



## Prevalence of HBV infection in suspected population of conflict-affected area of war against terrorism in North Waziristan FATA Pakistan

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### ABSTRACT

**Background:** Hepatitis B virus (HBV) infection is a major cause of severe liver diseases including fibrosis, hepatocellular carcinoma (HCC) and cirrhosis related end stage liver diseases (ESLD) in mankind. It is a common belief that infectious diseases have historically been responsible for the massiveness of war-related deaths, so the aim of this study was to estimate the prevalence of HBV infection and to demonstrate the various socio-economic, demographic and possible risk factors related to HBV infection among the conflict-affected peoples due to war against terrorism in North Waziristan.

**Methods:** Blood samples were collected from total 790 suspected individuals from the conflict-affected population of North Waziristan and were analyzed initially tested for the presence of HBsAg, HBeAg antigens, Anti-HBc and Anti-HBs antibodies using ELISA methods. All the positive samples were tested by real time PCR to confirm the presence of HBV DNA in ELISA positive specimens.

**Results:** Total of 126 (15.94%) samples were found positive for HBV DNA by real-time PCR. Among these positive subjects, 95 (75.5%) were males while 31 (24.5%) were females in a ratio of approximately 3:1. High HBV prevalence (41.26%) was observed among the subjects of subdivision Miran Shah relating to the high frequency of military activities against terrorism as compared to Mir Ali subdivision (35.7%) and Razmak subdivision (19.8%). Among the age groups, high prevalence (38.88%) was observed in age group 21–30 as compared to children and in older age groups. The modes of HBV transmission in this area was associated with re-uses of contaminated needles/syringes in medical care, barbers shops, sexual exposure and tattooing are the principal causal risks factors. Furthermore HBV infection was significantly higher in people with low socioeconomic status, in illiterate persons and in drivers.

**Conclusion:** Our results indicate high prevalence rate of HBV infection in young subjects obviously confirms the entire absence of any program to fight HBV. Mass Immunization programs, awareness campaigns and education efforts should be practiced immediately to reduce HBV transmission among young peoples of this conflict zone.

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### 1. Introduction

Hepatitis B virus (HBV) infection is a principal global public health issue in developing countries affecting 2 billion people worldwide with 400 million chronic carriers of this virus (Fiore and Neeman, 2004; WHO, 2011). HBV infection and its various complications including chronic hepatitis, cirrhosis and liver cancer accounts for 0.5–1.2 million deaths annually and is the 10th major source of death in globe (Lok, 2002; Mahoney, 1999).

Hepatitis B is highly endemic in Pakistani populace infecting 7–9 million people with a prevalence ratio of almost 3–5% (Ali et al., 2011).

HBV hepatic infection is transmitted percutaneous and per mucosal exposure to the body fluids of infected personnel (Hollinger and Liang, 2001). HBV is mainly found in sera and blood in high concentrations and its life time has been reported as several days on dried surfaces (Bond et al., 1977; Workowski and Berman, 2006). Other observed routes for HBV transmission are sharing of personal items, use of unsterilized medical instruments, barber risk, sharing needles with drugs addicts, reuse of disposable syringes and sexual contact with infected individual (Bukhari et al.,

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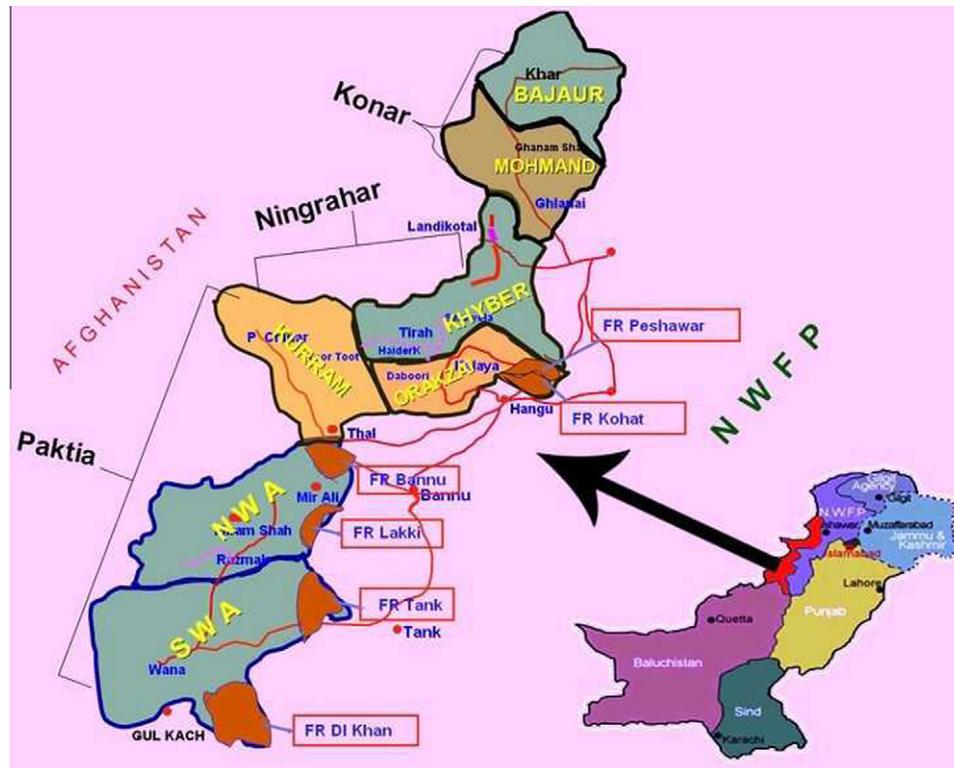


Fig. 1. Map of North Waziristan agency Pakistan bounded by Afghanistan. Adopted from: (www.defence.pk).

1999). As HBV is a blood borne virus, therefore, various specific proteins and corresponding antibodies are found in blood of infected people and these antibodies and proteins are used as markers for diagnosis of infection in blood tests (Bonino et al., 1987).

North Waziristan is a part of a rural backward area of Waziristan agency Federally Administered Tribal Area (FATA) of Pakistan. FATA is the northern western region of Pakistan comprised of about 3.4 million peoples with relatively low socioeconomic status and literacy rate. Waziristan agency is an extremely arid and mountainous region, divided into North Waziristan and South Waziristan. Especially North Waziristan is drastically affected by terrorist and anti-terrorist activities in last one decade. North Waziristan agency is bounded by Paktia Province of Afghanistan in north, and Shawal tribal region of Pakistan, Birmal area of Afghanistan and the South Waziristan Agency of Pakistan in the west, and the Frontier Region of Bannu District in the east (Fig. 1). As regards the ethnic origin some historians they are thought to be Semites or of Aryan origin. They are also thought to be the descendents of Karlan and Ghurghust due to their specific traditions. Moreover, Saidgis are also considered as an ethnic group. The North Waziristan Agency consists of three sub-divisions and nine Tehsils. The Miran-shah sub-division comprises the Miran Shah, Ghulam Khan, and Datta Khel tehsils. The Mir Ali sub-division contains the Mir Ali, Spinwam, and Shewa tehsils. The Razmak sub-division consists of the Razmak, Dossali, and Garyum tehsils. The terrorist actions completely destroyed the social infrastructures, health and education centers, consequently the health facilities and literacy rate is infinitesimal in agency (Global Security, 2011).

No such data or previous documented studies have been reported on HBV prevalence among the conflict-affected peoples of against war terrorism in North Waziristan and this is the first study to address this issue. Therefore the main objective of this study was to estimate the prevalence of HBV infection and the possible risk factors among conflict-affected people of North Waziristan.

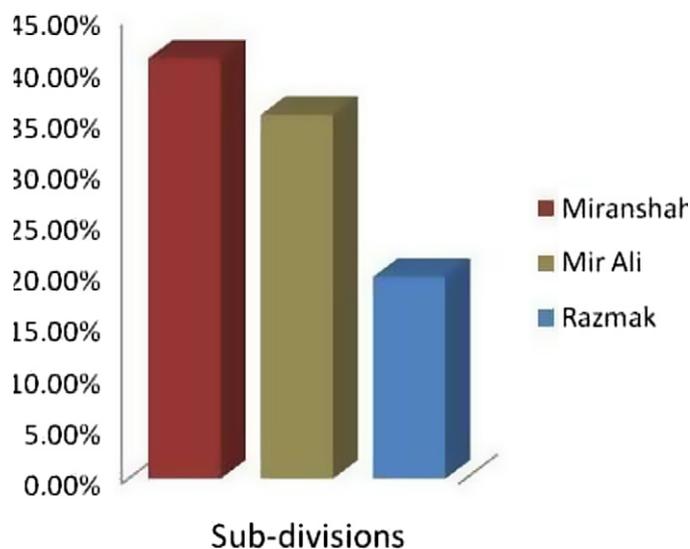
## 2. Materials and methods

### 2.1. Study design, sampling and data collection

The study was carried out in between 2010 and 2011 to determine the prevalence of HBV infection in suspected population and its possible modes of transmission. The study was conducted in two phases. In the first phase, areas affected with military operations were visited and primary data was collected from all the suspected hepatitis patients. Research scholars (RS) trained in data collection and sampling were sent to war affected areas of Waziristan.

RS explained the purpose and objectives of the present study in local Pashto language. Written informed consent in the local language was taken from subjects/head of family who agreed voluntarily to participate in the study. A printed questionnaire was completed by each participant before the blood sample was collected. The data sheet/questionnaire contained information on the subject's name, sex, age, socioeconomic status, history of jaundice, possible risk factors for viral transmission, educational level, type of health care provider for injections, history of any major or minor surgical procedures, dental procedure, tattooing, number of injections per year, diagnosis of HBV in the family and history of having been pricked with a needle and history of intravenous (IV) drug use. Five ml whole blood samples were collected from the suspected HBV patients in blood collection vacutainer tube. Centrifugation was done for sera separation within 6 h of collection, transported in cold chain and stored in three aliquots' at  $-20^{\circ}\text{C}$  for further processing. Volunteers, both males and females of all ages were eligible to participate in the present study. The study was approved by the ethical committee of the University.

In the second phase of study, the blood samples were thoroughly analyzed via ELISA and then by PCR to confirm the presence of HBV DNA in these collected samples.



**Fig. 2.** Observed prevalence of HBV infection in different subdivisions of North Waziristan.

## 2.2. Serological investigation

All the sera of 790 suspected individuals were subjected for serological HBV investigating markers including HBsAg, HBeAg, anti-HBsAg and anti-HBcAg using Sophisticated 3rd generation enzyme-linked Immunosorbent assays (ELISAs) (Asys Hitech GmbH Instruments, Austria) according to manufacturer's instruction specified in the instruction manual.

## 2.3. HBV DNA extraction and PCR amplification

For the confirmation of HBV DNA among the ELISAs positive subjects, DNA was extracted from serum samples with Precision Nucleic Acid Extraction kit (Primer Design, UK) according to the method given in the kit protocol. Real time PCR was carried out in the Department of Biotechnology University of Malakand, by using line Gene II RT-PCR (FQD-66A, Japan) with HBV qualitative and quantitative kits (Primer Design kit, UK) according to the kit protocol.

## 2.4. Statistical analysis

The available data was analyzed statistically by using SPSS version 20. The *P* value less than 0.05 were considered significant.

## 3. Results

### 3.1. Subjects enrolments and study disposition

In the current study total 885 individual who were willing to participate in the survey were interviewed for the enrolment of suspected HBV carriers. Out of these total 790 (89.3%) fulfilled the enrolment criteria. Of these, 485 (61.4%) were males and 305 (38.6%) were females. Samplings were done from each subject and sera were separated within 6 h and transported to clinical laboratory in proper cold chain. The age of subjects ranged from 3 to 60 years. These 790 sampled subjects were initially subjected to ELISA commercial kits for HBV screening. Of total 790 subjects, 461 (58.4%) were detected positive for Anti-HBs. Out of these anti-HBs positive cases, 324 (70.3%) individuals were also found positive for HBsAg while 137 (29.7%) were non-reactive for HBsAg

(naturally immunized/resolved cases). HBeAg were detected only in 64 (13.9%) of the HBsAg positive cases.

### 3.2. HBV DNA detection

Of the total 324 HBsAg reactive cases, 126 individuals were found positive by real time PCR for HBV DNA. The lower detection limit of the assay was 10 IU/mL. As the area under study (North Waziristan) has been divided into three subdivisions Miran Shah, Mir Ali, and Razmak, therefore, we had analyzed the PCR results area-wise. Our results indicate that HBV is highly prevalent in the Miran Shah subdivision (52/126; 41.3%), the second one is the Mir Ali subdivision (45/126; 35.7%) and in Razmak subdivision (29/126; 23.0%). Fig. 2 shows the area-wise results of the PCR-based study. The Miran Shah Subdivision is highly affected due to terrorist and anti-terrorist activities as compared to other two subdivisions. The high incidence may be due to medical deprivation and low socioeconomic position of residents in subdivision.

### 3.3. Age wise prevalence of infected population

Table 1 demonstrates age wise prevalence of active HBV diseased subjects. The data was distributed among 6 age groups such as <10, 10–20, 21–30, 31–40, 41–50 and >50 years. HBV prevalence was seen in 38.9% (49/126) in the age group-21–30 years as compared to age groups <10 having prevalence of 3.14% and in >50 group having 7.14% prevalence. Lack of immunization, illiteracy and unawareness are the casual factors, in consequence that young people are highly susceptible to HBV infection. Vaccination and awareness programs are very necessary on urgent basis to prevent HBV endemic between young group peoples.

### 3.4. Determination of HBV by selected parameters in the population of North Waziristan

Table 2 shows the presence of HBV infection that is significantly associated with gender, literacy, socioeconomic position and profession. The presence of HBV DNA was significantly higher in the male than female participants ( $P < 0.001$ ). Male patients 95 (75.39%) were more susceptible to HBV infection as compare to female 31 (24.60%). Illiterate patients appear to be more susceptible to HBV than literate patients ( $p = 0.035$ ). Moreover lower socioeconomic patients are significantly more affected as compare to middle class patients ( $p = 0.020$ ). A significantly higher HBV prevalence was observed in divers as compared to farmers and other professions. Drivers are significantly more susceptible to infection than farmers and others ( $p = 0.0005$ ).

### 3.5. Possible routes of HBV transmission among infected individuals

Table 3 shows related risk factors of HBV transmission that were observed during the data collection phase. The overall potential routes of HBV transmission found were; 81% due to the reuses of needles and disposable syringes, 42.06% due to sexual exposure,

**Table 1**  
Prevalence of HBV in diseased subjects according to different age group.

Age groups (years)	HBV PCR results	
	HBV carriers	Observed prevalence (%)
<10	4	3.17
10–20	11	8.73
21–30	49	38.88
31–40	24	19.06
41–50	29	23.01
>50	9	7.14

**Table 2**  
Distribution of HBV by selected variables in the subjects of North Waziristan.

Parameters		Total cases	HBV RNA positive	P value
Sex	Male	485 (61.39%)	95 (19.58%)	0.002
	Female	305 (38.60%)	31 (10.16)	
Literacy	Literate	112 (14.17%)	17 (15.17%)	0.035
	Illiterate	678 (85.82%)	109 (16.07%)	
Socioeconomic status	Middle	253 (32.02%)	27 (10.8%)	0.02
	Lower	547 (69.24%)	99 (18.33%)	
Profession	Farmers	309 (39.11%)	40 (12%)	0.0005
	Drivers	251 (31.77%)	62 (24.8%)	
	Others	230 (29.11%)	24 (10.43%)	

**Table 3**  
Risk factor/routes of transmission associated with HBV infection.

Variables	Mode of transmission			
	Reuses of needles/syringes	Sexual exposure	Barbers shops	Tattooing
Male	72	41	57	12
Female	30	12	–	27
Total	102	53	49	39
Observed prevalence (%)	81	42.06	45.23	37

45.23% due to barber risk and 37% due to tattooing. High prevalence was observed (81%) in patients with multiple uses and reuses of contaminated needles and disposable syringes.

#### 4. Discussion

In the present study we had evaluated the areas of North Waziristan, part of Waziristan agency affected with war against terrorism for prevalence of hepatitis B among the war affected people. The agency is a rural, socio-economically and educationally backward area of FATA Pakistan (CCS, 2011). According to a recent report, overall approximately 67.5% of the Pakistani population is living in rural areas (PCO, 2011). Studies have confirmed that HBV is mostly prevalent in low socio-economic rural areas (Akbar et al., 1997). A few sero-epidemiological surveys conducted in some rural parts of Pakistan are available with a very small number of samples for specific geographical regions. No study on HBV prevalence representing the war affected areas of North Waziristan regions of Pakistan is available; therefore, this study was carried out with the purpose of finding out the prevalence of HBV DNA circulating in this ignored region of the world.

According to the findings of our study, the overall HBV prevalence in the conflict areas of North Waziristan was 58.4% (anti-HBs reactive) with 41.01% HBsAg and 15.94% HBV DNA positivity. High prevalence (41.26%) was observed among the people of subdivision Miran Shah relating to the high frequency of military activities in this part. Similarly, a high HBV prevalence was reported in another major war affected area, i.e. Subdivision Mir Ali, which is approximately 35.7% as compared to 19.8% in Razmak subdivision. Miran Shah and Mir Ali subdivisions are mostly targeted in the war against the militants by Pakistani armed forces. High rate of prevalence in Miran Shah and Mir Ali subdivisions directly reflect that HBV infection is highly common in the subjects belonging to the low socioeconomic war affected setting of this part of the world. Subjects belonging to these areas were found low socioeconomic status and deprived of basic health facilities.

In the present setup males (75.4%) were more affected as compared to females (24.6%) the ratio of infection in male and female 3:1. Nearly similar results have been reported earlier from other

parts of Pakistan as high prevalence of HBV was observed in male gender (78.04%) as compared to female gender (21.95%) (Khan et al., 2011; Shazi and Abbas, 2006). Likewise in the neighboring countries the prevalence rate of HBV infection is significantly high in males as compared to female (Zhang et al., 2011; Zali et al., 1996). This high HBV prevalence rate may be associated to the fact that male in rural area of Pakistan is socially more active than female. Thus male gender is highly expose to the open environment, get infection more easily and are associated to many risk factors such as sexual exposure (homosexual men), shaving in community barbers shops and reuses of unsterilized needles/syringes as compared to female.

Our findings showed that low socioeconomic status, illiteracy, and profession do significantly facilitate HBV transmission. Several studies have been reported from developing countries that HBV infection is significantly high in patients with low socioeconomic settings, in illiterate or persons with low education level in farmers and in drivers (Sali et al., 2005; Akbar et al., 1997). The prolonged armed conflicts (From the last one decade) had caused considerable increases in poverty, medical deprivation, uncertainty and the breakdown of social structures in this part of the world that facilitated the transmission of blood born infectious agents including HBV.

It is clear from the findings of the current study that HBV infection was high in almost all age groups subjects, however, was very high (38.88%) HBV prevalence was seen in young subjects (from 21 to 30 years). This high prevalence of HBV carrier rates is in accordance with Khatkhat et al. (2009) published data where frequent prevalence was noted in young age peoples as compared to children and aged people. The possible reasons for this high prevalence of HBV disease among our young patients may be due to the lack of immunization against HBV, lack of education, barbers carelessness and presence of multiple sexual partners particularly men sex with men. It can be seen from the results of the present study that HBV infection is associated with different modes of transmission. The major route of transmission was noticed among injectable medicines users due to the reuses of unsterilized needles and disposable syringes (81%). It has already been well documented that in developing countries frequent route of HBV transmission is the use of contaminated needles/syringes in healthcare settings (Hauri et al., 2004) and similar results on HBV transmission are available from Pakistan (Jafri et al., 2006; Bari et al., 2001; Alavian et al., 2007). The existence of this main route of HBV transmission are multi-factorial such as a strong tendency in peoples preferring injectable medications over oral medications, lack of health education and health awareness among health care workers especially in health technicians and dispensers who are unaware the consequences of reuse the unsterilized contaminated needles and disposable syringes for general population of this area.

Sexual transmission is another common route of HBV transmission in several developed countries and it is important route for HBV infections in Pakistan (Mujeeb et al., 1998; Alavian et al., 2007) and in our neighbor country Iran (Vahid et al., 2005). According to our finding 42% patients were associated to sexual risk. In this context HBV infection was significantly high in drivers suggesting that majority of the drivers are uneducated and spending most of their life from outside the door makes multiple sexual partners and also some truck drivers do connect in homosexual contact to their young conductors/cleaners. Another risk factor evidently for male is barber risk (45.23%) majority of the barbers are uneducated and they have to reuse the contaminated blades and razors for the common peoples. Two national studies justified nearly the same barbers risk (47.6%) that barbers are major contributors in transmission of HBV infection (Janjua and Nizamy, 2004; Usman et al., 2003). Majority of the barbers are uneducated, poor and careless about reusing the blades. A large number of

males of this area visit to the community barber shops for face shaving. The last risk factor of the results indicated that 37% of HBV positive subjects besides had a tattoo. These findings supported the earlier findings that tattoo parlors and acupuncturists are playing important role in the transmission of HBV infection in healthy population (Khattak et al., 2009). Several limitations were observed during the interpretation of our findings. We do not have any data on sharing of personal items, on house hold contact, on dental medical and medical surgeries. Recently Khan et al. (2011) noted that the aforementioned risk factors that were strongly correlated with HBV infection in general populace of Pakistan (Khan et al., 2011).

The findings of this study suggest an alarming high rate of HBV infection among the conflict-affected people. These carries are not only at greater risk for severe complications such as chronic hepatitis end stage liver disease but it also makes them potential carriers of virus to the general healthy population. Dreadful actions of governmental, nongovernmental and health care teams are very necessary to vaccinate the naive public of this conflict area against HBV. Educative efforts should be very indispensable to reduce HBV infection to avoid reuses of contaminated needles/syringes, blades and unsafe sex. Finally to update national plan makers and line up leaders about inclinations in HBV rate and risk behaviors which are very helpful for developing appropriate precautionary measures and educational programs. Lastly the governmental strict enforcement regulations should be focused extensively on reducing the rate of HBV infection among conflict affected residents due to war against terrorism.

## 5. Conclusions

These findings of the current study show high prevalence of HBV infection in young individuals and among the injector medicines users. This alarming high prevalence of infection illustrates that there is no campaign or health strategy for combating and controlling HBV. It has been strictly recommended that these medically deprived areas affected with war against terrorism should be visited by the Governmental and Non-Governmental firms to provide medical facilities to HBV patients and finally to vaccinate the healthy population against HBV. Education, awareness programs and preventive measures should be taken to avoid reuses of contaminated needles/syringes, blades, razors and unsafe sex for further spreading of the deadly infection.

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