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Seroprevalence and Risk Factors of Hepatitis C Virus (HCV) in Tehsil Takht Bhai District Mardan, KPK, Pakistan

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Abstract

The assessment of Hepatitis C Virus based on seroprevalence with respect to its risk factors is carried out amongst the volunteers of Tehsil Takht Bhai district Mardan, Khyber Pakhtunkhwa, Pakistan. This type of study has never been carried out here in this region so far, so we done this research study to explore the prevalence ratio of HCV in this region. We used HCV Ab rapid test cassette serum/plasma (USA) kit to detect the antibodies produce by immune system in reaction to HCV in the serum/plasma of volunteers. Then ELISA was carried out for the ICT positive individuals and hence finally ELISA positive samples were reconfirmed by RT-PCR for Hepatitis C Virus. In every case of HCV socioeconomic details were noted apparently amongst the victims. The study population comprised of 640 volunteers, 385 (60.2%) males and 255 (39.8%) females. The overall seroprevalence was found to be 4.69% of HCV in Tehsil Takht Bhai district Mardan. This seroprevalence ratio was 5.19% in males and 3.92% in females. ICT positive samples were then refined by 3rd generation ELISA which indicated that 86.67% of the ICT positive volunteers had antibodies against HCV. Moreover to confirm the active infection, ELISA positive samples were investigated by real time PCR which showed that 65.38% of the ELISA positive volunteers had HCV RNA in their blood. Significant risk factors for acquiring HCV infection include contaminated reused syringes/blades, blood transfusion, surgical operations and dental procedures. Body weakness was common symptom in HCV positive volunteers. Generally we can protect ourselves from Hepatitis C Virus via blood screening before donation and to avoid injecting equipment i-e needles etc, sterilizing surgical equipments and following safe sexual activities.

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Introduction

Hepatitis C Virus (HCV) was identified in 1989 as a cause of Hepatitis C in human being (Ryan and Ray, 2004). It has a single stranded RNA genome of 9.6kb which transcribes in a single open reading frame (Kato, 200). Mainly HCV transmission occurs through whole blood or blood product transfusion (Akbar *et al.*, 1997; Wasley and Alter, 2000), surgical procedures (Esteban *et al.*, 1996), and dental procedures (Piazza *et al.*, 1995). According to WHO reports about 3% of the total world population which is almost equivalent to 200 million people worldwide are infected with HCV (Alter, 1997; Khan *et al.,* 2011).

The measurement of anti HCV antibodies or seroprevalence is normally used to monitor HCV burden on a population. Different studies have shown different seroprevalence in different populations like 2% in Spain (Sacristan *et al.,* 1996) 3.9% in Jakarta, Indonesia (Akbar *et al.*, 1997) and 1.25% in Zakinthos Greek (Sun *et al.,* 1999). Similarly, hospital based study of HCV reported seroprevalence of 5.9% in Mauritius (Shwarz *et al.,* 1994), 6.0% in Ethiopia (Frommel *et al.,* 1993) and 4.8% in South India (Bhattacharya *et al.,* 2003). WHO roughly calculated that about 10-20 million individuals are infected with HCV in India and a seroprevalence of 2.4% in Pakistan (Khan *et al.*, 2004) and 64 to 103 million prevalence of chronic HCV in children worldwide (Gower *et al.*, 2014).

Direct acting antiviral drugs against HCV were inaugurated in 2011. HCV protease inhibitors which are boceprevir and telaprevir used in combination with interferon alfa and ribavirin and were associated with complexity of treatment and increased toxicity (Dusheiko and Wedemeyer,2012) Only 3 years after approval, both first generation protease inhibitors are no longer recommended in many countries (Koh *et al.*, 2013).

In particular, WHO estimated HCV infections which were poorly characterized based on initial acute infection while associated burdens of chronic liver diseases are omitted (Global Burden, 2004; Kim, 2010). The aim of this study was to evaluate risk factors and to estimate seroprevalence of HCV in Tehsil Takht Bhai district Mardan, Pakistan.

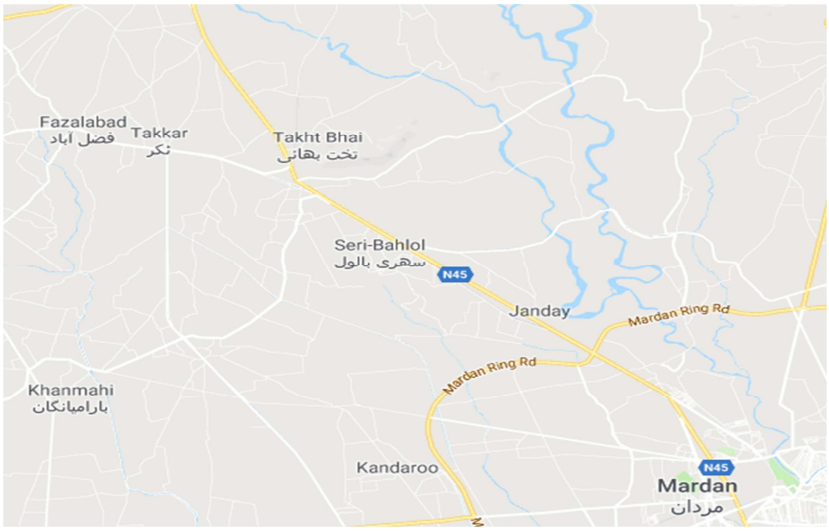

Fig. 1. Takht Bhai District Mardan, KPK, Pakistan.

Materials and methods

Study subjects

A total of 640 healthy volunteers including 385 (60.2%) males and 255 (39.8%) females belong to Tehsil Takht Bhai, Mardan Pakistan were enrolled in this study. Individuals already reported with any known liver diseases or complications were excluded from the study subject list. All volunteers were asked some specific questions about their general habits, risk factors and socioeconomic status and the data was noted on specially designed questionnaire. The Immuno Chromatographic Test (ICT) positive

individuals were than subjected to ELISA and real time PCR based method for confirmation. Approval for the study was taken from the ethical committee of Abdul Wali Khan University Mardan Pakistan and an informed consent form was signed from each individual participating in this study. Clinical and diagnostic tests were performed in Aziz Biotech Lab and Research Center Mardan, Paksitan.

Clinical investigations of the study subjects

All the study subjects were clinically investigated through different procedures like immuno chromatographic test (ICT), ELISA and real time PCR.

Immuno-chromatographic test (ICT)

ICT is the most important test for the detection of HCVAg indirectly from serum/plasma. All the samples were screened for HCVAg through HCV Ab Rapid Test Cassette Kit (USA).

Enzyme Linked Immune Sorbent Assay (ELISA)

This method is highly sensitive and easy to perform. The samples of the long-suffering individuals were investigated for antibodies with 3rd generation ELISA usingEMP-CR-201 ELISA kit following manufacturer instructions.

RNA Isolation and Real Time PCR

RNA was isolated from all ELISA positive HCV cases using GeneProof extraction kit and was then subjected to reverse transcriptase PCR (RT-PCR) (Sacace, Biotechnology, Italy) to prepare cDNA. All sero-positive samples were further analyzed and reconfirmed through real time PCR (Cepheid Smart Cycler).

Results

The study subjects were divided into different age groups that are from Group A to D and the error rate was assessed by repeating 10% of the samples. Out of the total 640 volunteers, 30 (4.69%) showed ICT positive result for HCV in which 20 (5.19%) were male and 10 (3.92%) were females (Table 1), while prevalence ratio was found 4.06 through ELISA and 2.66 through RT-PCR. Group B had high number of ICT positive individuals 14 of which 9 (4.26) were males and 5 (3.62%) were females. Out of 30 ICT positive individuals, 26 (86.67) %including 17 (56.67%) male and 9 (30%) female were found positive for HCV through ELISA (Table 2). Furthermore, 17 (65.38%) of the ELISA positive individuals were found positive through real time PCR as well (Table 3).

Table 1. Gender wise distribution of study subjects in different age groups and ICT results.

Age Groups (in years)	Number of volunteers (n=640)	Male Tested (n=385)	Male anti HCV + (%)	Females Tested (n=255)	Females anti HCV + (%)	Total Anti HCV
Group A (0-20)	93	57	2 (3.51%)	36	1 (2.78%)	3
Group B (21-40)	349	211	9 (4.26)	138	5 (3.62%)	14
Group C (41-60)	167	107	6 (5.61%)	60	3 (5.0%)	9
Group D (>60)	31	23	3 (13.04%)	8	1 (12.55)	4
Total	640	385	20 (5.19%)	255	10 (3.92%)	30 (4.69%)

Table 2. ELISA evaluation of ICT positive samples.

Total volunteers	Total Anti HCV +	Males Anti HCV+ (%)	Male ELISA + (%)	Females Anti HCV+ (%)	Female ELISA + (%)	Total ELISA + (%)
640	30 (4.69%)	20 (3.12%)	17 (56.67%)	10 (1.56%)	9 (30%)	26 (86.67) %

Table 3. RT-PCR evaluation of ELISA positive samples.

Total volunteers	Total ELISA+ (%)	Male ELISA + (%)	Male PCR + (%)	Female ELISA + (%)	Female PCR + (%)	Total PCR + (%)
640	26 (86.67%)	17 (56.67%)	13 (76.47%)	9 (30%)	4 (23.52%)	17 (65.38%)

Discussion

In our study the prevalence ratio of HCV were found to be 4.06% through ELISA and 2.66% through RT-PCR which is much lesser than the study previously conducted in Islamabad (Hashmi *et al.*, 2010). In 2004, the similar ratio was reported in a study conducted in the same district (Irfan *et al.,* 2004). However, other studies conducted in Islamabad and Sindh reported ratios of 5.31% and 9% respectively (Almani *et al.,* 2002; Khokhar *et al.,* 2004) . Individuals (both male and female) with the age ranges from 21-40 years are more affected in our study which is consistent with the previous investigation in Islamabad (Hashmi *et al.*, 2010).

In our study, we had 640 volunteers, out of which 385 (60.2%) were males and 255 (39.8%) were females. Blood screening through ICT devices showed that 4.69% the volunteers were positive for anti-HCV. The ratio of prevalence was more in male compared to female. The possible reason may be the travelling to abroad which is common in male while not common in females. ICT devices have problem of false positivity (Srivastava *et al.*, 2009; Grobusch *et al.*,1999) therefore ICT positive samples were subjected to 3rd generation ELISA which indicated that 86.67% of the ICT positive volunteers had antibodies against HCV.

Furthermore to confirm the active infection, ELISA positive samples were investigated by real time PCR which showed that 65.38% of the ELISA positive volunteers had HCV RNA in their blood. Absence of HCV RNA and presence of anti-HCV in the blood may be attributed to the self-limiting nature of the disease in some people or it may be due to the presence of antibodies against HCV in treated subjects. Similarly, prevention of active HCV was determined among 7148 blood donors in KP and FATA, Pakistan (Khan *et al.*, 2011). They have shown 3.13% anti HCV positivity through ICT, 1.89% through ELISA and 1.65 of active HCV through RT-PCR. Again, Seroprevalence can be different in different population. Ali, 2010 reported prevalence of active HCV in 400 individuals from District Mansehra, Pakistan.

Those subjects who were positive for anti HCV antibodies were subjected to PCR analysis and shown that 3.5% people from district Mansehra had active HCV infection. They further show 7% antibodies positive cases from general population. Several other studies from KPK and Pakistan reported prevalence of anti-HCV that is 3.13% and 5.31% to which our study is somewhat related (Khan, 2011; Khokhar, 2004).

Hence concluded that Takht Bhai District Mardan has 4.69% seroprevalence of HCV in general population, prominent risk factors were surgical operations and dental procedure and blood transfusion.

In the current study, cohorts of randomly selected 640 individuals were asked and filled a specific designed questionnaire. Moreover out of 640 volunteers, 89 volunteers had used contaminated reused blades/Syringes in which 9 volunteers showed positive result for Anti HCV, similarly 53 had blood transfusion history and 5 volunteers were positive for anti HCV, 137 volunteers had history of dental procedure in which 9 were positive for anti HCV, 217 volunteers had history of surgical operations in which 7 showed positive result for anti HCV. General weakness has strongest association with HCV showed by Logistic regression analysis.

Fatigue and general weakness was common symptom in HCV positive volunteers. Blood screening before donation avoiding unsterilized injecting equipments and safe sexual practices can lead to prevention of HCV, as there is no safety vaccine for it.

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