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Hepatitis B Virus Infection in the Healthy Volunteers: A Screening Campaign in Nowshera Khyber Pakhtunkhwa, Pakistan

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Abstract

Hepatitis B viral infection (HBV) is a genuine worldwide general medical issue. The aim of this study was to find the epidemiology of HBV infection with common risk factors among the people of Nowshera Khyber Pakhtunkhwa, Pakistan. A camp was conducted for HBV screening in Nowshera City (September 2018) in which 1180 volunteers participated. Blood (5ml) was taken from volunteers in medical camp and was transported to Aziz Biotech Medical Lab and Research Center Mardan, Pakistan. All the samples were initially screened for HBV surface antigen using ICT device kit (Accurate Diagnostics Canada). Positive samples were then subjected to Real time PCR to check active hepatitis B infection amongst positive ICT samples. Out of 1180 volunteers 58 (4.91%) were found positive including 22 (4.82%) females and 36 (4.97%) males. The ICT positive samples were than refined by real-time PCR for active hepatitis B virus out of that 26 (44.82%) were found active by PCR which comprises 8 (36.36%) females and 18 (50%) males. The HBsAg ratio was greater in the Age-limit 21-30 years (5.67%) and 41-50 years (5.20%). The Sero-prevalence of HBV infection is higher in Nowshera region. The prevalence ratio among males is greater than females and mostly infected females were married which shows that sexual interaction is the probable risk factor for HBV infection. The rural communities are illiterate and unaware of the causative agents, spreading and the consequences of HBV infection. Thus, to overcome the incidence of HBV infection, we must educate the ordinary citizens about Hepatitis B virus.

Keywords: HBV Infections, Nowshera, Pakistan, Risk Factors

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1. INTRODUCTION

Hepatitis B infection is a viral disease caused by hepatitis B virus (HBV) a DNA containing virus belonging to the family hepadnaviridae which is a noteworthy medical issue around the world. Hepatitis B virus (HBV)

influences the liver functional cells, the hepatocytes. Finding of HBV disease is affirmed by exhibiting antibodies or potentially antigen in serum of patients[1,2]. The illness may happen with constrained or no side effects, yet regularly prompts jaundice, poor hunger and disquietude. It is intense when goes for under a half year and unending when perseveres for longer time[3].

There are two routine diagnostic tests utilized in the laboratory for the identification of HBV disease. The main imperative laboratory test is the immune-chromatographic test (ICT) which is snappier, simple to perform and is more affordable and is utilized for deciding the counter hepatitis B surface antigen (HBsAg) positive volunteers will have dynamic disease of HBV. The other one is the PCR strategy which is costly, yet more exact and corroborative technique for active infection of HBV and HCV[4,5,6]. Hepatitis B virus (HBV) infection is a genuine worldwide general medical issue[7], especially in Africa, Asia, South Europe and Latin America[8]. Around 2 billion individuals are infected with HBV overall[9], and 400 million individuals are experiencing perpetual HBV infection[10]. Pakistan is to a great degree endemic territory for HBV with an expected 9 million individuals infected with HBV[11]. HBV infections are mainly blood borne disease which is mostly transmitted through unscreened blood transfusions from one person to another[12]. To overcome the HBV infections, it is vital to know the causative variables and the exact commonness of the sickness.

Protective estimates must be adjusted to maintain a strategic distance from Hepatitis B viral infections. The primary spreading factors are interacting with infected blood, unprotected sexual interactions, semen and vaginal discharges, the sharing of needles among injectable drug user (IDU's), sharing of personal equipment's like shaving razors, tooth brushes and hair brushes. In the developing nations the main risk factor for transmission of HBV infections are blood transfusion[13]. Our aim will to find out the prevalence ratio of HBV infection and its common risk factors among the people of Nowshera region KP, Pakistan.

2. MATERIALS AND METHODS

2.1 Data Collection

A medical Camp was conducted for HBV Screening in District Nowshera in which 5ml Blood samples were collected from each individual and were then transported to Aziz Biotech Medical Lab and Research Center Mardan. The serum was isolated from whole blood by centrifugation on 3000 rpm for five minutes. The serum was transferred to new Eppendorf tube and labeled the tube properly. All the samples were stored at -30ºC for further screening. A questioner was filled during the collection of blood samples from each individual and the HIV and HCV patients were excluded. The study was approved by advance studies and research board (ASRB) Abdul Wali Khan University Mardan Pakistan.

2.2 Anti HBV Screening

All the samples were initially screened for HBV antibodies using Immuno-chromatographic test (ICT) Kit (Accurate Diagnostics Canada) as described according to the manufacturer instructions. The ICT positive samples were further subjected to real time PCR to confirm active HBV infections amongst positive ICT samples.

2.3 HBV DNA Extraction

DNA was extracted from 75µl of serum sample by using Gene-Proof Extraction Kit (LOT No. 83092, Czech Republic) according to manufactures protocols.

2.4 HBV DNA Qualitative Detection

For the qualitative detection of HBV DNA, the Extracted DNA template is then amplified and detected on real time PCR (Cepheid-Smart Cycler) using primers and sequence specific probes of Anatolia, Turkey, HBV RT-PCR kit. The patients HBV DNA is detected on fluorophore florescence growth (FAM) channel. Control is incorporated for quality assurance. Internal control is detected on Cy3 channel. The PCR was run for 40 cycles in four batches.

2.5 Statistical Analysis

All the data were statistically summarized, analyzed and tabulated by using statistical package SPSS version 10.0 (SPSS Inc., Armonk, New York, USA) and Microsoft Excel version 2016. The percentages and ratios result for all variables were calculated.

3. RESULTS AND DISCUSSIONS

3.1 Gender-wise ratio

Out of the total 1180 volunteers 724 (61.35%) participants were male and 456 (38.64%) were females. For anti HBV screening we found 58 (4.91%) individuals reactive to ICT device kit which includes 36 (4.97%) male and 22 (4.82%) female individuals. Out of 58 ICT positive individuals we found 26 (44.82%) individuals reactive to qualitative PCR which includes 18 (50%) male individuals and 8 (36.36%) female individuals.

Table 1. Shows the gender wise prevalence on ICT device kit and PCR method**.**

Gender	Total Individuals = n (%)	ICT Positive = n (%)	PCR positive = n (%)
Male	724 (61.35%)	36 (4.97%)	18 (50%)
Female	456 (38.64%)	22 (4.82%)	08 (36.36%)
Total	1180 (100%)	58 (4.91%)	26 (44.82%)

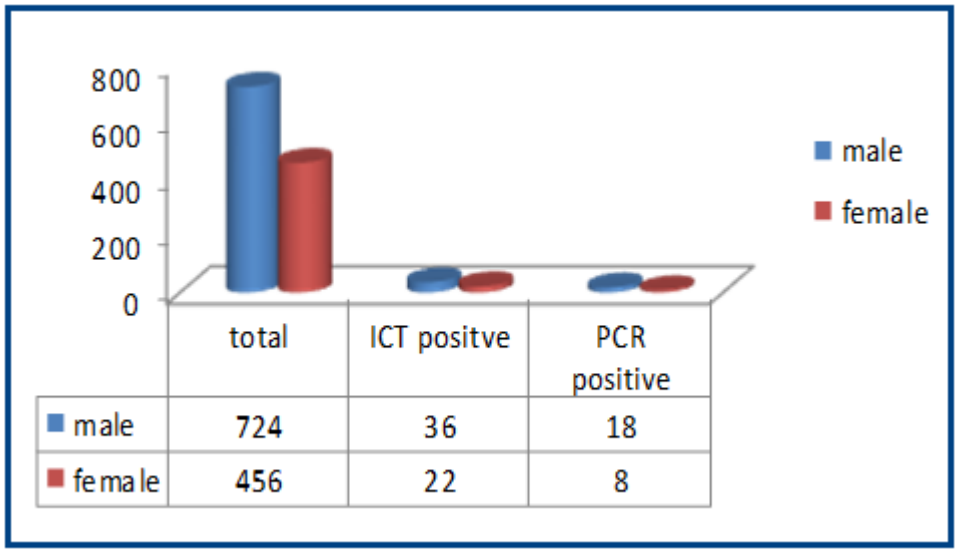


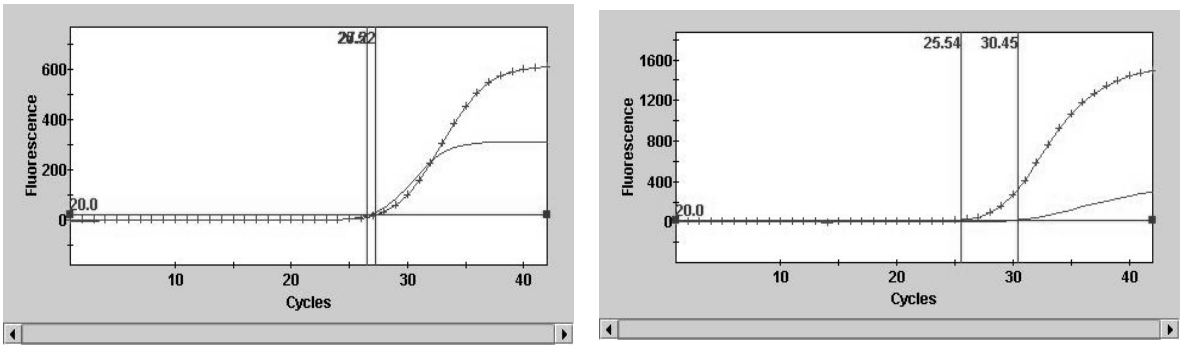
Fig. 1. Shows the overall prevalence of HBV infection s in district Nowshera KP, Pakistan.

3.2 Age-wise ratio

In the current study we classify the total 1180 volunteers in five Age-groups (A, B, C, D, E).

Table 2. Age-wise ratio in group A to E

Groups	Age groups (years)	Total individuals= n%	ICT positive= n%	PCR positive= n%
A	10-20	148 (12.54%)	6 (4.05%)	0 (0.0%)
B	21-30	388 (32.88%)	22 (5.67%)	10 (45.45%)
C	31-40	282 (23.89%)	12 (4.25%)	8 (66.66%)
D	41-50	192 (16.27%)	10 (5.20%)	4 (40%)
E	51-<	170 (14.42%)	8 (4.70%)	4 (50%)
Total	Total individuals	1180 (100%)	58 (4.91%)	26 (44.82%)



Patient samples **Positive control**

Fig. 2. This shows real time PCR result of patients with positive control samples. The graph shows the cycles of PCR.

Now a day's Hepatitis B viral infections is the major medical issue around the world including Pakistan as well[14]. Hepatitis B infections are mostly related to Blood borne diseases which is mainly transmitted through unscreened blood transfusions from one person to another. Pakistan has been rated as a great degree endemic territory for the HBV infections with an expected 9 million individuals tainted with HBV[11]. According to age-wise distribution we reported that the predominance of HBV on ICT in group-B (5.67%) was greater followed by group-D which were 5.20%. The group-E were 4.70% followed by group-C which was 4.25% and were less then group-B and group-D. The prevalence found in group-A were 4.05% which was less than all groups. As shown in the **Fig. 3.**

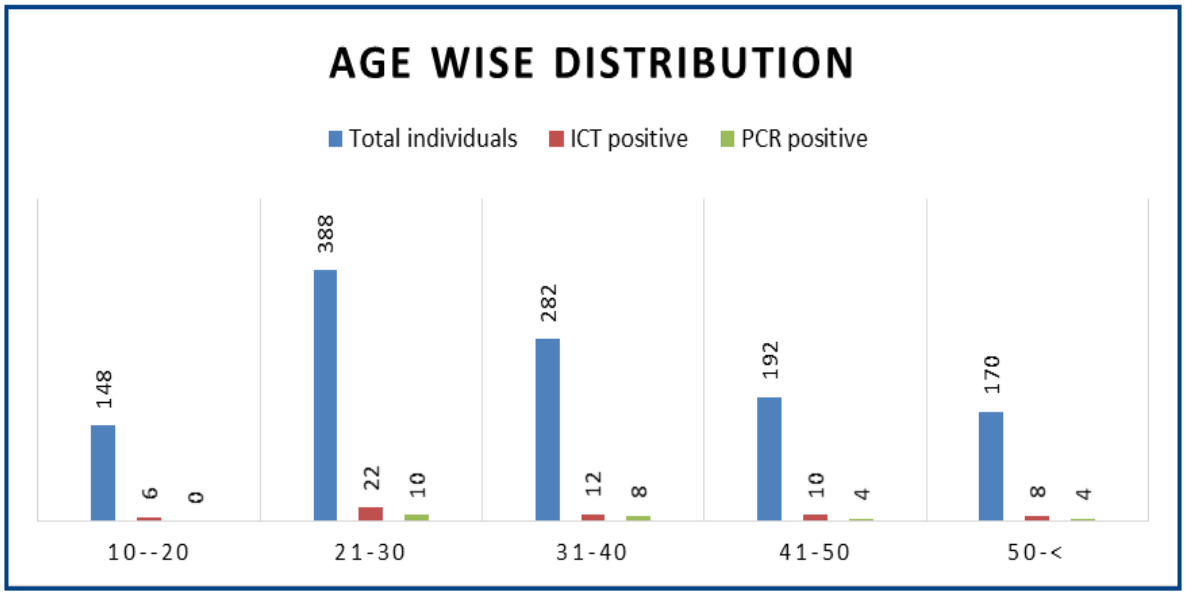


Fig. 3. Age-wise ratio of HBV infection in healthy volunteers.

Here we reported that the mostly infected individuals were in age limit 21-30 and 41-50 years. According to our findings we noted that the overall predominance of HBV infection among Nowshera peoples were 4.91% reported on ICT device Kit out of that 44.82% reported on real time PCR method. Our data comprises with other studies carried in different territories of Pakistan which was 4.33% (±1.64%) approximately[15]. Another study reported that in different territories of Punjab the HBV prevalence ratio reported in Rahim Yar Khan was 8% which was higher than Liaqatpur 6.99%, Pak Pattan 5.32% and Gujranwala 5%. The predominance detected in Dipalpur was 3% and Lahore were 2.3%[16]. Our data shows less predominance of HBV than Rahim Yar Khan, Liaqatpur, Pak Pattan and Gujranwala but shows greater predominance than Dilpalpur and Lahore. Similarly, the HBV prevalence ratio reported in Islamabad and Karachi were 5.3% and 6.5% respectively[17,18], while in Faisalabad the ratio was 4.5%[19]. In Khurram agency and Peshawar the HBsAg prevalence reported were 5.07% and 3.3% respectively[20,21]. Nowshera is situated at some distance from Peshawar and Khurram agency thus, we noted that the predominance of HBV infection in Nowshera region were 4.91% somehow related the prevalence found in Khurram agency and Peshawar which were 5.07% and 3.3% respectively. Our report shows the predominance of HBV infection is greater in males as compared with female individuals and the infected females were mostly married. The primary spreading factors responsible for HBV viral infections includes interaction with tainted blood, unprotected sexual interactions, semen and vaginal discharges, the sharing of needles among IDU's (injecting drug users), sharing of personal equipment's like shaving razors, tooth brushes and hair brushes. In the developing nation the main risk factor for the transmission of HBV infections are blood transfusion[18]. Other hazard factors are absence of screening of blood items[22], the reuse of syringes in some areas, underreporting of infected cases and needle stick injuries[23], absence of instruction and hazard mindfulness in rural and urban regions[24], and natural disasters like floods in the studied territories[25]. The study previously reported by Shabina Aziz and Rashid Iqbal in Nowshera shows that 17.6% peoples were aware that Hepatitis B were liver related infection and virus is its causative agent[26]. It shows that the rural communities are illiterate and unaware of the causative agents, mode of transmission and the consequences of HBV infections.

4. CONCLUSIONS

Serological prevalence of HBV infection is higher in Nowshera region. The prevalence ratio among males is greater than female individuals and the infected females were mostly married. The sexual interaction is the probable risk factor for HBV viral infection. It is proposed that the best consideration ought to be executed during surgical measures or medications and blood transfusions. The further mindfulness

development against Hepatitis B infections ought to be endorsed to educate the ordinary citizens on the hazard factors and defeat of transmitting particle with a specific end goal to diminish the rate of disease.

CONFLICT OF INTEREST

All the authors claim that there is no conflict of interest regarding the publication of this paper.

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