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A Cross-Sectional Study to Assess the Prevalence of Hepatitis B Serological Markers Among Individuals with A History of Jaundice

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Abstract

Background: Hepatitis B infection presents a significant global health challenge, with Pakistan experiencing particularly high prevalence rates. Moreover, jaundice is often regarded as a non-threatening metabolic condition by most individuals rather than an infectious concern. Given the strong association between hepatitis B and jaundice, this study aimed to investigate the prevalence of hepatitis B serological markers among individuals with a history of jaundice in the state of AJK, Pakistan.
Methods: A cross-sectional study was conducted among 100 participants from the University of Azad Jammu and Kashmir, Muzaffarabad, selected based on the history of jaundice within the last 6 months and aged over 18 years. Data collection included basic ethno-demographic information and screening for hepatitis B using immuno-chromatographic test (ICT) devices forHBsAg, anti-HBc, HBeAg, anti-HCV, and anti-HDV. HBV DNA was extracted for positive HBsAg cases and subjected to PCR amplification. We analyzed the data using GraphPad Prism V. 9.0.
Results: Among the participants, 2% tested positive for HBsAg, 1% for HBV DNA, and 1% for HBeAg. Fortunately, none tested positive for Anti-HCV or Anti-HDV. Age-based analysis showed a higher (11.8%) prevalence of hepatitis B markers among participants aged above 25 years. Male participants showed a higher prevalence of HBsAg compared to females (9.5% vs. 0%; $p = 0.0056; \chi 2 = 7.677$). District-wise prevalence of hepatitis B varied, with Bagh district showing the

highest rate of 10%, while some districts reported no cases. Job-based analysis showed higher prevalence among employees (28.6%) compared to students (0%). Tribal analysis revealed varying prevalence rates, with the Chaudhary tribe showing a positivity rate of 16.7%, and the Khan tribe exhibiting a positivity rate of 12.5% for hepatitis B markers.
Conclusion: The findings highlight the importance of recommending hepatitis B screening for individuals with a history of jaundice, especially among older age groups and males. Understanding the differences in hepatitis B prevalence among different ethnic groups and demographics is necessary for creating effective prevention and control plans in AJK state, Pakistan.

Keywords: Cross-Sectional Study; Hepatitis B Serological Markers; Prevalence of hepatitis B among jaundiced persons; Jaundice and hepatitis B; Control Strategies for hepatitis B; hepatitis B in AJK

1. Introduction

Chronic hepatitis B is a major health problem worldwide, particularly in Asia and sub-Saharan Africa, where it is most prevalent [1]. The incidence of hepatitis B virus (HBV) infection is closely associated with behavioral, host, and environmental factors [2]. Current global estimates indicate approximately 350-400 million individuals suffer from chronic HBV infection, with 80% of cases concentrated in the Asian population [3, 4]. In contrast, North America and Europe exhibit an HBV incidence of approximately 1 per 1000 in the normal population [4]. Annually, 10 to 30 million people worldwide, primarily children and teenagers, contract HBV [5].
The hepatitis B virus significantly contributes to both acute and chronic liver diseases, leading to substantial illness and death worldwide [2, 6]. In developing regions such as Africa and Asia, around 2 billion people display signs of current or previous HBV infection, with 15-25% developing chronic liver infections each year [7]. The ineffective control of HBV infection in developing nations is largely attributed to socio-economic factors and inadequately developed healthcare systems [8]. Transmission of HBV occurs through various means such as blood transfusion, close contacts, sexual activity, and shared syringes. Infection can cause severe conditions like liver cirrhosis and hepatocellular carcinoma, even without traditional parenteral risk factors [9, 10].
The rationale for investigating the prevalence of hepatitis B among jaundice cases lies in the imperative to address the substantial burden of hepatitis B within the population and identify individuals at risk of developing the disease. Jaundice, a common symptom of hepatitis B infection, serves as a potential avenue for mass screening to identify affected individuals and initiate timely treatment [11]. Martin et al. [4] identified that 24% of admitted jaundiced children aged 1 to 15 years had HBV infection.
Recognizing the risk factors linked to hepatitis B is important for developing focused prevention and control plans [12]. Similar studies conducted in the Middle East and Africa have aimed to determine hepatitis B prevalence among jaundice cases and identify associated risk factors. For instance, a study in the United Arab Emirates reported an 18.5% prevalence among patients with chronic liver disease [13], while another study in Nigeria found a prevalence of 22.5% among patients with chronic hepatitis [14].
Building on the findings of Kazmi et al. [15], who reported a history of jaundice in 1.4% (100 out of 7015) of their study participants, our current research, titled "A cross-sectional study to assess the prevalence of hepatitis B serological markers among individuals with a history of jaundice", adopts a focused approach. Specifically, we have chosen this subset of 100 participants for a detailed examination of hepatitis B prevalence, with particular attention to various serum markers and ethno-demographic characters.

2. Materials and Methods

2.1. Population description and inclusion criteria

In this cross-sectional study, participants from University of AJ and K Muzaffarabad were interviewed for consent and questioned about their jaundice history. Those with jaundice history in last 6 months and aged over 18 years were included. A total of 100 participants, with 79% males and 21% females, willingly participated in the study.

2.2. Data Collection

Basic ethno-demographic data of the participants were collected through consent and questionnaire method. Interviews were conducted to make the participants aware about the study background and objectives. Data were subjected to MS Excel and GraphPad Prism software V. 9.0 for analysis purpose.

2.3. Screening for viral hepatitis B, C, and D serological markers

Serum samples were tested to detect HBsAg, anti-HBc, anti-HCV, and anti-HDV using immuno-chromatographic test devices (ICT), following the guidelines provided by the manufacturer (Bio-line Diagnostics, India). For individuals testing positive for HBsAg, HBV DNA was isolated from their serum using the DNAzol method. For this, we followed the methodology of Rauf et al. [16]. The isolated DNA from the serum was then used to amplify the surface gene of HBV via PCR. Due to the low amount of HBV DNA in the serum, a two-round PCR amplification approach was used. The following primers were used in the first round of PCR:

"Forward: 5'-CATCCTGCTGCTATGCCTCATCT-3'"

"Reverse: 5'-CGAACCACTGAACAAATGGCACT-3'"

Primers used in the 2nd round of nested PCR were following:

"Forward: 5'-GGTATGTTGCCCGTTTGTCCTCT-3'"

"Reverse: 5'-GGCACTAGTAAACTGAGCCA-3'"

All the HBV DNA positive samples were further subjected for HBeAg detection through ICT method of same manufacturer as mentioned above.

2.4. Statistical evaluation

The data were analyzed using GraphPad Prism Version 9.0 for statistical analysis. Descriptive statistics were applied for age based prevalence and Chi-square test at $p<0.05$ with 95% CI was used to find the sex based correlation with the hepatitis B.

3. Results

3.1. Overall prevalence of hepatitis B and its co-infections among participants

Among the total of 100 participants, only 1 individual (1%) was vaccinated against HBV. HBsAg positivity was observed in 2 participants (2%), while no individuals tested positive for Ant-HBc. One participant (1%) tested positive for HBV DNA, which was further confirmed for HBeAg (1%). However, none of the participants tested positive for Anti-HCV or Anti-HDV as co-infections with hepatitis B as shown in Table 1.

Table 1. Incidence of various viral hepatitis markers among participants

Total Participants	Vaccinated Against HBV	HBsAg Positive	Ant-HBc Positive	HBV DNA Positive	HBeAg Positive	Anti-HCV Positive	Anti-HDV Positive
100	01	2	0	01	01	0	0

3.2. Age-based prevalence of hepatitis B Markers

Table 2 displays the prevalence rates of viral hepatitis markers categorized by age groups among the study participants. In the 19-25 age group, comprising 83 participants, the mean age was 21.05 ± 0.1939 years, with no individuals (0%) testing positive for HBsAg, HBV, or HBeAg. Conversely, in the age group above 25, consisting of 17 participants, the mean age was 35.35 ± 2.946 years. Within this group, 2 participants (11.8%) tested positive for HBsAg, 1 participant (5.9%) tested positive for HBV, and 1 participant (5.9%) tested positive for HBeAg.

Overall, among the total 100 participants, with a mean age of 23.48 ± 0.7456 years, 2 individuals (2%) tested positive for HBsAg, 1 individual (1%) tested positive for HBV, and 1 individual (1%) tested positive for HBeAg. The findings suggest a higher prevalence of hepatitis B markers among older age group. While the exact reasons remain unknown, increased exposure to risk factors with advancing age may be considered a contributing factor.

Table 2. Prevalence of viral hepatitis markers regarding age groups

Age groups	Participants count	Standard deviation	Mean age ± Std. Error	HBsAg positive participants	HBV positive participants	HBeAg positive participants
19-25	83	1.766	21.05 ± 0.1939	0	0	0
Above 25	17	12.14	35.35 ± 2.946	2	1	1
Total	100	7.456	23.48 ± 0.7456	2	1	1

3.3. Sex based prevalence of hepatitis B markers

From 79 female participants, none tested positive for HBsAg, HBV, or HBeAg. In contrast, among the 21 male participants, 2 individuals (9.5%) tested positive for HBsAg, 1 individual (4.8%) tested positive for HBV, and 1 individual (4.8%) tested positive for HBeAg. Table 3 and Figure 1 display the prevalence of hepatitis B serological markers categorized by sex of the participants. A positive correlation ($p = 0.0056$; $\chi^2 = 7.677$) of HBsAg prevalence with male sex was assessed through Chi-square test at $p<0.05$ with 95% CI.

Table 3. Prevalence of hepatitis B serological markers regarding sex

Sex	Total Participants	HBsAg Positive Participants	HBV Positive Participants	HBeAg Positive Participants	Chi-square test at P<0.05 with 95% CI	
					Chi-square Value	P value
Female	79	0	0	0		
Male	21	2	1	1	7.677	0.0056

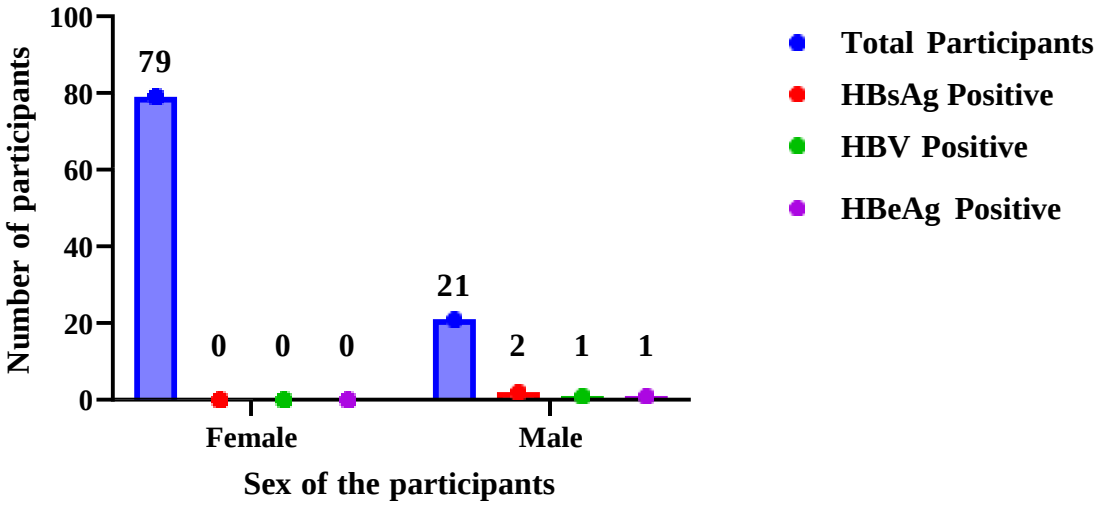

Figure 1. Sex-based prevalence of hepatitis B markers. The figure presents the prevalence of hepatitis B serological markers categorized by sex among the study participants.

3.4. District wise prevalence of hepatitis B

Table 4 presents the prevalence of hepatitis B with respect to demographic background of the participants. Among the districts, Bagh had 10 participants, with 1 individual (10%) testing positive for HBsAg. In Bhimber, Haveli, Jhelum Valley, Kotli, Muzaffarabad, and Neelum Valley districts, no individuals tested positive for any of the hepatitis B markers. In the Poonch district, out of 11 participants, 1 individual (9.1%) tested positive for HBsAg, and the same individual (9.1%) tested positive for HBV DNA and HBeAg as well.

Table 4. Prevalence of hepatitis B among various districts

Name of Districts	Total Participants	HBsAg Positive Participants	HBV DNA Positive Participants	HBeAg Positive Participants
Bagh	10	1	0	0
Bhimber	01	0	0	0
Haveli	03	0	0	0
Jhelum Valley	05	0	0	0
Kotli	10	0	0	0
Muzaffarabad	54	0	0	0
Neelum Valley	06	0	0	0
Poonch	11	1	1	1

3.5. Prevalence of hepatitis B based on the job status of the participants

Among employees, comprising 7 participants, 2 individuals (28.6%) tested positive for HBsAg, 1 individual (14.3%) tested positive for HBV DNA, and 1 individual (14.3%) tested positive for HBeAg. In contrast, among students, totaling 93 participants, none tested positive for any of the hepatitis B markers as shown in Table 5.

Table 5. Job status and the hepatitis B prevalence

Name of Districts	Total Participants	HBsAg Positive Participants	HBV DNA Positive Participants	HBeAg Positive Participants
Employees	07	02	01	01
Students	93	0	0	0

3.6. Prevalence of hepatitis B markers among various tribes

From the studied tribes, individuals from the Abbasi, Awan, Balti, Butt, Khawaja, Mughal, Qazi, Qureshi, Sardar, Sheikh, Syed, and Rajpoot tribes showed no cases of HBsAg, HBV DNA, or HBeAg positivity. However, among the Chaudhary tribe, out of 6 participants, 1 individual (16.7%) tested positive for HBsAg. Similarly, among the Khan tribe, consisting 8 participants, 1 individual (12.5%) tested positive for HBsAg, 1 individual (12.5%) tested positive for HBV DNA, and 1 individual (12.5%) tested positive for HBeAg as shown in Table 6.

Table 6. Tribe based prevalence of hepatitis B serological markers

Name of Tribes	Total Participants	HBsAg Positive Participants	HBV DNA Positive Participants	HBeAg Positive Participants
Abbasi	06	0	0	0
Awan	07	0	0	0
Balti	02	0	0	0
Butt	05	0	0	0
Chaudhary	06	01	0	0
Khan	08	01	01	01
Khawaja	10	0	0	0
Mughal	07	0	0	0
Qazi	02	0	0	0
Qureshi	01	0	0	0
Rajpoot	19	0	0	0
Sardar	07	0	0	0
Sheikh	04	0	0	0
Syed	16	0	0	0

4. Discussion

In a study conducted in Lahore, Pakistan, 280 children between the ages of 1 and 15, diagnosed with jaundice were enrolled. The average age of the children was 8.66±4.00 years. Among them, 179 (63.9%) were male, and 101 (36.1%) were female. Hepatitis B virus (HBV) infection was identified in 51 (18.2%) patients, with 38 (21.2%) males and 13 (12.9%) females tested positive for the hepatitis B virus antigen [17]. Similarly in Kenya, among individuals with jaundice, 50.6% were found positive for HBsAg, indicating hepatitis B virus infection. Moreover, 2.3% of the patients tested positive for IgM to the core protein, indicating a potential acute infection [18]. In Mongolia, IgM antibodies to hepatitis B core were detected in 34.5% patients and 27.3% were HBV carriers with detectable HBsAg [19]. Whereas in Ghana, among the 155 patients enrolled in the study, hepatitis B (54.2%) was identified as the most prevalent infection, with other viral hepatitis infections following closely [20]. In comparison, this current study aimed to investigate the prevalence of hepatitis B and its associated markers among participants across various demographic and geographic categories of Azad Kashmir, Pakistan. Among the total of 100 participants, HBsAg positivity wasobserved in 2 participants (2%), while no individuals tested positive for Ant-HBc. One participant (1%) tested positive for HBV DNA, which was further confirmed for HBeAg (1%). However, none of the participants tested positive for Anti-HCV or Anti-HDV as co-infections with hepatitis B.

In Lome, Togo, a screening for HBsAg was conducted on a total of 1,200 individuals from 2009 to 2011. The highest rates of HBV prevalence were found in people aged between 20 and 29 years, with a prevalence of 26.33%, and those aged between 30 and 39 years, with a prevalence of 21.67%. Conversely, the lowest prevalence, at 6.08%, was observed in individuals aged over 50 years [21]. In Punjab, Pakistan, the highest frequency of infection was recorded among individuals aged 21-30, accounting for 34.93%, followed by 23.83% in the 31-40 age bracket. The incidence decreased with age, with rates of 13.39% in the 11-20 age group, 16.13% in the 41-50 age group, and 7.09% in the 51-60 age group. Children aged 0-10 and individuals over 60 years old showed lower infection rates, at 1.49% and 1.65%, respectively [22]. In Turkey, a review reported that the outcomes of age-specific groups differed, ranging from 2.84% for individuals aged 0-14 years to 6.36% in the 25-34-year-old group [23]. Compared to previous studies, the results of the current study demonstrate that in the 19-25 age group, which comprised 83 participants, the mean age was 21.05 ± 0.1939 years, withno individuals (0%) testing positive for HBsAg, HBV, or HBeAg. Conversely, in the age group above 25, consisting of 17 participants, the mean age was 35.35 ± 2.946 years. Within this older age group, 2participants (11.8%) tested positive for HBsAg, 1 participant (5.9%) tested positive for HBV, and 1 participant (5.9%) tested positive for HBeAg.

From January 2017 to March 2018, a total of 6137 samples were collected from various Tehsils of district Buner, KPK, Pakistan. In a gender-based study, the highest prevalence of HBV was observed in the male population, with a rate of 1.66% [24]. Among the 463 participants in Nigeria, the analysis revealed that male gender ($p = 0.01$) and belonging to the reproductive age group ($p = 0.009$) were significant predictors of chronic HBV infection [25]. In a study conducted in Azad Kashmir, Pakistan, it was found that out of the participants, 150 individuals (2.13%) tested positive for the hepatitis B surface antigen, with 57.3% being male and 42.7% female [15]. In another study conducted in Muzaffarabad, AJK, Pakistan, it was reported that male participants were more likely to test positive for HBsAg, with an odds ratio of 4.026 and a p-value of 0.0074 [26]. Similarly, in the present study, it was observed that among the 79 female participants, none tested positive for HBsAg, HBV, or HBeAg. In contrast, among the 21 male participants, 2 individuals (9.5%) tested positive for HBsAg, 1 individual (4.8%) tested positive for HBV, and another individual (4.8%) tested positive for HBeAg.

In the current study, district Bagh of AJK state, Pakistan had 10 participants, with 1 (10%) testing positive for HBsAg. No individuals tested positive in Bhimber, Haveli, Jhelum Valley, Kotli, Muzaffarabad, and Neelum Valley districts. In Poonch district, 1 participant (9.1%) tested positive for HBsAg, HBV DNA, and HBeAg. Whereas, in KPK, Pakistan, Khan et al [27] found that 21.05% of 200 samples tested positive for HBV infection via PCR. High rates were observed among internally displaced persons from Malakand (31.10%) and Lower Dir (26%) compared to those from Swat (18%), Buner (15.90%), and Shangla (15.71%). Similarly, a cross-sectional survey conducted by Asghar et al [28] in Sindh, Pakistan, reported varying prevalence rates among districts for hepatitis B (ranging from 0.97% to 9.06%) and hepatitis C (ranging from1.61% to 29.50%). Umerkot emerged as the most prevalent district in rural Sindh, while Badin had the lowest number of seropositive individuals. Tando Allahyar was identified as the second most prevalent district, followed by Mirpur Khas.

Mayanja et al [29] conducted a study in Kenya and Uganda, finding that the highest HBV prevalence was among participants with primary level education (4.5%) and those involved in informal low-risk jobs (6.5%). Likewise, Tosun et al [30] found that individuals categorized as self-employed, business owners, and public sector workers were around 10% less likely to develop HBV infection in Turkey. Similarly, in the present study, among employees (7 participants), 2 individuals (28.6%) tested positive for HBsAg, 1 individual (14.3%) tested positive for HBV DNA, and another individual (14.3%) tested positive for HBeAg. Conversely, among students (93 participants), none tested positive for any of the hepatitis B markers.

The findings of our study showed variations among the surveyed tribes. Within the Chaudhary tribe, comprising 6 participants, one individual (16.7%) tested positive for HBsAg. Similarly, among the Khan tribe, consisting of 8 participants, one individual (12.5%) tested positive for HBsAg, while one individual each (12.5%) tested positive for HBV DNA and HBeAg. Moreover, no participants from other tribes included in the study exhibited positivity for hepatitis B markers. Whereas, according to Dwibedi et al [31], in India, the prevalence of HBsAg among tribes was as follows: Lodha (0.8%), Saora (0.9%), Khadia (0.9%), Juanga (1.7%), and Mankidia (3.7%). While, in a separate investigation by Ishida et al. [32], they explored the hepatitis B prevalence among rural ethnic communities in Northern Thailand. They found varying rates of HBV infection across tribes, with HBV ranging from 4.7% (Akha tribe) to 22.6% (Lahu tribe). Whereas, in AJK state, Pakistan, Kazmi et al [15] studied 13 distinct tribes and the highest prevalence of hepatitis B was observed in the Syed tribe (7.0%).

Conclusion and recommendations

The findings highlight the importance of recommending hepatitis B screening for individuals with a history of jaundice, especially among older age groups and males. Understanding the differences in hepatitis B prevalence among different ethnic groups and demographics is necessary for creating effective prevention and control plans in AJK state, Pakistan.

DECLARATIONS

Ethical approval

The study project was approved by the “BASR” at the University of Azad Jammu and Kashmir, Muzaffarabad, as outlined in Kazmi et al.'s study [33], under the project number “FBASR/-/16- 40/1669-71/-(41st M)”.

Conflict of Interest

The authors declare that they conducted the research without any potential conflicts of interest from commercial or financial relationships.

Author Contributions

SAK, AR, and MZL contributed to study design, sampling, experimentation, results analyses, and manuscript writing. TA assisted with sampling and manuscript writing. BS and SK contributed to methods setting, manuscript writing, and review. GR and IB were involved in manuscript writing and review. MT oversaw study design and management. ZA conducted data analysis and contributed to manuscript writing. FNK contributed to manuscript writing and review. MSS and SF participated in sampling and experimentation. JL contributed to field study and data collection.

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Date availability

The data is available and will be provided to the journal of suitable demand.

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