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EPIDEMIOLOGICAL INSIGHTS INTO THE PREVALENCE OF HEPATITIS B AND ASSOCIATED RISK FACTORS AMONG COLLEGE POPULATIONS

Syed Ayaz Kazmi[1*], Abdul Rauf[1], Muhammad Zahid Latif[2], Sundus Khawaja[3], Tasleem Akhtar[4], Beenish Shahid[1], Iqra Batool[1], Nuzhat Shafi[1], Zeeshan Anjum[3], Humaira Abid[5], Mohsin Nazir Awan[6], Ejaz Ahmad Khan[7], Faisal Farooq[1]

[1*]Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. ayaz.kazmi@ajku.edu.pk, dr.rauf@ajku.edu.pk, beenish.shahid@ajku.edu.pk, iqrabatool1909@gmail.com, nuzhatshafi@gmail.com, faisalraja826@gmail.com
[2]Community Medicine/ Public Health, Department of Medical Education, Director PGME Post Graduate Medical Education, Superior University Lahore, Pakistan. m.zahidlatif@superior.edu.pk
[3]Department of Biotechnology, Main Campus, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. sundus_kh@yahoo.com, drzanjum@gmail.com
[4]Department of Zoology, University of Poonch, Rawalakot, AJ&K, Pakistan. tasleemakhtar@upr.edu.pk
[5]King Edward Medical University, Mayo Hospital Lahore, Pakistan. abidhumaira86@gmail.com
[6]Department of Statistics, Main Campus, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. mohsinnazirawan@gmail.com
[7]Department of Mathematics, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. ejazkhan24@gmail.com

***Corresponding Author:** Syed Ayaz Kazmi
*Department of Zoology, King Abdullah Campus, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan. ayaz.biotech.uajk@gmail.com; ayaz.kazmi@ajku.edu.pk

Abstract

Background: As the prevalence of hepatitis B continues to rise in Pakistan, our study focused on educational institutions in the state of Azad Kashmir due to the complete absence of data on this scenario concerning college populations. We aimed to assess the prevalence of hepatitis B and identify associated risk factors among the population of postgraduate colleges in Muzaffarabad city, situated in Pakistan-administered Kashmir.

Methods: In this cross-sectional study, rapid immuno-chromatographic tests and real-time quantitative PCR methods were used to identify hepatitis B infection, while serum ALT levels were tested to assess their diagnostic associations with the infection level. The evaluation of risk factors was conducted through interviews using a questionnaire, and the obtained data were subjected to statistical analysis.

Results: The study revealed 1.3% of participants positive for HBsAg, 0.6% tested positive for HBV, and 0.1% tested positive for HBeAg. Male participants were found to be more likely to test positive for HBsAg (odds ratio of 4.026, p=0.0074), and participants aged 30 years and above exhibited a higher prevalence (p<0.0001) for hepatitis B. The average ALT level in the HBsAg positive

participants was significantly higher ($p<0.0001$). A significant association between HBsAg positivity and various factors including married status ($p=0.0005$), doing jobs ($p=0.0004$), getting cuts at hair salons or parlours ($p<0.0001$), hospitalization history ($p<0.0001$), blood transfusion ($p<0.0001$), and residence in hostels ($p=0.0015$) was observed. Only 0.4% participants were vaccinated against hepatitis B virus.

Conclusions: Our study revealed a significant prevalence of hepatitis B among postgraduate colleges in Muzaffarabad city, emphasizing the need for targeted awareness and prevention efforts. Identifying key risk factors such as blood transfusion, hospitalization, and residence in hostels underscores the importance of tailored initiatives. Considering the low vaccination rate, comprehensive actions, including routine screening, prioritized vaccination campaigns, and the implementation of multi-marker-based testing, are essential to mitigate the impact of hepatitis B.

Keywords: Hepatitis B; hepatitis B prevalence; risk factors of HBV; hepatitis B among college population; hepatitis B in Azad Kashmir; hepatitis B in Pakistan; hepatitis B in students; epidemiology of hepatitis B

1. Introduction

The HBV infection is a significant worldwide health issue that causes severe morbidity and mortality [1]. Globally in 2019, 296 million individuals had chronic hepatitis B infection with the increase of 1.5 million new infections yearly [2]. Men who engage in sexual activity with other males, intra-venous drug users, children born to infected mothers, haemodialysis patients, healthcare professionals, and household contacts with HBV-chronic patients are high-risk categories for HBV infection [3]. Pakistan and Egypt bear 80% of the global hepatitis B burden. In Pakistan, it is estimated that there are approximately 7-9 million individuals carrying the hepatitis B virus (HBV), resulting in a carrier prevalence ranging from 3-5% [4]. However, in the year 2021, a study reported that 25.2% of total population had contracted hepatitis B in District Bannu, Khyber Pakhtunkhwa, Pakistan [5]. Whereas the Punjab province, along with the interior of Sindh, and regions affected by war, experienced the highest infection rates in 2020 [6]. During 2019, the general prevalence of hepatitis B in Punjab was found 8.4%. Among the various districts, Muzaffargarh exhibited the highest seroprevalence of hepatitis B at 26%, while the Rajanpur district followed closely behind with a seroprevalence of 20.3% [7]. The HBV prevalence in Pakistan has a rapid increase despite the initiative of a government program for its control and prevention [8]. One important hurdle to managing and preventing disease spread is illiteracy or ignorance regarding risk factors and modes of transmission of HBV [9]. Hepatitis B transmission in different areas of Pakistan was commonly associated with several risk factors, including but not limited to blood transfusion, needle accidents, barber shop shaving, dental extractions, body piercing, injection drug abuse, unsafe surgical procedures, and seeking treatment from unqualified practitioners [10].

A lack of detailed research on hepatitis B among students and staff in educational institutions in Pakistan makes it difficult to determine the actual prevalence and risk factors. Further studies are needed to address this gap, although multiple studies on the topic are being conducted globally. In 2021 among Ethiopian medical students, hepatitis B virus surface antigen seroprevalence was 11.5%. After multivariate analysis, poor understanding of universal precaution guidelines, a history of needle stick injury, and having never been vaccinated for HBV were revealed to be significantly linked with hepatitis B positivity [11]. Similarly among Nigerian students, the HBsAg seroprevalence was 31.5%, males (43%) having a higher rate than females (27%), with no statistically significant variation across age groups [12]. While in Saudi Arabia, 0.41% HBsAg prevalence was reported among Majmaah University students and the prevalence was higher in males contrary to females [13]. Though in University of Lome, Togo, the HBsAg prevalence was 4.6% but it was also higher (5.8%) in males than females (3.1%). Male gender and a sexual relationship were found the significant risk factors [14]. HBV infection was found in 6.7% of medical students in Ghana. Torn gloves (43.0%) and splashes of blood (28.0%) were the risk factors. HBV vaccination rate among students was 43.3% [15]. Research studies on viral infectious diseases among educational institutes are exceptionally rare

in the state of Azad Kashmir, Pakistan. Moreover, there is a complete absence of data on this scenario concerning college populations. In this study, we examined the prevalence of hepatitis B and associated risk factors among the populations of postgraduate colleges, including both male and female colleges, located in Muzaffarabad city, Azad Kashmir, Pakistan.

2. Methodology

2.1. Population size and data collection

The research was conducted to determine the prevalence of hepatitis B and identify the related risk factors among the populations of postgraduate colleges, encompassing both male and female participants, in Muzaffarabad city of Pakistan-administered Kashmir. The study also aimed to disburse awareness about the viral hepatitis B infection and its preventions. In April 2018, the Raosoft calculation tool was used to ascertain an appropriate sample size for the study, drawing from the 2017 census data for the Muzaffarabad city in Azad Jammu and Kashmir (AJ&K). According to the census, the human population of the Muzaffarabad city was reported to be 149,913. With a confidence interval (CI) set at 95% and a 5.0% error margin, the assumed response distribution was established at 50%. Subsequent analysis using the Raosoft tool indicated that, based on these parameters, a minimum sample size of 384 was recommended for the study. A comprehensive survey was conducted, involving interviews with a total of 1,359 participants aged 20 years and above. During these interviews, participants provided valuable data regarding their demographics, medical history, and the risk factors.

2.2. Sample collection and hepatitis B serological markers

Each individual contributed a blood sample of five millilitres. Following the collection, the blood samples were processed for centrifugation, leading to the separation of serums. These serums were subsequently stored at -20 degrees Celsius until needed for further use. Hepatitis B surface antigen (HBsAg) was tested using immuno-chromatographic test (ICT) devices, following manufacturer instructions (Bio-line Diagnostics, India) and the DNAzol method was used to extract hepatitis B virus (HBV) DNA from positive samples. PCR amplification was done twice on extracted samples as reported by Rauf et al [16]. The PCR conditions involved initial denaturation at 94^{o}C for 5 minutes, followed by 30 cycles with annealing at 53^{o}C for 40 seconds and extension at 72^{o}C for 30 seconds, concluding with a final extension step lasting five minutes.

In the initial round of PCR, the following primers were employed:

Forward: “5'-CATCCTGCTGCTATGCCTCATCT-3'”

Reverse: “5'-CGAACCACTGAACAAATGGCACT-3'”

The primers employed in the second round of nested PCR were as follows:

Forward: “5'-GGTATGTTGCCCGTTTGTCCTCT-3'”

Reverse: “5'-GGCACTAGTAAACTGAGCCA-3'”

Hepatitis B envelope antigens (HBeAg) was detected using ICT devices (SD BIOLINE, Abbott Laboratories Karachi, Pakistan) to identify high blood infectivity and active replication of HBV among HBV DNA positive participants. The participants' serum alanine transaminase or alanine aminotransferase (ALT) levels were also measured using the Randox Laboratories Clinical Chemistry Reagents ALT kit with the Merck Microlab-300 analyzer.

2.3. Risk factor assessment and awareness disbursement

Prior to sampling, participants were interviewed through self-administered questionnaire regarding the basic demographic information and the data about risk factors. Awareness seminars about hepatitis B during the campaign were arranged in various settings of the colleges. These sessions aimed to inform and educate participants about the virus, its transmission, and preventive measures, contributing to an enhanced understanding of the prevalence and associated risk factors.

2.4. Statistical evaluation

The data were analyzed using GraphPad Prism Version 7.0 for statistical approaches. Various statistical tests like Chi-square test, T-test, and Fisher's exact test were performed to analyze the data regarding general prevalence, diagnostic associations, and the risk factors.

2.5. Hospital-based survey regarding hepatitis B testing procedures

To suggest a workable control policy, a survey was conducted among hospitals of the Muzaffarabad, Azad Kashmir Pakistan. This was based on assessing the methods commonly used for hepatitis B screening before blood transfusion and hospitalization.

3. Results

3.1. Prevalence of HBV infection in the study population

The study investigated the prevalence of HBsAg, HBV, and HBeAg among the 1359 participants. 1.3% of participants tested positive for HBsAg, 0.6% tested positive for HBV, and 0.1% tested positive for HBeAg. Table 1 shows the gender-based prevalence of hepatitis B in the present study. Among the 632 male participants, 14 (2.21%) were HBsAg positive, 6 (0.95%) were HBV positive, and 1 (0.16%) was HBeAg positive. In contrast, among the 727 female participants, 4 (0.55%) were HBsAg positive, 2 (0.28%) were HBV positive, and none were positive for HBeAg. These results underscore the gender-specific variations in the prevalence of hepatitis B related markers, highlighting the importance of considering gender in understanding the epidemiology of Hepatitis B.

Table 1. Gender-based prevalence of HBV, HBsAg and HBeAg.

Gender (N=1359)	HBsAg Positive (N=18)	HBV Positive (N=8)	HBeAg Positive (N=1)
Male (N=632)	14	06	01
Female (N=727)	04	02	0

3.2. Characteristics of the participants and their correlation with HBsAg

Table 2 demonstrates the correlation between participant demographics and the prevalence of HBsAg in a cross sectional study of 1359 individuals. Among males (46.5% of the participants), 2.21% tested positive for HBsAg, exhibiting a significant association with a Chi-square value of 7.171 and a p-value of 0.0074. In contrast, females (53.5% of participants) had a lower prevalence of 0.55%. Vaccinated individuals (0.4% of participants) showed no positive cases (0%), with a statistically significant protective association (Chi-square = 0.08089, p = 0.7761). Participants aged 30 years and below (90.4% of participants) had a prevalence of 0.65%, while those above 30 years (9.6% of participants) exhibited a higher prevalence of 7.63% (Chi-square = 44.15, p < 0.0001). Students (88.2% of participants) showed a prevalence of 0.91%, with a significant association, while employees (11.8% of participants) had a higher prevalence of 4.34% (Chi-square = 12.77, p = 0.0004). Married individuals (12.2% of participants) showed a prevalence of 4.21%, significantly associated with a Chi-square value of 12.10 and a p-value of 0.0005, while those not married (87.8% of participants) exhibited a lower prevalence of 0.92%. High school-educated participants (11.8% of participants) had a prevalence of 4.34%, and graduates (88.2% of participants) exhibited a lower prevalence of 0.91%, both statistically significant (Chi-square = 12.77, p = 0.0004).

Table 2. Correlation between participant characteristics and the prevalence of HBsAg.

Demographic Characters (N=1359)	Number of Participants (N); (%)	HBsAg Positive Participants	Association with HBsAg Prevalence (p< 0.05 and 95% CI)			Fisher's Exact Test (p<0.05 and 95% CI)			
			Chi-square	Odds ratio	P-value	Relative Risk	Sensitivity	Specificity	P-value
Gender									
Male	632 (46.5)	14 (2.21%)	7.171	4.095	0.0074	4.026	0.7778	0.5391	0.0084
Female	727 (53.5)	04 (0.55%)							

Vaccination Status									
Vaccinated	06 (0.4)	0 (0%)	0.08089	5.553	0.7761	0.0000	0.0000	0.9955	1.0000
Non Vaccinated	1353 (99.6)	18 (100%)							
Age									
30 years and below	1228 (90.4)	08 (0.65%)	44.15	0.07934	<0.0001	0.08534	0.4444	0.09023	<0.0001
Above 30 years	131 (9.6)	10 (7.63%)							
Job Status									
Student	1198 (88.2)	11 (0.91%)	12.77	0.2039	0.0004	0.2112	0.6111	0.1148	0.0029
Employee	161 (11.8)	07 (4.34%)							
Marital Status									
Married	166 (12.2)	07 (4.21%)	12.10	4.731	0.0005	4.573	0.3889	0.8814	0.0035
Not Married	1193 (87.8)	11 (0.92%)							
Education Level									
High School	161 (11.8)	07 (4.34%)	12.77	4.905	0.0004	4.735	0.3889	0.8852	0.0029
Graduate	1198 (88.2)	11 (0.91%)							

3.3. Hepatitis B in different districts

In the present study, the HBsAg prevalence varies across the 10 districts of Azad Kashmir, Pakistan. The lowest prevalence of hepatitis B was in districts Poonch (0%), Haveli (0%), Sudhnoti (0%), Bhimber (0%), and Mirpur (0%). While the highest prevalence of hepatitis B was in district Bagh (9.5%), as shown in Table 3.

Table 3. District-wise hepatitis-B infection prevalence.

Name of Districts	Participants; %	HBsAg positive; %	HBV positive; %
Muzaffarabad	1010 (74.3)	7 (0.69)	3 (42.8)
Bagh	21 (1.5)	2 (9.5)	1 (50)
Jhelum Valley	39 (2.8)	2 (5.1)	0
Neelum Valley	59 (4.3)	4 (6.7)	2 (50)
Poonch	17 (1.2)	-	-
Haveli	10 (0.7)	-	-
Sudhnoti	03 (0.22)	-	-
Kotli	189 (13.9)	3 (1.6)	2 (66.7)
Bhimber	06 (0.4)	-	-
Mirpur	03 (0.2)	-	-

3.4. Evaluation of serum ALT level

The average ALT level in the HBsAg-positive group was significantly higher ($p<0.0001$). For comparison of mean ALT levels of the groups an unpaired t-test (two-tailed) was used, and results showed a significant difference with a t-test value of 36.40 ($p<0.0001$). The variances of the groups were also found to be significantly different ($p<0.0001$) indicating that individuals with HBsAg positivity have higher ALT levels compared to those who were HBsAg negative as shown in Figure 1.

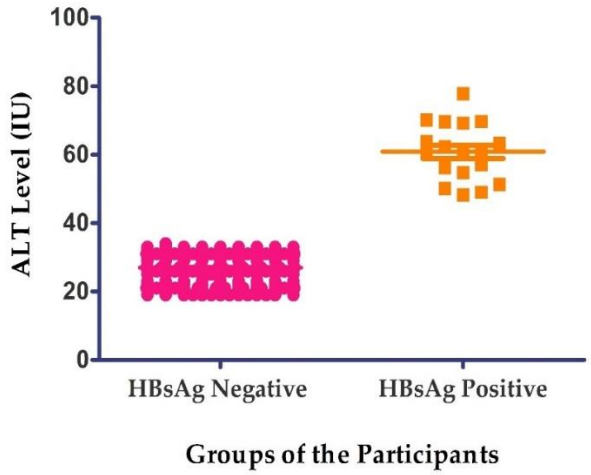

Figure 1. ALT level of the participants. The ALT levels of the HBsAg positive participants were high. The t-test (two tailed; p<0.0001) and the F-test (p<0.0001) were significant for the comparative analysis.

3.5. Viral load of the HBV positive participants and its relation with HBeAg prevalence

The results of a real-time quantitative PCR analysis in the study showed that of the HBV-positive participants, 7 had a below 10,000 IU/mL viral-load, whereas, 1 had a higher viral load. A Chi-square test found a significant correlation between viral load and HBeAg (P=0.0047, 95% CI), but the small sample size led to non-significant Fisher's exact values (P=0.1250, 95% CI) as shown in Table 4.

Table 4. Load of HBV DNA and the correlation with HBeAg prevalence

Viral Load	Included Participants (N=8)	HBeAg Positive	HBeAg Negative	Chi-square vs Fisher's Exact test
<10,000 IU/mL	7	0	7	
>10,000 IU/mL	1	1	0	P=0.0047; P=0.1250

3.6. Frequency of hepatitis B and age-wise distribution of the participants

The age-wise data of 1359 participants was examined, finding a coefficient of variation (28.05%), standard deviation (6.878), and mean age (24.52 years). The results indicated that the 20-29 years age group had the lowest prevalence of HBsAg (0.66%) and the 40-49 years age group had the highest (9.1%). The highest prevalence of HBV (5%) was also found in the 40-49 years age groups. The details of the analysis are shown in Table 5.

Table 5. Age base statistics from the study

Age groups	Participants count	Standard deviation	Median -age	Mean age ± Standard Error	HBsAg positive participants	HBV positive participants
20-29	1208	2.999	21.00	22.54±0.08629	8 (0.66%)	3 (0.24%)
30-39	80	3.055	34.00	33.69±0.3415	5 (6.25%)	0 (0%)
40-49	44	3.161	43.50	43.68±0.4765	4 (9.1%)	4 (5.0%)
50 and above	27	3.512	54.00	54.52±0.6759	1 (3.7%)	1 (3.7%)
Total	1359	6.878	22.00	24.52±0.1866	18 (1.32%)	8 (44.4%)

3.7. Assessment of the risk factors regarding hepatitis B among the participants

The association of HBsAg positivity with risk factors such as previous viral hepatitis infection (P=0.0062), sharp cuts from barbers/beauticians (P<0.0001), blood transfusion (P<0.0001), and hospitalization history (P<0.0001) was found significant in the study (Table 6). Participants with these risk factors had a higher probability of being HBsAg positive, as per the Odds ratio and relative risk values. However, no significant association of hepatitis B prevalence was found with jaundice (P=0.8126) and the surgery history (P=0.5882). Moreover, the results of age based risk factor assessment are shown in Table 7.

Table 6. Risk factors and their correlation with HBsAg

Assessed Risk Factors	No. of Participants	Response Options	Response of HBsAg positive	Response of HBsAg negative	Chi-square (Two tailed) test with P<0.05 and 95% CI				
					Odds ratio	Relative Risk	Sensitivity	Specificity	P-value
Previous viral hepatitis infection	55	Yes	3	15	4.958	4.298	0.05455	0.9885	0.0062
		No	52	1289					
Jaundice	60	Yes	1	17	1.278	1.263	0.01667	0.9869	0.8126
		No	59	1282					
Sharp cut from barber or beautician	17	Yes	4	13	29.19	22.55	0.2222	0.9903	<0.0001
		No	14	1328					
Blood Transfusion	13	Yes	5	8	64.09	39.82	0.2778	0.9940	<0.0001
		No	13	1333					
Hospitalisation history	32	Yes	11	21	98.78	65.17	0.6111	0.9843	<0.0001
		No	7	1320					
Any surgery	105	Yes	2	103	1.502	1.493	0.1111	0.9232	0.5882
		No	16	1238					

Table 7. This table shows the Age-based risk factors assessment among the participants of the study

Age Groups (Years)	No. of Participants	Risk Factors; %					
		Previous viral hepatitis infection	Jaundice	Sharp cut from barber or beautician	Blood Transfusion	Hospitalisation history	Any surgery
20-29	1208	49(4.0%)	52(4.3%)	17(1.4%)	6(0.49%)	10(0.82%)	70(5.8%)
30-39	80	3(3.7%)	2(2.5%)	0(0%)	4(5.0%)	10(12.5%)	10(12.5%)
40-49	44	1(2.2%)	5(11.4%)	0(0%)	3(6.8%)	10(22.7%)	15(34.0%)
50 and Above	27	2(7.4%)	1(3.7%)	0(0%)	0(0%)	2(7.4%)	10(37%)

3.8. Association of HBsAg with the residence of the participants

The current study found a significant association between HBsAg prevalence and residence of the participants. Two groups were formed: those living in hostels with roommates (447 participants) and those living with family members at home (894 participants). The HBsAg prevalence among hostel dwellers was 2.9% and among those living at home was 0.55%. Chi-square and Fisher's exact tests showed a significant correlation between HBsAg prevalence and residence of the participants (P=0.0005; O.R=5.200) as shown in Table 8.

Table 8. The table shows the information about participants' residence in the study area and the hepatitis B prevalence

Residence or Accommodation	HBsAg Positive	HBsAg Negative	Correlation at P<0.05 with 95% CI			
			Chi-square (two tailed) Test		Fisher's-Exact Test	
			O.R.	P-Value	O.R.	P-Value
Hostel (N=460)	13	447				
Home (N=899)	5	894	5.200	0.0005	5.200	0.0015

3.9. Awareness dissemination regarding hepatitis B

During the campaign, awareness lectures were conducted for the students and staff (teaching and non-teaching) of post graduate colleges. Banners about hepatitis B virus and its prevention were displayed for the whole month around the buildings and the class rooms. Awareness brochures were distributed among the participants so that they can carry the written material to their homes and hostels. Oral questioning was done before and after the lectures about knowledge of viral hepatitis B among participants. Teachers were sensitized to arrange quizzes for the students about common infectious diseases in the community.

3.10. Hospital-based survey regarding hepatitis B testing procedures

We also sought to determine the methods used to screen the hepatitis B before blood transfusion and hospitalization. It was found that among all blood banks and hospitals in the state of AJ and K, the only mechanism used for the initial diagnosis of hepatitis B was HBsAg testing. If the blood tested negative for HBsAg, the transfusion was allowed, but it was prohibited if the blood tested positive for HBsAg. There was a lack of awareness about the importance of testing for anti-HBc and anti-HBs, and these markers were never used. Awareness sessions were conducted among blood bank and hospital staff regarding the occult hepatitis B infection and to educate them about the importance of multi-marker-based testing before blood transfusion. The authorities from the Hepatitis Control Program in AJ&K were also sensitized about the importance of implementing multi-marker-based testing for hepatitis B as part of the hepatitis control policy in the state.

4. Discussion

HBsAg seroprevalence was 11.5% among Ethiopian medical students and it was found associated with the poor understanding of universal precaution guidelines, a history of needle stick injury, and having never been vaccinated [11]. Similarly in University of Lome, Togo, the HBsAg prevalence was 4.6%, while male gender and a sex were found the significant risk factors [14]. In Ghana, the HBV infection was found in 6.7% among medical students. The risk variables were torn gloves (43.0%) and blood splashes (28.0%), whereas 43.3% of the participants were confirmed to have already vaccinated against HBV. [15]. In contrary, the present study reveals 1.3% HBsAg prevalence among the college participants including 77.7% males and 22.3% females. The vaccination status was very low (0.44%) as compared the assessments in Ghana [15]. However, the common key risk factor responsible for the spread of hepatitis B was the blood transfusion.

1.81% HBsAg prevalence was among the general population of Morocco. Age, gender, dental treatment, sex, and surgery were the significant risk factors [17]. In Mexico, USA, the HBsAg prevalence was 0.8% among the participants from general population and it was found associated with tattooing and multiple sex partners [18]. According to a meta-analysis, China has a 6.89% prevalence of HBV between 2013 and 2017. Adult males from rural area s showed a greater frequency of HBV infection. [19]. In another study from Russia reported 1.2% HBsAg prevalence associated with age 30–34 years, more than 2 sex partners, and lack of vaccination [20]. While in a population-based survey from India, 1.4% HBsAg prevalence was reported and found associated with the male gender, older age, and rural livelihood [21]. In a national prevalence review from Pakistan, the HBsAg was 1.98%. The highest prevalence of HBV was found among healthcare professionals, following individuals with a history of IDPs. Interior Sindh and Punjab were the most afflicted areas in Pakistan

[6]. Conversely among the population of education institutes, it was found that the male gender (P=0.0074), age (P<0.0001), job status (P=0.0004), education level (P=0.0004), marital status (P=0.0005), previous viral hepatitis infection (P=0.0062; OR: 4.958), sharp cut from barber or beautician (P<0.0001; OR: 29.19), blood transfusion (P<0.0001; OR: 64.09), hospitalisation history (P<0.0001; OR: 98.78), high ALT level (P<0.0001), and accommodation in hostels (P=0.0005) were significantly associated with the 1.3% HBsAg prevalence in Muzaffarabad, Azad Kashmir, Pakistan.

1.3% prevalence of hepatitis B in our study is low as compared to the reported prevalence in Ethiopian medical students [11], university students Togo [14], medical students of Ghana [15], general population of Morocco [17], general population of China [19], population-based survey in Indian [21], and national prevalence review in Pakistan [6]. Whereas, the current prevalence is higher from the reported prevalence in general population of Mexico, USA [18] and population based cross-sectional study of Russia [20].

In the current study, the highest prevalence of HBsAg (9.5%) was among the participants of district Bagh followed by district Neelum Valley (6.7%), district Jhelum Valley (5.1%), district Kotli (1.6%), and district Muzaffarabad (0.69%). Whereas, in district Mardan KPK, the prevalence of hepatitis B was 13.00% [22], and 8.09% was in district Sargodha, Punjab [23].

For present study analysis, 4 groups of the participants were made, aged between 20 to 60 years. The age group 40 to 49 years of age was found with highest prevalence of both HBsAg (9.1%) and HBV (5.0%) among all other age groups. After this, 6.25% HBsAg and 0% HBV prevalence was found in 30-39 years of age group. Following this, there was 3.7% prevalence of each HBsAg and HBV was found among the participants of 50 and above year's age group. The lowest prevalence of HBsAg (0.66%) and the second lowest prevalence of HBV (0.24%) was among the participants of 20 to 29 years age group. Conversely, the lowest prevalence was found in the age-group of 01-20 years, representing only 10% of cases. The age-group of 21-40 years demonstrated a moderate prevalence of 13.33% in the district of Bannu, KPK, Pakistan. [5]. However, in China, the highest prevalence of HBV was found in ages 60-69 (9.3%). Risk factors for those between 15 to 59 years were male gender, surgery, a positive HBsAg family member, and lack of vaccination. A previous history of blood transfusions was a risk factor for those over 59 [24]. The current study found the highest percentages of previous viral hepatitis infection history (7.4%) and history of surgery (37%) among participants 50 and above, while participants 40-49 had the highest percentages of jaundice (11.4%), blood transfusion (6.8%), and hospitalisation history (22.7%). Sharp cuts from barbers or beauticians were only found among participants 20-29, accounting for 1.4% of that age group.

In current study, we observed viral load below 10,000 IU/mL in 7 participants, denoting a low level of risk. Conversely, it was exceeding 10,000 IU/mL in one participant, suggesting a higher level of risk. A correlation between HBeAg and viral load was not found to be significant. However, in China, a high viral load was found in 31.9% of cases, but not related to HBeAg prevalence [25]. In Russia, a study reported that low viral load was typically correlated with low HBsAg levels, but some patients with high viral load also exhibited elevated HBsAg levels [26].

Pido and Kagimu [27] after conducting a study at Makerere University, reported that more than 90% of medical students surveyed had not received hepatitis B vaccination and 11.0% of the participants were found positive for HBsAg. Whereas in the present study, 1.3% were HBsAg positive and only 0.44% were completely vaccinated including 16.7% males and 83.3% females. All the vaccinated participants were unmarried, 33.3% were home accommodated and 66.7% were hostel living.

Conclusions and recommendations

Our study revealed a significant prevalence of hepatitis B among postgraduate colleges in Muzaffarabad city, emphasizing the need for targeted awareness and prevention efforts. Identifying key risk factors such as blood transfusion, hospitalization, and residence in hostels underscores the importance of tailored initiatives. Considering the low vaccination rate, comprehensive actions, including routine screening, prioritized vaccination campaigns, and the implementation of multi-marker-based testing, are essential to mitigate the impact of hepatitis B.

Author Contributions:
The authors' contributions and responsibilities are summarized as follows: S.A.K. and A.R. were involved in conceptualization; S.A.K., A.R., Z.A. and T.A. contributed to the study design; S.A.K., M.N.A., E.A.K. and M.Z.L. were responsible for results analysis through software; validation was performed by S.A.K., A.R., S.K., T.A., M.Z.L. and H.A. formal analysis was conducted by S.A.K., A.R., S.K, Z.A., T.A. and M.Z.L.; investigation was carried out by S.A.K., B.S. and A.R.; M.Z.L., A.R., and S.A.K. provided resources; S.A.K. curated the data; S.A.K., T.A., Z.A., I.B., F.F. and S.K. prepared the initial draft; S.A.K., B.S., F.F., I.B. and Z.A. participated in writing, reviewing, and editing; visualization was done by S.A.K., M.N.A., N.S. and A.R.; A.R. and N.S. provided supervision; project administration was handled by A.R.; funding acquisition was secured by A.R. ALL authors have seen and approved the final manuscript.

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Institutional Approval: Ethical permissions were obtained from Board of Advanced Studies & Research at the University of Azad Jammu and Kashmir Muzaffarabad.

Consent to Participate: An official communication was sent by the authorities at the University of Azad Jammu and Kashmir, Muzaffarabad to the heads of different colleges, encouraging their active engagement in a research study. In response, college principals instructed both students and employees to attend informative sessions aimed at raising awareness about viral hepatitis B. These sessions also involved collecting samples for testing purposes. As a result of the motivating pre-screening awareness sessions, a considerable number of individuals willingly participated in the study. To ensure accurate documentation and inclusion in project reports, photographs were taken during the entirety of the sessions.

Data Availability: Data regarding videos and audios were prohibited. However, images of the conducted camps and the data in excel sheet are available.

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