ORIGINAL ARTICLE

Risk factors and molecular epidemiology of HBV and HCV in internally displaced persons (IDPs) of North Waziristan Agency, Pakistan

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Abstract

Objective: To investigate the prevalence and transmission risks of hepatitis B virus and hepatitis C virus infections among internally displaced persons.

Methods: This study was conducted in district Bannu, Khyber Pakhtunkhwa, Pakistan, from August 2014 to April 2015, and comprised internally displaced people from North Waziristan Agency residing in camps or settled in towns. Potential subjects were initially screened through immunochromatographic test devices. Samples detected positive were confirmed through enzyme-linked immunosorbent assay. Confirmation of the presence of viral nucleic acids and genotyping was done via real-time polymerase chain reaction. SPSS 21 was used for data analysis.

Results: Of the 1,000 participants, 570(57%) were males and 430(43%) females. Seroprevalence of hepatitis B virus among the participants was 45(4.5%) and hepatitis C virus was 52(5.2%). Viral nucleic acids were present in 41(4.1%) participants in case of hepatitis B virus and 39(3.9%) in case of hepatitis C virus. No co-infection with both of these viruses was found. Dominant hepatitis B virus and hepatitis C virus genotypes were genotype-D 19(46.3%) and 3a with 20(51.2%), respectively.

Conclusions: High prevalence of hepatitis B and C virus infections was found among internally displaced people.

Keywords: HBV, HCV, IDPs, Prevalence, NWA, Risk. (JPMA 68: 165; 2018)

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are posing obvious threat to public health worldwide due to their high prevalence and mortality rates.[1] The above statement can be clearly justified by taking into consideration the high number of people infected with HBV and HCV. It has been estimated that 350 million people are persistently infected by HBV and 185 million by HCV worldwide. Each year HBV and HCV together cause 1.3 million deaths worldwide.[2] People with persistent infection with HBV and HCV are at incessant risk to progression into end-stage liver diseases like fibrosis, hepatocellular carcinoma (HCC) and cirrhosis.[3-5] Until now 10 HBV genotypes (A-J) and 7 HCV genotypes have been recognised that exhibit geography-based distribution.[6,7]

In Pakistan, the 6th most populous country in the world, a steady rise has been seen in HBV/HCV infections. More than 9 million people in our country are living with HBV and 10 million with HCV.[8] The knowledge of infecting virus genotype is indispensable for effective treatment. In Pakistan HBV genotype-D and HCV genotype 3a.[9,10] are reported to be highly prevalent.

Complex emergence due to war often affects large civilian populations. People displacement due to conflicts further fuels the spread of infectious diseases.[11] Risks associated with the acquisition and transmission of infectious diseases is common in displaced people. Moreover, displacement adversely affects mental health and use of injectable drug increases among displaced people.[12] In June2014 a military operation by Pakistan Army in North Waziristan Agency (NWA), the 2nd largest agency in Federally Administrated Tribal Areas (FATA), compelled the people of NWA to leave their places of habitual residence and live as internally displaced persons (IDPs). Neighbouring districts, specifically Bannu and Dera Ismail Khan, remained the main hosting districts for IDPs from NWA.[13,14]

In hosting districts the environmental and social conditions in which IDPs have to live are highly conducive to the acquisition and transmission of infectious diseases.[15] IDPs are allowed to avail public health facilities, provided both at camp sites and in the hosting districts, but they sometimes face the social trauma of discrimination when they have to visit hospitals located in cities for complicated cases.[16] The present study, the first of its kind, was planned to investigate the prevalence and transmission risks of HBV and HCV infections among IDPs. The study also aimed at providing time-sensitive information about the effect of displacement on HBV and HCV infection. The key to get rid of HBV/HCV and the ailments that they inflict is timely detection, diagnosis

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and treatment. To achieve this, a clear understanding of HBV and HCV epidemiology is needful. Also, the risks of HBV/HCV transmission need to be critically reviewed in IDPs. Our intent was also to stimulate thinking about the potential threat of increased HBV/HCV transmission among displaced people, with particular reference to IDPs from NWA, and to encourage timely interventions to prevent a worsening HBV and HCV epidemic both in IDPs and hosting communities.

Subjects and Methods

This study was conducted in district Bannu, Khyber Pakhtunkhwa (KP), Pakistan, from August 2014 to April 2015, and comprised IDPs from NWA residing in camps or settled in Bannu. After approval from the institutional bio-safety and ethics committee, the screened population was divided into 5 age groups with the difference of 9 years between them. Other than determining molecular epidemiology of these infections, factors responsible for acquisition of these diseases were investigated using structured questionnaire based on personal interviews. Sampling was done in two phases. In phase-I, a total of 600 IDPs were sampled randomly and in phase-II deliberately 400 IDPs were sampled in order to chart our near to accurate dynamics of HBV and HCV infections.

Non-laboratory data on personal demographics and risks factors for HBV/HCV infection among IDPs was collected through questionnaires written in English. The questionnaire was explained to all participants in local Pashtu language and filled in accordingly after having their informed consent. Data was analysed using SPSS 21. Blood samples of all participants were taken. To draw blood from volunteers, 5cc syringes were used.

Samples were first screened through immunochromatographic test devices (ICT) devices for HBV (sensitive to HBV surface antigen [HBsAg]) and HCV (sensitive to anti-HCV antibody). ICT positive samples were confirmed via relatively higher specific and sensitive 3rd generation enzyme-linked immunosorbent assay (ELISA) kit (AntecDiagnostic's products, United Kingdom) for HBsAg and anti-HCV, according to the recommendations of manufacturers.

Samples indicated positive by ELISA were then subjected to real-time polymerase chain reaction (RT-PCR) to check the presence of viral nucleic acids. Total nucleic acid was extracted from all ELISA-positive samples using GF-RD (Rapid detection) deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) extraction kit (Vivantis, Malaysia). RT-PCR (MiniOpticon, Bio-Rad, United States) and SYBR Green PCR master mix (Life Technologies) was used in this study. After confirmation of the presence of viral DNA in case of HBV, multiplex PCR was performed for genotyping according to protocol proposed by Naito et al.[17] Similarly, after confirmation of the presence of HCVRNA using universal primers, genotyping was done according to protocol elucidated by Idrees M.[18]

Results

Of the 1,000 participants, 570(57%) were males and

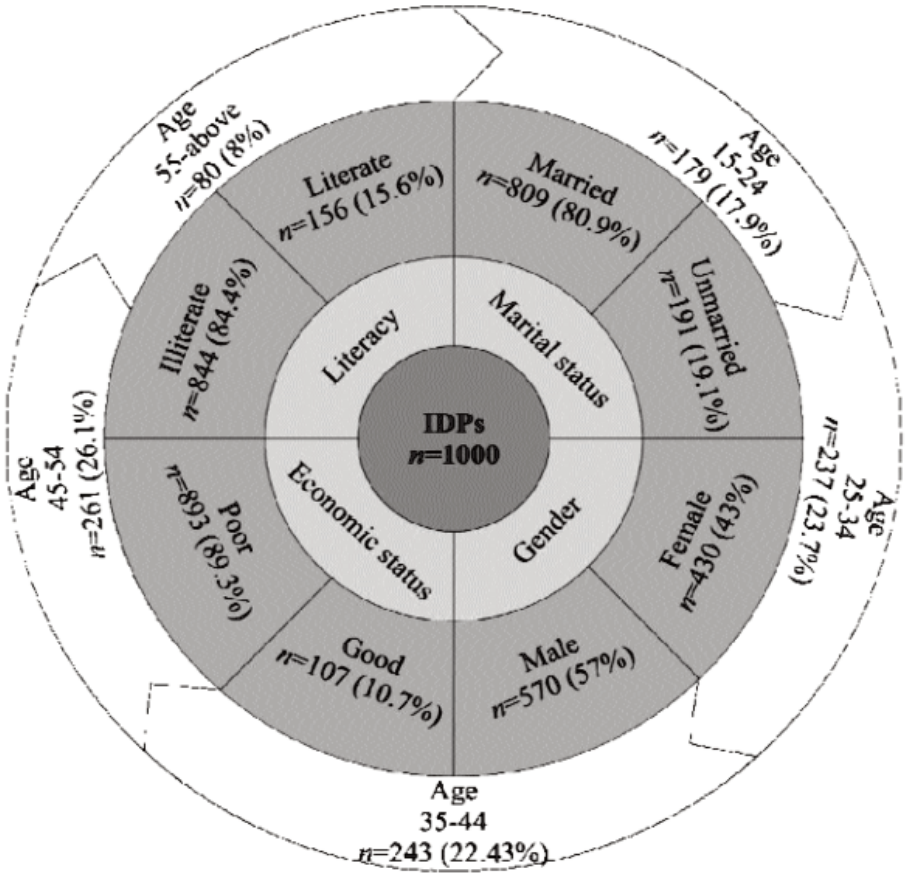
Table-1: Overall prevalence of HBV and HCV among IDPs.

	ELISA Positive	PCR Positive
HBV	n= 45 (4.5%)	n= 41 (4.1%)
HCV	n= 52 (5.2%)	n= 39 (3.9%)
Total	n= 97 (9.7%)	n= 80 (8%)

Table-2: Major risk factors in sampled population and odds ratio estimation for HBsAg and anti-HCV seropositivity by risk factor.

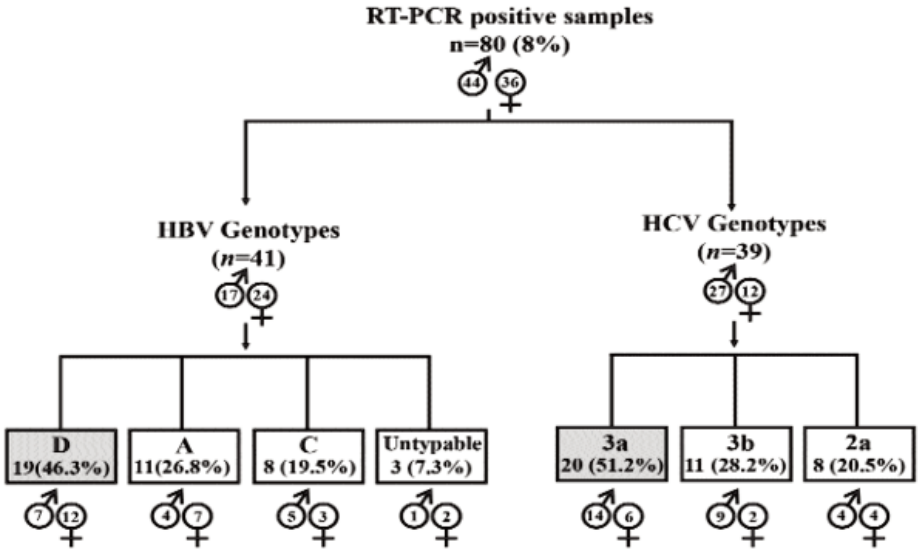
Risk Factor	Total (%)	HBsAg positive		Anti-HCV positive	
		n (%)	OR (95% CI)	n (%)	OR (95% CI)
Undergone blood transfusion?					
Yes	157 (15.7)	15 (33.3)	.116 (.098 - .139)	21 (40.4)	.111 (.092- .133)
NO	843 (84.3)	30 (66.7)		31 (59.6)	
Barber shop Shaving?					
Yes	230 (77)	27 (60)	.535 (.372 - .771)	25 (48)	.327 (.257 - .417)
NO	770 (23)	18 (40)		27 (52)	
Multiple injections?					
Yes	413 (41.3)	32 (71.1)	.585 (.455 - .517)	35 (6.3)	.581 (.551- .613)
No	587 (58.7)	13 (28.8)		17 (32.7)	
General/dental surgery?					
Yes	493 (49.3)	36 (80)	.596 (.438 - .601)	41 (78.8)	.565 (.534 - .598)
NO	507 (50.7)	9 (20)		11 (21.2)	

HBV: Hepatitis B virus
HCV: Hepatitis C virus
HBsAg: Hepatitis B surface antigen
CI: Confidence interval.



IDPs: Internally displaced persons

Figure-1: Baseline demographic characteristics of the study population.



RT-PCR: Real-time polymerase chain reaction. HBV: Hepatitis B virus. HCV: Hepatitis C virus. IDPs: Internally displaced persons

Figure-2: Genotypic distribution and gender specific stratification of the RT-PCR positive samples. Most dominant genotypes of HBV and HCV among IDPs are represented in shaded boxes.

430(43%) were females. Overall, 179(17.9%) participants were aged 15-24 years, 237(23.7%) 25-34 years, 243(24.3%) 35-44 years, 261(26.1%) 45-54 years and 88(8.8%) participants were aged 55 years or above. Besides, 893(89.3%) participants were poor, 844(84.4%) illiterate and 809(80.9%) were married (Figure-1).

The prevalence of HBV and HCV was 45(4.5%) and 52(5.2%), respectively, based on ELISA and 41(4.1%) and 39(3.9%) based on PCR (Table-1).

There was a significant decrease in HBV and HCV false positive cases with increase in specificity and sensitivity of detection technique. Not a single case of co-infection with HBV+HCV was found in our subject population. Gender-based stratification of the total infected individuals showed high HBV infection among females, and high HCV infection among males (Figure-2).

PCR-based genotyping for HBV revealed that the most dominant HBV genotype among infected IDPs was genotype D with 19(46.3%) prevalence, followed by genotype A 11(26.8%) and genotype C 8(19.5%), while 3(7.3%) cases did not fall into any type according to the method used for genotyping. Genotype 3a was found to be most dominant HCV RNA among IDPs with 20(51.2%) prevalence, followed by genotype 3b 11(28.2%) and genotype 2a 8(20.5%).

The awareness level among IDPs regarding these infections and modes of spread was alarmingly low. Major risk factors for acquiring infections were found to be unsafe dental and surgical procedures, multiple injections and unhygienic conditions at local barber shops. Most importantly,

more than half of infected individuals (HBV/HCV) had a history of blood transfusion (Table-2).

Discussion

The armed conflict between Pakistan Army and multiple militant groups in FATA has resulted in internal displacement of hundreds of thousands of people.[19] NWA is the second-largest agency of FATA with poor socio-economic conditions as well as deplorable health and education infrastructure.[20] Mass population movement, resettlement in temporary locations, overcrowding in camps, poor health facilities, malnutrition, unhygienic food and water, poor sanitation and lack of awareness are the factors that interact synergistically in transmission of communicable diseases.[21]

Present study was conducted with the objective to investigate the overall prevalence, baseline epidemiology and genotypic distribution of both HBV and HCV among IDPs of NWA residing in district Bannu. This study differs from previous studies on IDPs in Pakistan mainly due to comparatively large population size, epidemiology on molecular level and determination of major risk factors.[20,22-24] The overall prevalence of these viruses in apparently healthy adults based upon prevalence studies conducted so far is reported to be 2.4% for HBsAg and 3.0% for anti-HCV in Pakistan.[25] Our results exhibit significantly high HBsAg and anti-HCV seroprevalence as the population of our study is not only subject to forced displacement but are deprived of basic health and sanitation facilities in camp settings. In the present study, HCV was reported to be more prevalent then HBV among IDPs, which is in accordance with the results of a study conducted by Rauf et al. among IDPs hailing from Swat.[22] The seroprevalence of HBV (4.5%) and HCV (5.2%) among apparently healthy IDPs residing in district Bannu is comparatively low then previously reported (9.66%).[22] However, the discrepancy may be due to the fact that the present study's population size is approximately double the population size (n=590) of the afore-mentioned study. Similarly, studies from various population groups in Pakistan have reported higher prevalence of HCV infection as compared to HBV.[26] Vaccination against HBV has resulted in a substantial decrease in infection in developing countries. Moreover, HCV is more easily transmitted horizontally as compared to HBV.[27]

The knowledge of causative HBV genotype is of immense importance from both clinical and epidemiological points of view. The type of causative genotype determines the disease pattern and response to interferon therapy.[28] In the present study, the most frequent DNA of HBV in the studied population was genotype D, followed by genotype C, genotype A and untypable genotypes. Our results are supported by the findings of Ali et al. that about 63.71% of HBV patients in Pakistan carry genotype D.[29] PCR-based genotypic determination in the present study revealed that the most frequent HCV RNA among the studied population is genotype 3a, followed by genotype 3b and genotype 2a. Similar results were reported by Saleha et al. in their studied population of district Bannu.[30] These results are also in agreement with the results of another study in which genotype 3a was reported to be most prevalent among all observed genotypes followed by genotype 3b.[28]

As far as prevalence in the hosting community of district Bannu is concerned, Majid et al. reported a rising prevalence of HBV and HCV in district Bannu. The combined seroprevalence rate of HBV and HCV reported by them was 5.2%. They also reported higher HCV seroprevalence than HBV. Not a single case of co-infection was reported in their studied population. Our results show significantly high HBV and HCV infection in IDPs than the hosting community of district Bannu[31] as they have arrived from NWA with poor health facilities.

One of the objectives of the study was evaluating the major risks leading to HBV and HCV infection in the population subjected to study. Our data depicts that a majority of HBV- and HCV-infected IDPs had undergone dental or general surgery in the near past, making it the most obvious exposure risk for acquiring infection among IDPs. Our findings are in conformity with the results of Awan et al., reporting strong association between previous history of dental procedure with HCV infection.[23]

A significant proportion of infected individuals in our study indicated exposure to multiple injections. Similarly, Ali et al. reported strong association between the exposure to multiple injections and HBV transmission among conflict-affected NWA inhabitants. It might be because people in these areas prefer injectable medication over oral medication.[20] A study aimed at evaluating the multiple risks leading to HBV and HCV infections among chronically infected HBV and HCV patients in Pakistan evaluated that general and dental surgery, multiple injections and blood transfusion are the common risks leading to HBV and HCV infections. In our study, similar risks leading to HBV and HCV infections were evaluated.[32]

Conclusions

Like general population of the country, considerable prevalence of HBV and HCV infections was found among IDPs. The practice of sharing space and resources with the hosting community with already fragile health conditions

may increase virus transmission and acquisition. To prevent the onset of HBV and HCV epidemics among IDPs and local communities, tailored awareness and screening programmes are needed.

Declarations: The study was performed with the approval of institutional bio-safety and ethics committee of Quaid-i-Azam University with informed consent of volunteers who provided samples for this study. This work was M-Phil research project of Mr. Adeel Khan.

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Conflict of Interest: None.

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