

## Molecular Prevalence and Risk Factors of Hepatitis B Virus Infection Among the Patients Attending the Human Medical Laboratories in Kabul, Afghanistan

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### ABSTRACT

**Background:** Hepatitis B virus (HBV) can cause acute and/or chronic hepatitis, responsible for over one million deaths annually. We aimed to estimate the prevalence of hepatitis B and its risk factors among the patients attending the Human Medical Laboratories (HML) in Kabul, Afghanistan.

**Methods:** This retrospective study was conducted on samples received between January 2022 and September 2023. We tested 2062 blood samples using a Geneproof Amplification Kit and Rotor-Gene Real-Time PCR. Descriptive and analytical statistics (Chi-square test and logistic regression) were analyzed using SPSS version 26.

**Results:** The prevalence of HBV was 51.2% (1056/2062), with 59.3% males and a mean (SD) age of 32.0 (14.9) years. The majority of the HBV-positive patients (792/1056 or 74.8%) were from the age group of 16–45 years. The highest and lowest prevalence of HBV was observed in Kandahar (29/48 or 60.4%) and Nangarhar (107/235 or 45.5%), respectively. The mean overall HBV viral load was 39,587,427.2 IU/mL, with the highest and lowest mean viral load observed in patients from Takhar (71,940,575.8 IU/mL) and Kandahar (1,419,819.0 IU/mL), respectively. The statistically significant risk factors associated with HBV were blood transfusion, dental extraction, and syringe sharing.

**Conclusion:** HBV is highly prevalent among the Afghans attending HML in Kabul, Afghanistan. The Afghanistan Ministry of Public Health and international donor agencies should help in conducting more studies of HBV in all 34 provinces of Afghanistan. This will help in finding the real burden and risk factors of HBV in different regions and ethnicities of Afghanistan.

**Keyword:** Hepatitis B Virus, Afghanistan, Prevalence, Polymerase Chain Reaction.

## Introduction

Human hepatitis B virus (HBV) is a membrane-enveloped DNA virus classified as a member of the Hepadnaviridae family. According to the WHO, an estimated 254 million individuals were afflicted with chronic hepatitis B infection as of 2022, with approximately 1.2 million new infections annually (1). In 2022, approximately 1.1 million deaths were attributed to hepatitis B. These deaths were predominantly resulting from complications such as cirrhosis and hepatocellular carcinoma, which can be the result of chronic liver disease following persistent HBV infection (1, 2). The latest available data highlight that viral hepatitis, particularly hepatitis B, remains a significant public health challenge. Also, the goal of its elimination by 2030 seems to be a long way off (3).

Many patients are asymptomatic carriers of HBV. On the other hand, since most HBV cases are asymptomatic, they are mostly diagnosed during routine blood screenings (4). Asymptomatic carriers of HBV can still spread the virus and potentially develop complications over time. So, to prevent transmission and complications, early diagnosis and management of these cases is important. Patients are often asymptomatic for years but may unknowingly transmit infection through perinatal, percutaneous, or sexual exposure, or close person-to-person contact (e.g., open cuts and wounds) (4). An estimated 15%-40% of HBV-infected patients may develop complications, such as liver cirrhosis, liver failure, and hepatocellular carcinoma. These complications are the most common causes of HBV-related deaths (5).

HBV infection cannot be differentiated from other diseases based on clinical symptoms alone. The final diagnosis depends on the serological test results. The serological markers of HBV infection depend on whether the infection is acute or chronic. HBsAg is the most widely used test for diagnosing acute HBV infection or identifying carriers. However, real-time PCR is

a relatively new quantitative testing technique that is a more accurate, sensitive, and highly reproducible method for the detection of HBV (6).

Afghanistan, a culturally diverse and landlocked country in South Central Asia. It is expected to have a population of 41,454,761 in 2023 (7). The Afghan healthcare system operates under the significant strain of endemic infectious diseases such as tuberculosis, malaria, and viral hepatitis (HBV and HCV). This public health challenge is exacerbated by systemic poverty, rendering many citizens unable to afford the higher-quality care typically available in the private sector. Consequently, the population's most vulnerable groups—particularly children and pregnant women—face disproportionate risks due to their reliance on an under-resourced public health infrastructure that provides limited services (7). After decades of political instability, protracted war, and other challenges, Afghanistan is facing a huge humanitarian crisis. IDPs from different parts of the country have built overcrowded camps in the displaced areas of Kabul, with a lack of cleanliness and hygiene. These camps and critical situations may pose a significant risk of disease transmission to the general public (8). Establishing a relationship between the burden of infectious diseases and the capacity of the health system is important. It is important to know the real burden of HBV in Afghanistan. However, studies on the burden of HBV infection in Afghanistan are very limited. So, the main objective of this study was to estimate the prevalence of hepatitis B and its risk factors among the patients attending the human medical laboratories in Kabul, Afghanistan.

## Materials and Methods

### *Study design, study area, and study period*

This was a retrospective study conducted during the 20-month period from 12<sup>th</sup> January 2022 to 12<sup>th</sup> September 2023. This study was conducted in Human Medical Laboratories (HML) in Kabul, Afghanistan. HML is a well-equipped and standard laboratory that receives samples from all over Afghanistan. Inclusion criteria for this study consisted of all the patients in all groups who attended HML for HBV examination during the study period. Exclusion criteria consisted of incomplete data and patients who were not Afghan citizens and were from other nationalities.

### *Study samples*

Our sample size consisted of all the patients who were referred to HML during the 20-month period. A total of 2062 blood samples from both male and female participants, aged between 2 and 90 years, were sent by physicians for HBV DNA confirmation.

### *Sample collection*

From each study participant, 3 ml of venous blood were collected using a sterile disposable syringe and then transferred into a gel tube. The serum/plasma was separated by centrifugation,

collected in serum cups, and stored at  $-25^{\circ}\text{C}$  in the Molecular Diagnostic Department.

### *HBV DNA Detection by Quantitative Real-Time PCR*

HBV DNA was extracted from all samples using the Qiagen mini-DNA Extraction Kit. The extracted HBV nucleic acid was then amplified using Rotor-Gene real-time PCR. The amplification process utilized the HBV Geneproof Amplification Kit, the amplification or thermal cycler profile as mentioned in **Table 1**, which targets specific conserved DNA sequences of the "P" gene. The master mix included uracil-DNA glycosylase (UDG) and deoxyuracil triphosphate (dUTP) to eliminate carryover contamination. This method ensures the detection of all HBV genotypes (A-H) with optimal sensitivity, down to 13.9 IU/ml.

For the quality control assessment in HBV PCR testing, strict measures were taken during the procedures of sample preparation, amplification, and analysis. To prevent contamination and ensure accurate results, errors were minimized by physical separation of pre-PCR and post-PCR areas, using specialized, disposable, and pre-sterilized materials, as well as using separate equipment for each stage.

**Table 1:** Amplification or thermal cycler profile for amplification of targets of HBV

<i>Step</i>	<i>Temperature (<math>^{\circ}\text{C}</math>)</i>	<i>Time</i>	<i>Data collection</i>	<i>Cycles</i>
Hold	37	2 minutes		1
Hold	95	10 minutes		1
PCR	95	5 seconds		45 cycles
	60	40 seconds	FAM+HEX	
	72	20 seconds		

FAM, Fluorescein amidite; HEX, Hexachlorofluorescein

### *Statistical analysis*

All the collected data were double-entered and cleaned in Microsoft Excel 2021 and then im-

ported into the SPSS version 22 (IBM Corp., Armonk, NY, USA) for statistical analysis. To summarize demographic characteristics, de-

scriptive analyses were used, such as frequency, percentage, mean, and standard deviation (SD). The chi-square test (using crude odds ratio [COR]) was used to assess the binary association between categorical variables. All statistically significant variables in the univariate analyses were assessed for independence in a multivariate logistic regression (using adjusted odds ratio [AOR]) to determine the real risk factors associated with positive HBV. All analyses were two-sided, and a *P*-value <0.05 was considered statistically significant.

#### **Ethics approval and consent to participate**

Formal approval for the conduct of this study was obtained from the officials of Human Medical Laboratories in Kabul, Afghanistan. The study was conducted in accordance with the principles of the Helsinki Declaration. The data were anonymized to ensure confidentiality.

## **Result**

**Table 2:** Gender-wise positive hepatitis B virus infection in patients from different provinces of Afghanistan.

<i>Province</i>	<i>HBV Positive</i>	<i>Male</i>		<i>Female</i>	
		<b>Frequency (n)</b>	<b>Percentage (%)</b>	<b>Frequency (n)</b>	<b>Percentage (%)</b>
Kabul	491	313	63.7	178	36.3
Faryab	163	85	52.1	78	47.9
Khost	152	87	57.2	65	42.8
Nangarhar	107	66	61.7	41	38.3
Balkh	58	25	43.1	33	56.9
Takhar	33	14	42.4	19	57.6
Kandahar	29	22	75.9	7	24.1
Ghazni	23	14	60.9	9	39.1
Total	1056	626	59.3	430	40.7

HBV, Hepatitis B virus

#### **Age-based HBV detection prevalence**

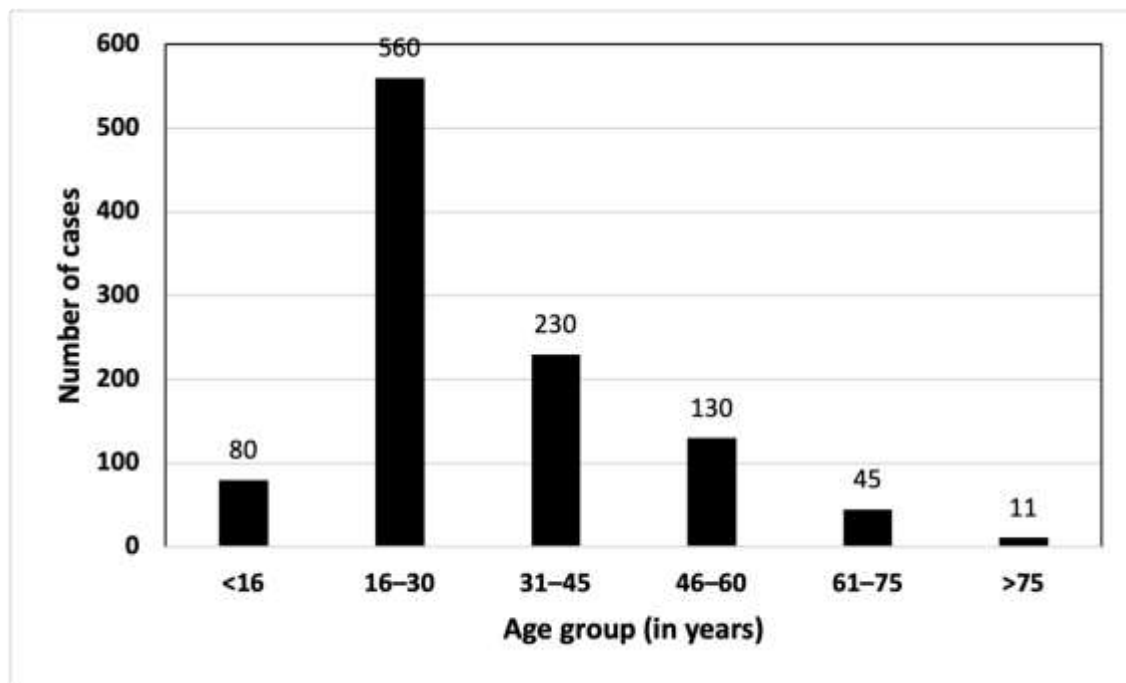
The highest prevalence of HBV was observed in the age group of 16–30 years (560/1056 or 53.0%), followed by 31–45 years (230/1056 or

All 2062 suspected patient samples sent to the human laboratory were collected. After running the PCR, HBV DNA was detected in 1056 patients. Among the study participants, the prevalence of HBV was 51.2% (1056/2062). The mean (SD) age of the HBV-positive patients was 32.0 (14.9) years. The minimum age was 2 years, while the maximum age was 90 years in the study participants.

#### **Gender base HBV Detection prevalence**

Among the 1056 HBV-positive patients, 626 (59.3%) were males. More males were HBV-positive from Kandahar (22/29 or 75.9%), Kabul (313/491 or 63.7%), and Nangarhar (66/107 or 61.7%) provinces. However, more HBV-positive females were observed in samples from Takhar (19/33 or 57.6%), Balkh (33/58 or 56.9%), and Faryab (78/163 or 47.9%) provinces (**Table 2**).

21.8%). Among these patients, 80/1056 (7.6%) were <16 years of age, while 11/1056 (1.0%) were having an age of >75 years (**Figure 1**).



**Figure 1:** The presence of hepatitis B infection in patients of different age groups.

#### ***Province-based HBV detection prevalence***

Approximately half (953/2062 or 46.2%) of the study participants were from Kabul Province. The highest prevalence of HBV was observed in Kandahar Province (29/48 or 60.4%), followed by Khost (152/272 or 55.9%) and Balkh

(58/106 or 54.7%) provinces. The lowest prevalence of HBV was observed in Nangarhar Province (107/235 or 45.5%), followed by Faryab (163/339 or 48.1%) and Ghazni (23/46 or 50.0%) provinces (**Table 3**).

**Table 3:** Prevalence of hepatitis B virus among the study participants from different provinces of Afghanistan

<i>Province</i>	<i>Total Cases (Frequency, n)</i>	<i>HBV Positive, (Frequency, n)</i>	<i>HBV Negative (Frequency, n)</i>	<i>HBV Prevalence (Percentage, %)</i>
Kandahar	48	29	19	60.4
Khost	272	152	120	55.9
Balkh	106	58	48	54.7
Takhar	63	33	30	52.4
Kabul	953	491	462	51.5
Ghazni	46	23	23	50.0
Faryab	339	163	176	48.1
Nangarhar	235	107	128	45.5
Total	2062	1056	1006	51.2

HBV, Hepatitis B virus

#### ***Viral load of HBV***

Among the HBV-positive patients, the mean (SD) viral load was 39,587,427.2 (203,347,965.3) IU/mL, respectively. The high-

est mean (SD) viral load of the HBV was observed in patients from Takhar Province (71,940,575.8 [180,608,425.9] IU/mL), followed by Kabul Province (54,406,285.2

[279,845,477.0] IU/mL). However, the lowest mean (SD) viral load of the HBV was present in the patients from Kandahar Province

(1,419,819.0 [6,096,429.3] IU/mL), followed by the Nangarhar Province (10,360,369.6 [27,605,029.0] IU/mL) (**Table 4**).

**Table 4:** Viral load of the hepatitis-B positive patients in the study

Residence	Viral load (IU/mL)			
	Mean	Standard deviation	Minimum	Maximum
Takhar	71,940,575.8	180,608,425.9	250	935,550,000
Kabul	54,406,285.2	279,845,477.0	250	3,015,713,250
Ghazni	43,628,163.0	117,509,888.1	250	413,158,750
Balkh	41,074,017.2	110,314,116.5	250	497,746,250
Khost	37,083,414.5	112,385,389.2	250	780,750,250
Faryab	15,611,313.2	72,419,080.4	50	557,907,500
Nangarhar	10,360,369.6	27,605,029.0	250	154,037,750
Kandahar	1,419,819.0	6,096,429.3	250	32,173,500
Total	39,587,427.2	203,347,965.3	50	3,015,713,250

### Risk factors of HBV

In this study, the statistically significant risk factors associated with HBV were as follows: (a) blood transfusion, dental extraction, and sy-

ringe sharing. However, 44.9% (463/1032) of the HBV-positive study participants did not report any risk factor that could have caused the HBV infection acquisition (**Table 5**).

**Table 5:** Prevalence of HBV risk factors among the study participants

Risk Factors	HBV positive, Frequency (%)	HBV negative, Frequency (%)	COR (95% CI)	AOR (95% CI)	P-value
Gender					
Male	626 (51.6)	587 (48.4)	1.0 (0.9–1.2)	0.8 (0.6–1.4)	0.733
Female	430 (50.6)	419 (49.4)			
Residence			1.0 (0.9–1.2)		
Kabul province	491 (51.5)	462 (48.5)		0.8 (0.7–1.5)	0.795
Other provinces	565 (50.9)	544 (49.1)			
Blood transfusion	230 (64.4)	127 (35.6)	1.7 (1.1–4.3)	1.6 (1.2–3.5)	0.030
Dental extraction	157 (60.4)	103 (39.6)	1.5 (1.2–3.7)	1.4 (1.1–2.4)	0.039
Shaving at barber-shop	118 (52.0)	109 (28.0)	0.5 (0.1–1.1)	0.7 (0.2–0.9)	0.684
Sharing personal belongings*	69 (48.6)	73 (51.4)	0.3 (0.2–0.8)	0.2 (0.1–0.9)	0.832
Syringe sharing	19 (70.4)	8 (29.6)	2.1 (1.7–5.1)	1.9 (1.5–4.2)	0.018
Unknown (no data)	463 (44.9)	569 (55.1)			
Total	1056 (51.2)	1006 (48.8)			

\* Examples are razors, shavers, toothbrushes, and nail clippers.

### Discussion

The prevalence of HBV was 51.2% in the patients attending HML. Nearly half (46.2%) of

the people infected with HBV were from Kabul province. This could be because the HML, i.e., study site, is located in the center of Afghanistan (Kabul). Also, due to the dense population



in the center, Kabul is more prone to HBV than the northern, eastern, western, and southern regions of Afghanistan. The prevalence of carriers among different population groups varies greatly from 0.1% in developed countries to 20% in developing countries. In general, this level is lowest in countries or regions with a high standard of living (such as Australia, North America, and Northern Europe) and highest in countries or regions with a low socio-economic level (such as China, Southeast Asia, and South America) (9). The very high prevalence (51.2%) in our study is not comparable with other studies. This is due to the fact that our study was in a laboratory, not a community. Most of our study participants were referred by doctors and were patients who were suspected of HBV infection.

HBV infection is a major global health crisis. Approximately 350 million people worldwide are chronically infected, with more than 1 million deaths per year from HBV-related liver disease (10)(11). Several epidemiological and molecular studies have shown that chronic HBV infection is an important risk factor for the development of hepatocellular carcinoma (12-14). Although HBV does not cause direct cytopathic effects under normal conditions of infection (15,16), liver damage (fibrosis, cirrhosis, and possibly hepatocellular carcinoma) results from persistent immune responses and persistent inflammation (17,18). The prevalence of chronic HBV infection varies geographically: high (>8%), moderate (2–7%), and low (<2%) (19). Inadequate HBV vaccination coverage, sharing of blood-contaminated equipment among users of injecting drugs, unsafe blood transfusions, and inadequate hygiene precautions are the contributing factors and the greatest risk of infection with HBV (20). Molecular analysis was the best choice to assess the prevalence of HBV infection in our study. Blood samples from different sexes and ages were collected and analyzed using molecular PCR to detect HBV DNA.

In our study, males were more likely (59.3%) to be infected with HBV than females (40.7%). This study is in line with a study conducted in Khyber Pakhtunkhwa, Pakistan, which reported that more males were infected with HBV than females (21). Similarly, another study on HBV infection in Pakistan reported a higher prevalence in males (68.15%) than in females (31.85%) (22). A study from Nigeria also reported a significantly higher (79.2%) infection rate in males as compared to females (20.8%) (23). Another study determined the frequency of HBV infection among children with chronic liver disease and found a higher prevalence (54 %) in males than in females (46%) (24). Another study from Pakistan also reported a higher (59.1%) prevalence in males than in females (40.9%) (25,26). These studies also reported a higher prevalence of HBV infection in males than in females (27,28). Contrary to our study, more females than males were infected with HBV in studies conducted in Pakistan (29) and Iran (30).

Higher infection rates in males may be due to their frequent exposure to risk factors such as injecting drugs and multiple sexual partners, because of their employment away from their homes. As the majority of the female participants in our study were housewives, they were less exposed to various risk factors. In these infected areas of Afghanistan, barber shaving, involvement in blood transfusion practices, drug use, and cross-border activity with neighboring countries are very common, which strengthens the argument for the high prevalence of HBV in males.

Our study showed that almost all the age groups were infected with HBV. The prevalence among children aged <16 years was 7.6% which increased to 53.0% among those aged 16–30 years. After that, it decreased to 21.8% and 12.3% among the patients aged 31–45 years and 46–60 years, respectively. Patients aged >60 years were less likely to be infected with HBV (5.3%). These results are comparable

to those of another study that also reported a similar trend of higher prevalence among younger people compared to children and/or older age groups. A study in India also reported a high prevalence (32.7%) in the population aged 15-20 years (31). In stratified age groups, a higher prevalence was recorded in the younger population than in older people and/or children (29). A study from Iran reported a high prevalence in the age group 25-34 years (32). Studies from Pakistan reported the highest prevalence (86%) in the age group 20-40 years (33). A study conducted in Pakistani Punjab revealed that the highest rate of HBV infection was 34.93% in the age group 21-30 (22). This indicates that age affects the prevalence of HBV infection. Long-term exposure to HBV infection may be due to increased exposure and interactions in the community compared to children and the elderly. Based on the data from our study, the *P*-value was 0.2216, which exceeds the significance level of 0.05, confirming that there is no significant association between age and HBV.

There are several other risk factors for HBV infection, such as shaving, blood donation, dental risk factors, and sharing personal belongings with others. The shaving risk in men is significantly higher (10.65%) (34). The presence of HBsAg in the saliva of HBV-infected patients and infected dental instruments also plays an important role in HBV infection (35). A history of dental procedures was a significant risk factor for hepatitis B in our study population because of the low income and economic crisis; the people did not use disposable equipment, and some of them had even visited unqualified practitioners. A study from Pakistan pointed out that dental procedures are a significant risk factor for HBV infection (36).

In our study, blood transfusion was a statistically significant risk factor of HBV infection. A study in Pakistan also reported that the incidence of HBV infection in blood donors was 3.1% (37). In Afghanistan, blood transfusions

played an important role in the transmission of HBV due to the unawareness of blood-borne diseases. Also, during the four-decade war in Afghanistan, patients needed an urgent supply of blood, and the blood screening test was not done properly, or due to the unavailability of proper equipment or facilities, so the HBV infection was transferred easily from the infected person.

HBV also affects doctors, paramedical staff, and health care workers at the time of surgical practice, and these people may deal with hospitals acquiring blood-borne disease from the patients on whom they operate. Surgeons have a higher risk than other specialists do (38). According to a previous report, injuries have been estimated to occur in approximately 7% of operations, and as many as 87% of surgeons are estimated to experience a percutaneous cut, prick, or injury at the same point in their career (39).

In our study, needle sharing was a statistically significant risk factor of HBV infection among the study participants. Similarly, a study from Nigeria also observed needle sharing as a significant risk factor (*p*-value 0.062) (40). An epidemiological study in Anhui Province of China showed a higher prevalence among married (7.9%) than unmarried (7.4%) individuals (41). In Afghanistan, it is strictly prohibited to have unmarried sexual partners; however, our study found that unmarried participants had lower HBV positivity rates than married participants. Married couples are more affected than unmarried couples. This may be due to non-adherence to preventive methods during sexual meetings and unawareness of HBV transmission from infected partners.

Our study had several limitations. First, it was a retrospective study. Therefore, we were unable to follow up with the patients to determine the HBV viral load and its progress on different occasions. Second, we did not have funding for this study. So, we could not do other lab exams and an ultrasound to confirm the complications



and other comorbidities in these patients. Third, this was a single-center study. So, we cannot generalize the results to the whole of Kabul or Afghanistan.

## Conclusion

The findings highlighted the very high burden of HBV among patients attending the HML in Kabul, Afghanistan. This study emphasized the essential role of routine blood screening for HBV in identifying asymptomatic carriers. The Afghanistan Ministry of Public Health and international donor agencies should help in conducting more studies of HBV in all 34 provinces of Afghanistan. This will help in finding the real burden and risk factors of HBV in regions and ethnicities living in Afghanistan. The absence of a comprehensive vaccination program, inadequate government policies, and systemic issues further exacerbate the spread of this disease. Given these findings, the WHO and other donor agencies should help the Afghanistan Ministry of Public Health in reducing the burden of HBV in Afghanistan. Special public health awareness programs, especially focusing on the prevention of HBV risk factors, should be implemented in all the provinces of Afghanistan. Without proper awareness and intervention, this contagious infection could escalate into an epidemic in Afghanistan in the future.

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## Conflict of Interest

The authors hereby declare that there is no conflict of interest.

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