contact between high-risk persons is the predominant mode of transmission; in places where HBV endemicity is high, perinatal transmission is the primary mode of infection. HBV has been split into seven genotypes, or A to G, based on the divergence of the complete genome sequence. Each genotype has a distinct geographic distribution. Three main strategies have been found to be successful in preventing HBV infection. They include behaviour modification, passive immunoprophylaxis, and active immunization. Since 1991, many countries have greatly decreased the prevalence of HBV infection and HCC as a result of the WHO's advice to conduct a mass immunization program against HBV.

The pooled prevalence of cirrhosis was 4.1% (95% CI 2.6-6.4) in primary care or general population settings, but it was 12.7% (95% CI 8.6-18.3) in referral or tertiary care facilities (adjusted hazard ratio 0.29, 95% CI 0.15-0.56). Age, gender, fibrosis test utilised, or co-infection with HIV do not correlate with cirrhosis. Conclusions: 4% to 13% of HBV-infected people in SSA have cirrhosis and require rapid antiviral medication, depending on the situation. When deciding on HBV treatment plans and allocating resources, these estimates should be taken into account (Suria *et al.*, 2021).

Regarding the clearance of HBV infection, chronic hepatitis B virus (HBV) infection caused by vertical transmission continues to be a major challenge. The hepatitis B vaccine, which is the main concerning point for preventing transmission throughout pregnancy and during pregnancy, has decreased the prevalence of HBV by more than 80%. In countries such as China and the US, it is normal practice to give hepatitis B immune globulin and the hepatitis B vaccine to children whose mothers test positive for hepatitis B surface antigen. This is because these interventions effectively prevent vertical transmission. Growing data regarding the effectiveness and safety of antiviral prophylaxis during pregnancy suggests that the World Health Organization's 2030 target of eradicating hepatitis is likely to be achieved. We go over the vertical transmission's transmission pathways, diagnostic standards, and preventative measures in this review. "Perfect strategies" to eradicate vertical transmission can be found in a preventive program that involves screening prior to conception, antiviral prophylaxis during pregnancy, and post pregnancy immunoprophylaxis. The effectiveness and necessity of HBIG, particularly in mothers who test negative for hepatitis B envelope antigen, and the inadequate coverage of timely birth dose vaccinations are two examples of the significant gaps that still exist between "perfect strategies" and practical implementation. Specifically, a thorough long-term safety profile of antiviral prophylaxis is obviously needed. Thus, it is necessary to identify preventive measures that are both practical and affordable across regional boundaries. In order to satisfy the requirements for prophylaxis and prevalence targets, access must also be increased(Liu *et al.*, 2021).

3. Research Design

This research employed a quantitative as well qualitative methodology to delve into the intricate facets of health awareness and lifestyle practices among residents in Multan, Pakistan, with a specific focus on prevalence and preventive guidelines of hepatitis B. Utilizing survey data, we explored the geographic distribution and demographics of respondents, shedding light on the diverse perspectives emanating from different localities and age groups. The study investigates participants' awareness of healthy living, barriers to seeking preventive measures, and their understanding of specific health conditions,



particularly hepatitis B. By unraveling these insights, we aimed to contribute to a nuanced understanding of the community's health landscape, paving the way for targeted interventions and initiatives tailored to their needs. To assess the reason about lack of interest of people towards preventive guidelines of hepatitis B asked such questions given in table to the people who are facing hepatitis B but not taking any preventive measure.

3.1.1: Sampling techniques

HBsAg identification using ICT Using ICT strips (Acon USA), HBsAg antibodies were found by following the manufacturer's recommendations. After removing the strip from the foil bag, it was laid out on a clean, dry surface. Then, two drops of a buffer were dispensed together with 5 μ L of serum each for HBsAg detection that had been decanted in the strip. The results were interpreted based on the emergence of colour bands after 15 minutes. There was also a control group that is used to verify the validity of the test strip. The membrane of the strip took acquired a purplish-red hue in both the test and control bands, indicating a favourable outcome. In the control zone (C), a single red line could be seen in the strip's layer. A negative result was indicated by the test area's lack of a red line. As directed by the company, HBsAg was identified using a third-generation ELISA (EASE BN-96 TMB, Taiwan). Three wells that have each been pre-coated with anti-HCV and HBsAg antigens were removed and placed in a holder. 50 microliters of specimens, together with a positive and negative control, were added to their designated wells. Next, each well (except from the blank) receives 50 μ L of horse-reddish peroxidase conjugate (HRP- conjugate), which was introduced and mixed by gently patting. The plate was sealed with an adhesive slip and incubated for one hour at 37°C. Following the incubation period, the adhesive slip was removed from each well and diluted buffer was used five times to wash it. In each well, including the blank, 50 μ L of chromogenic solution A and 50 μ L of chromogenic solution B were added.

The mixture was then created by gently patting the plate for 15 seconds. After that, the plate was incubated for 15 minutes at 37°C without shaking in the dark. To halt the reaction, add 50 μ L of stop-solution. Using a spectrophotometer set to 492 nm, the absorbance of the specimens and controls was obtained in 15 minutes on plate. Prior to the addition of the stop solution, the enzymatic reaction between the HRP-conjugate and



chromogenic solutions produces a blue colour in the positive control and HBsAg positive sample wells. The blue colour of the HBsAg positive wells and the positive control well changed to yellow when the stop solution was added; the negative samples looked like clear water both before and after the stop solution was dispensed. For HBsAg, a sample was deemed reactive if its absorbance value was more than or equal to the cut-off value, or 2.00. A sample that was less than the cut-off value was deemed HBsAg negative.

3.1.2 Study setting and population

This cross-sectional study examines HBV prevalence in men and women aged 18–60 from the public. Patients with chronic diseases like diabetes, chronic kidney disease, and tuberculosis and those with Hepatitis B vaccination records were excluded. The WHO sample size calculator was used to calculate the study's sample size based on prior research's expected HBV prevalence of 0.855% (Shah *et al.*, 2023), a 95% confidence level, and a 5% absolute precision. A non-probability convenience sampling method was employed. The study was carried out within healthcare facilities, including hospitals, clinics, and laboratories, located in District Multan, ensuring a representative sample from this region. Detailed information was collected, including the individual's name, age, gender, marital status (married/unmarried), education level (illiterate/literate), and risk factors including previous hospitalizations, surgeries, blood transfusions, needle injuries, reused syringes, shaving practices at barbershops (for males), visits to beauty parlor's (for females), dental extractions, sexual behavior, intravenous drug use, tattooing or body piercing, occupational exposure, household contact with HBV-infected individuals or recent travel to regions with high HBV prevalence. Subsequently, a trained phlebotomist will draw approximately ten milliliters of blood from each participant using a serum separator tube for blood sample collection.

3.1.3 Sampling technique

This research study involved a sample of 375 individuals, comprising participants suffering from hepatitis B virus infection and medical precautions. The first group, consisting of participants, provided quantitative data, while the second group of positive individuals yielded qualitative data. For the initial group, researchers utilized the chi-square test to assess the awareness of risk factors which causes hepatitis by comparing



participants' health conditions before and after the intervention. As a corroborative measure, serological tests are conducted on all participants before and after acquiring about history. The findings provide a comprehensive understanding on the prevalence and risk factors of hepatitis B virus infection.

3.1.4 Sample Selection

3.1.4.1 Inclusion Criteria

Individuals whose ages are between 18-60 were selected for participation in survey. The people residing in urban areas of District Multan were chosen to play their role in survey. The medical records of all participants were reviewed to determine their eligibility for the research, taking into account history of any type of hepatitis as well as those who were diagnosed with hepatitis B at the time of the survey.

3.2.4.2 Exclusion Criteria

People below the age of eighteen were not included in the study. The survey does not include those who did not live in Multan. Those who are incapable of giving informed consent were not included. Analysis not include participants whose information was not dependable. People who live outside of cities were not included in the study in order to keep the focus solely on the urban population. The study did not include pregnant women.

2.1.5 Data Collection

Blood was extracted into two vacutainers from the donor. The vacutainers containing ethylene diamine tetra-acetic acid (EDTA) anticoagulation was used to measure the contributors' hemoglobin, haemoglobin level, count of platelets, and blood types. Using the other vacutainer that was not including an anticoagulant serum is obtained in order to screen for HBV. Only well-groomed donors of blood who visited the health facility and met the WHO and SBTG contributor criteria for eligibility were eligible to give blood. A structured interview was conducted with the participants to collect data on their medical history, demographics, and possible causes for concern for HBV infections. Blood samples are used in serological testing to determine the frequency of HBV was obtained.

3.2.6 Screening of Samples

The blood samples are centrifuged at 3000 rounds per minute for 20 minutes to facilitate serum separation. A test kit named immunochromatographic test was used for

serologic testing for hepatitis B surface antigen. Further, two techniques were employed for confirmation of results. Those techniques were PCR and enzyme linked immunosorbent assay techniques. Individuals whom were tested positive for hepatitis B virus after applying (ICT, ELISA and PCR) techniques were further focused in data analysis. Moreover, individuals whom were tested for HBV, advised to take medical treatment.

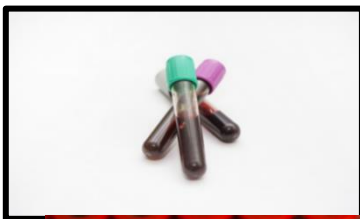


Fig 2.1: Diagrammatic representation for screening of sample

3.2.7 Statistical Analysis

Statistical analysis using SPSS ver.27.0 was use for collecting data related to this study or project. Numerical value i.e. age as mean and standard deviation is presented by using descriptive analysis, while the variables (gender, marital status, education and causing agent) that were categorical variable was present in term of percentage and frequency. Thematic analysis was use for analysis of qualitative data, in which supplementary file were guide for sub themes and identified themes. Chi square test was applied to evaluate association between factors like gender, marital status, education, causing agent and positive HBV. $p \leq 0.05$, a significance level considered to be significant statistically.

4. Results

This chapter delves into a comprehensive examination of the analytical procedures employed, the quantitative data collection techniques utilized, and the outcomes derived from this phase of the study. A detailed exploration of the results was presented, drawing upon references to recent literature as outlined in previous section furnishes details about the research participants' characteristics, while the methodology chapter has previously expounded upon the data analysis process. The ensuing sections of this study wereshowcasing the assessment of both measurement and structural models, coupled with an in-depth analysis of each variable and hypothesis. The chapter concludes by consolidating all analytical findings.

4.1 Reliability Analysis

The reliability of the study's constructs was assessed through their internal consistency, gauged by reliability. When the Alpha (α) value surpasses 0.70, the construct was deemed reliable. The evaluation of construct reliability was conducted utilizing Cronbach's Alpha in this study.

Table 1: Reliability Statistics Table

	Response of risk factors	Response of serological test
Chi-Square	94.593	87.206 ^a
Difference frequency	2	2
Asymptotically Sig. (Alpha Value)	0.0001	0.0001

Table 2.1 shows that our data's overall reliability statistics was 0.819, which was good for analysis. This questionnaire has two sections including risk factors section and serological testing in all which were then separated into other sections. 374 Individuals comprised our sample. Almost all the findings were reliable.

4.2 Demographic Profile

To ascertain if the study participants effectively represented the broader target population for generalization, it was crucial to gather demographic information about them. Typically detailed in the methods section of a research report as independent variables in

the research design, demographics or characteristics of research participants play a key role. Given that demographic variables were inherent traits that cannot be altered; they were inherently classified as independent variables.

43: Descriptive analysis

Descriptive analysis serves as a valuable tool for interpreting all respondent data, enabling researchers to gain a deeper understanding of the selected sample population, including mean values, and the demographic characteristics of respondents. Additionally, descriptive analysis provides a succinct summary description of both respondents and their observations. In the context of this research, demographic variables include age, gender, race, job status, and years of working (Kemp *et al.*, 2018).

4.4: Description of the size of sample

This involved providing information about the number of participants or data points in study. It helped to contextualize the subsequent analyses and indicates the generalization of findings.

4.5: Age Distribution

The data provides a demographic breakdown of participants in an event or group, categorized into eight age groups ranging from 18-23 to 54-60. The data shows that the most common age groups are 36-41 (16%), 30-35 (14.7%), and 24-29 (14.7%), while the least common age groups are 54-60 (6.7%) and 48-53 (12%). The distribution of participants across age groups appears bell-shaped, with a concentration in the middle age ranges and fewer participants in younger and older age groups. The data could be used in educational institutions, workplaces, or surveys targeting specific age groups or populations. However, a definitive interpretation is difficult without more contexts. The data could be used to analyze the age distribution of students in a course or program, the age demographics of employees in a company, or in a survey or event.

Table 2: Age Distribution

Age Group	Number of participants (percentage)
18-23	45 (12%)
24-29	50 (13.3%)
30-35	55 (14.7%)
36-41	60 (16%)
42-47	55 (14.7%)
48-53	45 (12%)
54-60	25 (6.7%)

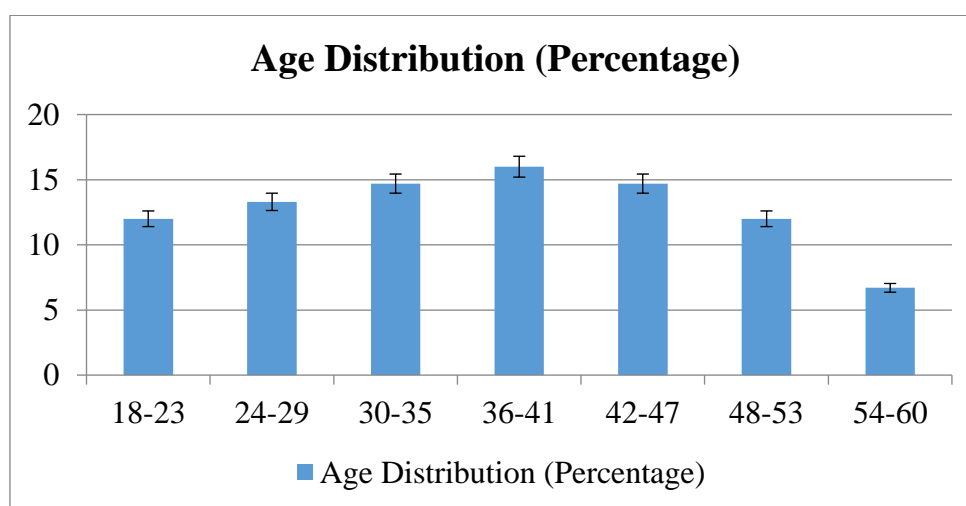


Fig 1: Demographic profile (Age) of individuals

4.6: Gender Distribution

The pie chart shows a gender distribution of a specific group, with 35% being male and 65% being female. This indicates a higher proportion of females than males, with a female-to-male ratio of approximately 1.86:1. This data can be used in various fields, such as demographics, marketing, and social research, to inform government policies, tailor products and campaigns, and study gender differences in education, employment, and health.

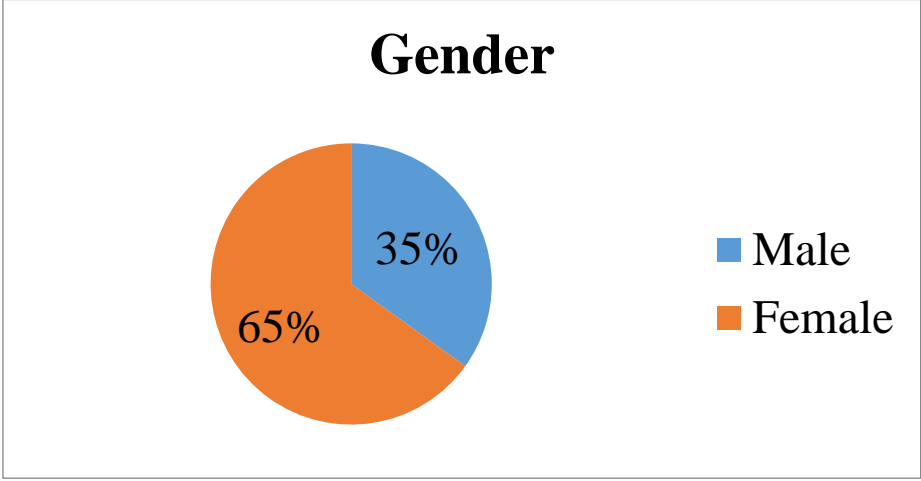


Fig 2: Demographic profile (Gender) of individuals

4.7: Prevalence of Hepatitis

The pie chart shows the prevalence of Hepatitis B Virus (HBV) in a population, with 4% positive and 96% negative. This indicates a small percentage of the population (4%) infected with HBV, but it's crucial to note that HBV is highly contagious and can cause serious health consequences like liver damage and cancer. This data can be used in public health, healthcare, and research to develop effective prevention and treatment strategies, identify individuals at high risk, and study factors contributing to HBV transmission for new prevention methods.

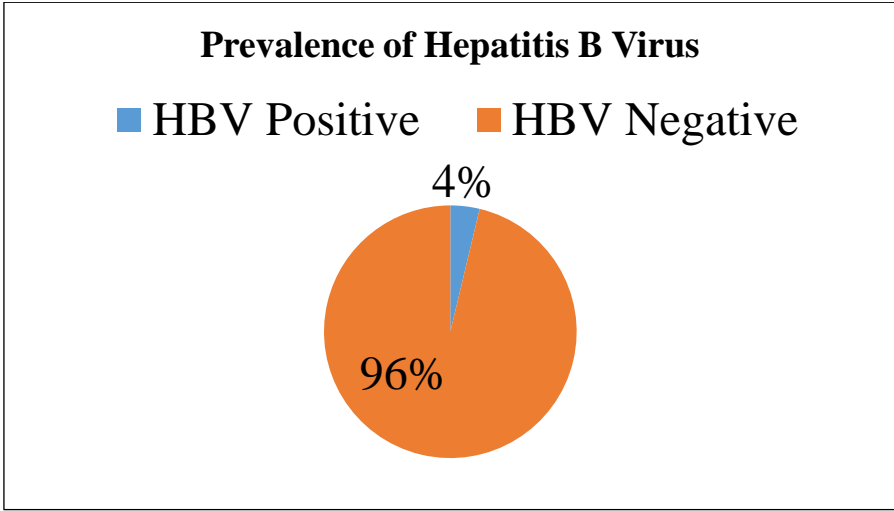


Fig 3: Prevalence of Hepatitis B Virus

4.8: Analysis of Hypothesis

4.8.1. Chi-square (Quantitative)

Certainly, our explanation provides a clear overview of the use of the Chi-Square test in assessing associations among categorical variables. The Chi-Square test was a widely used method for evaluating associations among categorical variables. It was a common practice to examine correlations between categorical data using the Chi-Square statistic. The null hypothesis of the Chi-Square test posits that there was no association between the categorical variables in the population, indicating that they were all independent of each other.

When conducting a cross-tabulation, the Chi-Square statistic was frequently employed to assess Tests of Independence, also known as a bi variate table. In this type of table, the intersections of the categories of the categorical variables are presented in the cells, along with the distributions of the two category variables. The test of Independence evaluates whether there is a link between the two variables by comparing the observed pattern of cell responses with the expected pattern if the two factors were truly independent of each other. This analysis is particularly useful in determining associations and dependencies among categorical variables.

Table 3: Chi-square test on quantitative data

	ICT	ELISA
Chi-Square	94.593	87.206
Difference frequency	2	2
Asymptomatically Sig. (Alpha Value)	0.0001	0.0001

Interpretation

The p-value (probability value) was used to compare the results. The p-value, which determines whether or not our null hypothesis is true, was an exact probability number. It was comparable to the alpha value, which measures the level of significance. Current study results were significant, and they draw the conclusion that the variables had significant

relationships between them if the value of p was less than alpha value. So, p-value 0.0001 was smaller than 0.05 so we rejected our null hypothesis.

2.3.8.2: Chi-square test (Gender)

The table displays the distribution of Hepatitis B Virus (HBV) positivity among males and females in a population. Out of 131 males, 5 tested positive, representing 3.8% of the male population. Out of 244 females, 9 tested positive, representing 3.7% of the female population. Overall, 14 out of 375 individuals tested positive for HBV. The chi-square test ($X^2 = 0.007$, p-value = 0.933) showed no significant difference in HBV positivity between males and females, suggesting that the observed difference could be due to chance. The data and chi-square test indicate no significant difference in HBV positivity between males and females in this population. However, the small sample size may limit the statistical analysis's power.

Table 4: Chi-square test on Gender

	HBV Positive	HBV Negative	Total
Male	5	126	131
Female	9	235	244
Total	14	361	375
	$X^2 = 0.007$	p-Value = 0.933	

4.8.2. Paired T-Test

The paired samples *t* Test measures the difference of two measurements taken from the two dependent groups. Serological test applied to compare the progress in hepatitis B infectious patients.

4.8.3 Socio-Demographics of the Study Population

The research had 375 participants, yielding a 100% response rate. Of the participants, 131 (34.93 %) were male and most are between the ages of 20 and 29. Another 244 (65.07 %) were between the ages of 30 and 39, and 29 (7.7%) are 40 years of age or older. 61.6% of the women lived in rural areas. In terms of education, 167 (44.5%) of the women completed secondary school or higher, 111 (29.6%) only completed elementary school, and 97 (25.8%) never attended any formal schooling. Housewives make up 209

(55.7%) of all pregnant moms, followed by workers (166, 44.2%). 177 (47.2%) of the contributors reported a family scheduled earnings of Rs 15000 or less, followed by 143 (38.1%) with a family scheduled earnings of Rs 15000–20000, and 55 (14.6%) with a family monthly income of Rs 20000 or more.

Table 5: Socio-demographics of population

Characteristics		Total n (%)	HBV Infection	
			+n (%)	p-value
Residential status	Urban	145 (38.6)	4 (2.7)	0.017
	Rural	230 (61.6)	10 (4.3)	
Scholastic level	Illiterate	97 (25.8)	5 (5.2)	0.048
	Primary	111 (29.6)	6 (5.4)	0.124
	Secondary and above	167 (44.5)	3 (1.8)	
Family monthly Income (Rs0)	Lower	177 (47.2)	7 (3.9)	0.863
	Middle	143 (38.1)	5 (3.5)	0.963
	Highr	55 (14.6)	2 (3.6)	
Occupation	Employed	166 (44.2)	5 (3.0)	0.528
	House wives	209 (55.7)	9 (4.3)	

Ten risk factors for HBV were examined in this studied, including a history of drug used, body tattoos, dental extractions, abortions, eyebrow shaving, previous deliveries at medical facilities, surgery, hospital admissions, blood transfusions, and foreign travel history. Out of the 375 participants in the studied, 2 (3. 5%) of the HBV female and 2 (3. 5%) of the HBV male had a history of sexually communicated diseases (STDS); 3 (5. 2%) of the HBV female and 1 (1. 7%) of the HBV positive male had tattoos on their bodies; 4 (19. 1%) of

the HBV female and 1 (3. 8%) of the HBV positive male had having dental extractions done. One (5. 5%) HBV female and one (5. 5%) HBV positive man had a history of abortion and infertility, respectively. Two (5. 7%) HBV females and 0 (0. 00%) HBV positive males often shave their eyebrows. Table 3.2 shows that there was no correlation between HBV seropositivity and the history of foreign travel for women. Specifically, 1 (2. 7%) of HBV females and 4 (10. 8%) of HBV positive males had had surgical procedures; 4 (7. 1%) of HBV females and 2 (3. 5%) of HBV positive males had had hospital admissions; and 5 (14. 2%) of HBV females and 1 (2. 8%) of HBV positive males had had blood transfusions.

Table 6: Risk factors associated to HBV infection and seropositivity status

Risk factors	Response	HBV Sero-status		X ²	p-value
		+V n (%)	-V n (%)		
History of drugs	Yes	2 (3.5)	55 (96.5)	0.01	0.922
	No	12 (3.7)	306 (96.2)		
Tattooing on body	Yes	3 (5.2)	55 (94.8)	3.08	0.079
	No	11 (3.4)	307 (96.5)		
Dental Extraction	Yes	4 (19.1)	22 (84.6)	10.55	0.001
	No	10 (2.8)	339 (97.1)		
Abortion (females)	Yes	1 (5.5)	17(94.4)	0.17	0.676
Infertility (males)	No	13 (3.6)	344 (96.3)		
Shaving eyebrows (female)	Yes	2 (5.7)	50 (96.1)	0.03	0.961
Shaving (male)	No	12 (3.7)	311(96.2)		

Surgical procedure	Yes	1(2.7)	36(97.3)	0.12	0.729
	No	13(3.8)	326(96.1)		
Hospital admission	Yes	4(7.1)	52(92.8)	2.13	0.144
	No	10(3.1)	309 (96.8)		
Getting blood transfusion	Yes	5(14.2)	30(85.7)	11.96	0.0005
	No	9(2.6)	331(97.3)		
History of holiday at abroad	Yes	0(0.00)	8 (100)	0.32	0.573
	No	14(3.8)	353 (96.1)		

Rate of prevalence for HBV infection

The total prevalence of HBV identified by ELISA and ICT was 3.7%. It was discovered that the other parameters do not suggestively correlate with the frequency of HBV. It was discovered that a strongly linked risk factor with HBV sero-positivity was the surgical operation ($P = 0.0001$) (Table 3.1). In a similar vein, only women in the 20–29 age range are seropositive for HBV. According to statistical analysis, there was a statistically noteworthy difference in the affiliation between HBV seroprevalence and female age ($P = 0.033$) (Table 3.2). The remaining risk variables, however, did not significantly affect HBV seropositivity.

Serological Testing

In both clinical trials and clinical practices, serological indicators of HBV infection such as HBsAg, HBeAg, anti-HBe, and HBV DNA level have been widely acknowledged as valuable instruments for the diagnosis and follow-up of HBV illness.

Table 3.4: Serological testing in patients with HBV infection

	Acute HBV	Chronic HBV	Cleared HBV	Vaccination
HBcAb IgM	+	-	-	-
HBcAb IgG	+	+	+	-
HBsAg	+	+	-	-
Anti-HBs	-	-	+	+
HBeAg	+	+/-	-	-
Anti-HBe	-	+/-	+/-	-
HBV DNA	High/Low	Low/High	-	-

6. DISCUSSION

A serious health issue that affects a lot of nations large population is considering HBV surely. This study is showing that the prevalence of HB infection is rising generally and that the 25–44 age range is the main area where these infections are found. Ninety-four percent of the participants test positive for HBsAg, and patients who are married, had children, or lived in an urban environment are more likely to have these infections. A antiquity of surgical treatment is the most typical risk aspect among the participants, taking into account 41.02% of cases (Hou et al., 2005). The disparity in the years between the two studies may be one cause of this disagreement. An rise in intravenous drug users (IDUs) can be justify by the rising trend of HCV infection. As a result of IDUs, HBV infection is really becoming more common in our nation. An rise in incidence may be correlate with an increase in annual blood donations, caesarean deliveries, and surgical procedures. It is important to highlight that there is no statistically significant rise in trends. The low frequency of HBV contamination in people under 25 is accredited to immunity and widespread immunisation against HBV infection, which began in 1993 among newborns. The age group of 25–44 years old have the highest prevalence of HBV during this investigation. One possible explanation for the low incidence rate of HBV in this age range is the common occurrence of drug dose among vaccination consumers. It should be highlight that while a small percentage of participants indicate a history of drug use as a risk factor, around 57% of them decline to respond to this inquiry.



The majority of them, including the sex workers, were reluctant to respond to this question since injecting drug usage is prohibited and stigmatized. Despite their low frequency, health planning should take into account the high incidence rate of HBV and HCV infections in the over 65 age group. In their home, more than 16% of individuals found to be positive cases of HBV. Research has indicates a comparatively elevated frequency among family members of patients who test positive for HBV. Fortunately, National vaccination program's guidance stated, high-risk groups, including healthcare staff, other family members of positive patients, and consumers of blood products, are being immunized against HBV infection. One reason may be that they live in the same area. There is no significant variation in the disease's incidence between males and females. This may have to do with the fact that women are more likely than males to undergo screening procedures due to the examinations they undergo during pregnancy. In exchange, males are more likely than females to be incarcerated and to share needles among intravenous drug users. The findings of a population-based study conducted in three Iranian provinces revealed that whereas HBV did not significantly correlate with sex, HBV is more frequent in men. Males are shown to have a greater prevalence of HBsAg in certain investigations.

According to residence, this study found that metropolitan regions had greater rates of HBV infection. One explanation for this is because the majority of married individuals is not covered by national immunisation programmes and is therefore not immune to the virus; couples' sexual activity is another factor. The first study restriction relates to the data that is gathered via a passive monitoring method; as a result, incomplete and underreported data are unavoidable in this investigation. The second restriction is that, because HBV infections usually affect prolonged patients asymptomatic or unintentionally identified during blood contribution, pregnancy screening, or as a result of a positive case in another family member, the results strongly suggest underestimation. Passive surveillance systems have drawbacks, such as low sensitivity, but they are also inexpensive, simple to use, and helpful in tracking trends over time and supplying vital data for community health monitoring, all of which align with the goals of our investigation. In summary, our findings demonstrated that the middle age group is the primary location of HBV infections. Further

research is necessary in this area because, even though the hepatitis B vaccine is effective, trends in HBV infections in blood products are rising, suggesting that other risk factors may be important in the development of new infections. Improved blood donor screening and improved sterilisation practices are also important. More emphasis should be placed on immunising high-risk individuals against HBV infection, such as healthcare professionals, other family members of positive patients, and recipients of blood products who have never had a vaccine.

According to the current study, the majority of HBV-infected patients, or 70% (n = 115), are between the ages of 18 and 30, indicating that authorities should be more watchful in their efforts to stop the proliferation of hepatitis virus. The current study's age groups of 31 to 45 years old had a greater the frequency of HBV infection than those in another study. The frequency of HBV-positive donors between the ages of 18 and 30 is shown in a survey conducted in Multan. The identification of HBV infection in the age categories of 31–45 years is similarly reported in the Multan City report. The results of the current study agree with those of other studies from Nigerian literature. In order to establish a healthy society, stakeholders and legislators should carefully consider taking action to stop the spread of all blood-borne infections (Zafar, 2014).

The findings shown in our analysis are a positive consequence of the measures that the local Punjab Blood Transfusion Authority (PBTA) is now putting into effect. However, it is also clear that there is a gradual or sluggish progress being made towards reaching particular outcomes. Healthcare departments are faced with a significant difficulty due to the large patient population and state rules that prohibit diseases. For those who want a safe blood supply, including patients, doctors, legislators, and the public, the rise in disease burden is a serious worry (Aslam et al., 2016). The hepatitis B virus (HBV) is one of several viruses that can cause acute and chronic illness. The national prevalence rate of hepatitis B infection in pregnant women is unknown, despite the fact that it is the most common serious liver infection in the world and has a high risk of morbidity and mortality. Pregnant moms who are infected with HBV should be particularly concerned since knowing their status will help prevent the virus from being passed on to their unborn child during delivery, which will ultimately reduce the baby's risk of having chronic hepatitis (Mahmood et al.,



2016). 49 studies revealed a comparatively large disparity in the reported frequency of HBV infection among Ethiopian pregnant women, from 2.311 to 11.3.12, in this systematic review and meta-analysis. The subgroup analysis revealed substantial regional variances in the frequency of HBV infection in Ethiopia's subnational areas. According to this subgroup analysis, Addis Ababa had the lowest prevalence of HBV at 3.52%, while Somalia had the highest incidence at 7.36. Different cultural practices, behavioral differences in expectant mothers, and variations in socioeconomic status could all be contributing factors to the reason. Furthermore, there are just two studies in the Somalia region that are considered. Subgroup analysis revealed that the year of publishing had a substantial impact on the frequency of HBV infection. The subgroup analysis showed that after 2017, the highest HBV prevalence is seen. One likely reason is that, in order to achieve elimination of viral hepatitis by 2030, the national policy for prevention and control emphasizes the importance of improving early diagnosis of the illness (Mehmood *et al.*, 2020).

Women who were sexually active, particularly those who have a background of several sexual companions, are more likely to become infected with the virus since sexual intimacy can operate as a means of transfer. Additionally, this study demonstrated that expectant mothers with tattoos were more likely to contract HBV than those without. The present discovery aligns with research carried out in Sudan, Rwanda, Nigeria, Cameroon, India. This may be the result of a flaw in the materials used to clean and sterilize the area, which would explain why contaminated blood surfaces, for example, can harbor the HBV for up to seven days. Furthermore, the risk of developing HBV is approximately 4.5 times higher in expectant mothers who had previously had a tooth extracted (Jadoon *et al.*, 2009).

HBV can spread by direct contact with contaminated objects, such as instruments, gadgets, and surfaces, or through contact with physiological fluids like blood or saliva. These routes may result in the disease spreading from one patient to another as well as from dental professionals to patients and vice versa. Furthermore, poor infection control practices in the dental field could provide a major channel for the spread of blood-borne infections like hepatitis B (Ali *et al.*, 2009).

The incidence of HBV infection is approximately 5% in Pakistan. The highest annual rate of therapeutic intramuscular injections per person is found in Pakistan. Barbers'

shaving is becoming identified as an additional means of disease transmission, in addition to the well-known risk factors of blood transfusions, syringe reuse, and inadequate sterilisation of invasive medical devices. Since there is no vaccination to prevent it, hospitals and blood banks that violate standard operating procedures and best clinical practices for sterilising and disposing of medical waste must face legal repercussions. The clinical importance of HBV serotypes is crucial for understanding how the disease progresses and responds to therapy. Types A and D of the HBV serotypes are linked to cirrhosis of the HBV serotypes, type C is linked to liver cancer and type A and D to liver cirrhosis (Waheed *et al.*, 2021).

Studies from Punjab reveal greater HBV genotype C, which is linked to the development of cirrhosis and HCC as well as a worse response rate to interferon or nucleoside analogue therapy as compared to genotype B. Studies from Sindh show a higher prevalence of genotype D. There are six known serotypes of hepatitis C. Studies conducted worldwide have revealed that serotype 3, which has an 80% cure rate, is the most treatable. The most prevalent serotype in Pakistan is 3 (Lin and Kao, 2011). Most research indicates that interferon therapy can produce a 50–70% sustained viral response over a six-month period. The primary causes are not covered by national statistics. According to a Peshawar study, liver illnesses accounted for 23% of all gastroenterology admissions in a given year. 181 examined admission data spanning a year to determine the reason behind 283 deaths out of 8529 admissions. 160 deaths are attributed to medical causes, with chronic liver disease accounting for 33 deaths (20.6%) of all deaths. In order to lower the prevalence and incidence of the disease, the Prime Minister of Pakistan, in partnership with the Ministry of Health and the provincial health departments, launched a hepatitis prevention and control program (Mehmood *et al.*, 2020).

7. Conclusion

The study's reliability was assessed through their internal consistency, gauged by reliability. The overall reliability statistics were 0.819, which was good for analysis. The questionnaire had two sections including risk factors section and serological testing in all, which were then separated into other sections. 374 individuals comprised the sample, and almost all the findings were reliable.



To ascertain if the study participants effectively represented the broader target population for generalization, it was crucial to gather demographic information about them. Demographic variables are inherent traits that cannot be altered; they were inherently classified as independent variables. Descriptive analysis serves as a valuable tool for interpreting all respondent data, enabling researchers to gain a deeper understanding of the selected sample population, including mean values, and the demographic characteristics of respondents. In the context of this research, demographic variables include age, gender, race, job status, and years of working.

The data provided a demographic breakdown of participants in an event or group, categorized into eight age groups ranging from 18-23 to 54-60. The most common age groups are 36-41 (16%), 30-35 (14.7%), and 24-29 (14.7%), while the least common age groups are 54-60 (6.7%) and 48-53 (12%). The distribution of participants across age groups appears bell-shaped, with a concentration in the middle age ranges and fewer participants in younger and older age groups. The data could be used in educational institutions, workplaces, or surveys targeting specific age groups or populations.

The pie chart shows a gender distribution of a specific group, with 35% being male and 65% being female. This indicates a higher proportion of females than males, with a female-to-male ratio of approximately 1.86:1. This data can be used in various fields, such as demographics, marketing, and social research, to inform government policies, tailor products and campaigns, and study gender differences in education, employment, and health.

The prevalence of Hepatitis B Virus (HBV) in a population was found to be 4% positive and 96% negative. This indicates a small percentage of the population (4%) infected with HBV, but it is crucial to note that HBV was highly contagious and can cause serious health consequences like liver damage and cancer. This data can be used in public health, healthcare, and research to develop effective prevention and treatment strategies, identify individuals at high risk, and study factors contributing to HBV transmission for new prevention methods.

Ten risk factors for HBV were examined in this studied, including a history of drug used, body tattoos, dental extractions, abortions, eyebrow shaving, previous deliveries at



medical facilities, surgery, hospital admissions, blood transfusions, and foreign travel history. Out of the 375 participants, 2 (3.5%) of the HBV female and 2 (3.5%) of the HBV male had a history of sexually communicated diseases (STDS), 5 (2.2%) of the HBV female and 1 (1.7%) of the HBV positive male had tattoos on their bodies, 4 (19.1%) of the HBV female and 1 (3.8%) of the HBV positive male had having dental extractions done, one (5.5%) HBV female and one (5.5%) HBV positive man had a history of abortion and infertility, respectively.

The total prevalence of HBV identified by ELISA and ICT was 3.7%. It was discovered that a strongly linked risk factor with HBV seropositivity was the surgical operation ($P = 0.0001$). In a similar vein, only women in the 20–29 age range are seropositive for HBV. According to statistical analysis, there was a statistically noteworthy difference in the affiliation between HBV seroprevalence and female age ($P = 0.033$). The remaining risk variables, however, did not significantly affect HBV seropositivity.

This research offers a scientific foundation for future treatments aimed at lowering the occurrence and frequency of HBV infection in this severely underprivileged population, as well as for the adaptation and design of healthcare services. More thorough examining in especially major risk locations and efficient treatment and preventative service planning can be created with a better knowledge of the regional heterogeneity of HBV as well as Warning signs for getting sick, rates, and viraemic frequency. Implementing focused programs geared towards harm reduction techniques is especially necessary. These programs should educate the public, official, and informal healthcare practitioners about the risks associated with HBV, especially those related to reusing injection equipment. In addition, this will advance Pakistan's national HBV elimination objectives. The highest rate of hepatitis illness is found in Pakistan. At the current rate, eradicating hepatitis seems unattainable. The number of tests and treatments can be increased by introducing the birth dose of the hepatitis B vaccine and by making testing more easily accessible and reasonably priced. Funds must be raised both domestically and internationally to help the eradication of illness.



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Journal of Medical & Health Sciences Review

VOL-2, ISSUE-1, 2025

Online ISSN: 3007-309X Print ISSN: 3007-3081

<https://jmhsr.com/index.php/jmhsr>



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