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Seroprevalence of Hepatitis C Virus Infection in Kohat Division, KhyberPakhtoonkhwa, Pakistan

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ABSTRACT

The current study was conducted to investigate prevalence of Hepatitis C virus (HCV) infection in Kohat division. A total of 270 ICT (Immunuochromatography test) positive male and female subjects were included in this study and divided into three groups according to their age. Blood samples were collected and processed to confirm the anti-HCV status and data was collected on pre-tested questionnaires to identify the risk factors. Out of 270 ICT positive patients, 224 were confirmed positive by ELISA (Enzyme Linked Immunosorbent Assay). Results revealed that male population was more affected (46.29%) as compared to female (36.66%). The people with age group 15-40 years were found to be more affected (87.03%) followed by above 40 years (30.37%).The mean age group of HCV positive for male was 37.57±7.85 while for female *i.e.*, 38.26±8.47. Infection was related to different risk factors and it was observed that 69.25% patients have the history of hospitalization, 50.37% piercing, 39.25% blood transfusion, 17.40% dental treatment, 31.85% surgical procedure, 22.22%have shaving practices at barber shops while 14.44% have tattooing on body. Statistical analysis shows that both educational and occupational status is significantly associated with anti-HCV antibody. Other factors associated significantly with infection were blood transfusion (p-value <0.0001, OR (Odds Ratio) =0.021, CI (Confidence Interval) =0.0050-0.0913), dental treatment (p-value=0.0059, OR=0.367, CI=0.176-0.764) and diagnosis in family member (p-value=0.0012, OR=0.3508, CI=0.183-0.6710). Most of the factors associated significantly with infection are demographic and hospital associated, reflecting the fact of unawareness of disease in public, improper sterilization of instruments in hospitals and poor blood screening facility.

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Authors' Contribution
AAS and FH designed the study. MU executed the experimental work and wrote the article. N helped in statistical analysis. MN and SUR collected the samples. MSA, SSZZ and MSR processed the samples.

Key words
Hepatitis C virus, Seroprevalence, risk factors, ELISA, ICT, statistical analysis

INTRODUCTION

Acute Hepatitis C virus (HCV) infection is mostly asymptomatic which converts to chronic hepatitis, a major reason of severe infection that leads to deaths all over the world (Shepard *et al*., 2005). HCV is a single stranded ribonucleic acid virus associated significantly with blood transfusion (Alter *et al*., 1999). It was discovered in the year 1989 and considered the main agent responsible for liver infection (Mukhopadhya, 2008). HCV is a member of Flaviviridae family with genus Hepacivirus. The genome of HCV composed of single stranded RNA with at least 10 different structural and non-structural proteins NS2, NS3, NS4A, NS5A, NS5B (Sharma, 2010).

HCV infection is a global health problem that can be transmitted by blood or body fluids like semen, saliva or vaginal secretions (Weiss *et al*., 2005). Most of the HCV infected patients are asymptomatic (Sampietro *et al*., 1998) and these can be of great danger for spreading this infection to the society and medical related individuals particularly (Choudhary *et al*., 2005).It has been estimated that globally 250,000 to 300,000 deaths each year occur because of severe chronic HCV infection and almost 3-4 million new individuals are diagnosed to be HCV positive per year (Higuchi *et al*., 2002; Ray, 2012). However, not all the infected individuals reach to the chronic stage of infection. It is observed that up to 85% of infected individuals got chronic infection while remaining 15% recovered from the infection spontaneously (Seeff, 2002).

According to World Health Organization (WHO) 2009 report, 180 million people are affected by HCV infection. The infection rate is reported to be increased every year with more cases of severe liver infection leading to death (WHO, 2009). HCV infection is considered to be responsible for above 70% carcinoma cases and is the major reason of transplant globally (WHO, 2009). Liver cirrhosis due to HCV infection ultimately leads to death, it is ranked at position eight globally (WHO, 2008). The prevalence rate of infection

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is different for different regions of world. The variation may be due to the difference in socioeconomic status, exposure rate etc. It has been reported that 2.2-3% individuals are affected globally by HCV. The prevalence rate in the United State and countries of Europe varies from 1-2% (Lavanchy, 2009).The general seropravalence of HCV infection in Pakistan was reported to be 4-10% (Akbar *et al.*, 2009).The data from different parts of the country represented variation regarding HCV infection. One of the studies has documented high prevalence rate of anti-HCV and active HCV infection from different parts of KPK (Akhtar *et al*., 2013). It was reported approximately 09% in district Mardan (Khan *et al.*, 2004), 13 to 14% in D. I. Khan (Mashud *et al.*, 2004), nearly 18% in Faisalabad (Nafees *et al.*, 2007) and 4-6% in Karachi (Kazmi *et al*., 1997). Different types of diagnostic tools are developed and used for screening and identification of HCV infection, however screening for anti HCV antibody assay system is the most widely used diagnostic tool and considerable improvement has been made to increase its specificity and sensitivity. The most commonly used diagnostic tests includes antibody testing with immunochromatography test (ICT), enzyme linked immunosorbent assay (ELISA) or molecular genome detection by polymerase chain reaction (PCR) (WHO, 2001). In the current study, the prevalence of HCV was investigated in Kohat Division among the people of different age group. The active infection was confirmed through ICT and ELISA techniques.

MATERIALS AND METHODS

The present report was an epidemiological study, conducted at National Institute of Health (NIH) Islamabad. Total of 270 HCV infected/suspected patients were included along with a complete record of patient history from three districts (Karak, Kohat, and Hangu) of Kohat division. Specially designed questionnaire was filled for each patient during sample collection. The questionnaire was composed of community and hospital associated risks factors and the biochemistry (ALT/AST) level of patients.

Blood samples were collected from each patient in EDTA (Ethylene diamine tetraacetic acid) tube. The blood samples were then centrifuged at 4000 rpm for 5-7 min for separation of serum. Each patient's serum sample was labeled in specialized tubes with specific number and store at -20ºC at NIH till further processing.

An indirect ELISA method was used to detect antibody against HCV. Broadly speaking it was two-step incubation procedure used to detect antibody against HCV.

Data were statistically analyzed through graph pad prism version 5.All the data were presented in the form of frequency, percentage and mean ± SD. Chi-square was used to see association of risk factors to anti-HCV of patients. Odd ratio was calculated for assessment of risk regarding certain factors. A p-value<0.05 was considered as statistically significant.

RESULTS

A total of 270 ICT (immunochromatography technique) positive samples *viz.* 83 from district Karak, 121 from district Kohat and 66 from district Hangu were included in this study. Of these 72 (86.74%) out of 83 samples from district Karak, 102 (84.29%) out of 121 from district Kohat and 50 (75.75%) out of 66 samples from district Hangu were confirmed positive by ELISA. Overall 224 out of 270 (82.96%) sample were ELISA positive.

Both male and female subjects were included in this study and classified into different age groups. The highest prevalence rate was 87.03% among patients of age group 16-40, followed by 30.37% in age group 41 and above. While 3.61% prevalence was observed among patients of age group 1-15 years. Male population was highly affected (46.29 %) as compared to female (36.66%).

HCV infection has been linked with different risk factors and it has been observed that hospitalization has the leading factor associated with HCV with 69.25% followed by body piercing (50.37%) and blood transfusion (39.25%). The percentage for other risk factors was 17.40 % for dental treatment, 31.85% for surgical procedure, 22.22% for shaving at barber shop and 14.44% for tattooing on body.

Statistical analysis (independent t-test) shows that there is no significant difference (p-value>0.05) present among the age group of reactive and non-reactive patients. Mean age among reactive and non-reactive male was 37.57±7.85 and 36.66±8.28, respectively. Similarly mean age among reactive and non-reactive female patients was 38.26±8.47 and 36.52±6.45, respectively. In terms of p-value no significant association was observed as shown in Table I.

Table I.- Distribution of reactive (positive) and non-reactive (negative) HCV patients according to gender.

Gender	Anti- HCV Status	Number	Age (Mean ± SD)	p-value
Male	Reactive	125	37.57 ± 7.85	0.59
	Non-Reactive	17	36.66 ± 8.28	
Female	Reactive	99	38.26 ± 8.47	0.53
	Non-Reactive	29	36.52 ± 6.45	

Independent t- test applied

Table II.- Prevalence of hepatitis C virus reactive and non-reactive patients according to demographic characteristics.

Demographic characteristics		Anti-HCV status		P value	Odds ratio	95% CI
		Reactive	Non-reactive			
Gender	Male	125	17	0.49	2.154	1.12 4.14
	Female	99	29			
Educational status	Literate	101	33	0.001	0.323	0.16-0.64
	Illiterate	123	13			
Occupational status	Private	181	19	0.0001	5.982	3.04-11.7
	Public	43	27			

Table III.- Hepatitis C and hospital associated risk factors.

Hospital associated risk factors		Anti-HCV status		P value	Odds ratio	95% CI
		Reactive	Non-reactive			
Hospitalization	Yes	153	34	0.966	1.014	0.525-1.975
	No	71	16			
Blood transfusion	Yes	72	44	<0.0001	0.021	0.0050-0.0913
	No	152	02			
Dental treatment	Yes	31	14	0.005	0.367	0.176-0.7648
	No	193	32			
Surgical procedure	Yes	73	13	0.566	1.227	0.6093-2.472
	No	151	33			

Chi-square test was applied and $p<0.05$ at 95% CI was considered significant.

Table IV.- Hepatitis C and community associated risk factors.

Community associated risk factors		Anti-HCV status		P value	Odds ratio	95% CI
		Reactive	Non-reactive			
Family member died of liver disease	Yes	26	06	0.7837	0.875	0.388-2.265
	No	198	40			
Diagnosis in family member	Yes	62	24	0.001	0.3508	0.183-0.671
	No	162	22			
Shaving at barber shop	Yes	48	12	0.488	0.772	0.371-1.606
	No	176	34			
Dental extraction at home	Yes	54	04	0.602	3.335	1.143-9.729
	No	170	42			

Chi-square test was applied and $p<0.05$ at 95% CI was considered significant.

Table II elucidates the association of anti-HCV to demographic risk factors. It was observed that both occupational and educational status is significantly associated with anti-HCV antibody. For occupational status p=0.0001, OR=5.982 95% and CI=3.04-11.7, while for educational status p=0.001, OR=0.323 and 95% CI= 0.16-0.64 was observed. In Table III, the association of anti–HCV with hospital associated risk factors is shown. Among 224 reactive patients, 187 have hospitalization, 116 have blood transfusion, 45 have dental treatment and 86 have surgical procedure. In terms of p-value, blood transfusion (p=<0.0001, OR=0.021, 95%CI=0.005-0.0913) and dental treatment (p=0.0059, OR=0.367, 95%CI=0.176-0.764) were significantly associated with HCV infection. Hospitalization and surgical procedures were insignificantly associated with HCV infection.

The association of community associated risk factors with anti-HCV status of patients were also studied (Table IV). Out of 224 reactive patients, 32 have the history of family member died of liver disease, 86 with positive HCV diagnosis in family member, 60 with history of shaving at barber shops and 58 patients have

the practice of dental extraction at home. Diagnosis in family members (p=0.0012, OR=0.3508, 95%CI=0.183-0.6710) was significantly associated with antibody to HCV. Others factors like family member died of liver disease (p=0.7837, OR=0.875, 95% CI=0.338-2.265), shaving at barber shops (p=0.488, OR=0.772, 95% CI=0.3718-1.606) and dental extraction at home (p=0.6204, OR=3.353, 95% CI=1.143-9.729) were not significantly associated with anti-HCV.

DISCUSSION

The current study revealed that the prevalence of HCV was higher in male as compared to female population. It was observed that 46.29% of male and 36.66% female population were affected by this disease. The mean age for male reactive and non-reactive was observed 37.57 and 36.66, while for female it was 38.26 and 36.52, respectively. This finding coincide up to some extent with that of study conducted by Suliman *et al.* (2013) in which they conclude that male population is more affected (50%) as compared to female population (49%). Similar kind of studies was conducted in different parts of country to evaluate infection (Mashud *et al*., 2004; Khan and Siddiqui, 2007; Farooqi and Farooqi, 2000) and the results coincide with the current findings. Khan *et al*. (2006) has also reported higher prevalence rate in male compared to female population. The high prevalence in male may be because of the reason that exposure of male to different risk factors is higher as compared to female. Rajesh *et al*. (2012) concluded from their study in Yemen that female population is more affected as compared to male, which contradict our findings.

The second finding of the current study is that patients with age group from 15-40 was highly affected (87.03%) with HCV, followed by the patients with age group above 40. The age group from 1 to 15 was rarely found positive for HCV infection. According to Sharma *et al.* (2007), Bhattacharya *et al*. (2003) and Chandra *et al*. (2003), the maximum sero-positivity was seen in age group 11-20 years (9%) followed by 21-30 years (6.1%), 0-10 years (5.1%) and >40 years (3.5%). Various studies have demonstrated high seroprevalence of IgM anti-HCV among the adult population. These findings contradict our findings and the difference may be because of socioeconomic status and exposure to different risk factors. However Bhattacharya *et al.* (2003) also found seroprevalence rate of 8.5% in 661 patients with acute viral hepatitis within 20-39 years of age group, which absolutely coincide with our findings. Devi *et al*.(2004)reported maximum seropositivity in the age group of 30-40 years (46.6%). The high prevalence rate seen in young adults (15-40 years) are due to cumulative risk of exposure in these age groups.

The most important aspect of the current study is the statistical analysis and association of different risk factors. The data were analyzed by mean of Chi square test and it was observed that both educational and occupational status were significantly associated with anti HCV status. Others factors associated significantly with infection include blood transfusion, dental treatment and diagnosis in family members. Results of a study carried out in Thailand revealed that blood transfusion and surgery are highly linked with transmission of HCV infection (Lakkana *et al.*, 2004). Study of Rajesh and Sadiq Al-Mohani (2012) conclude that educational level has great impact on disease transmission and statistically found significantly associated with disease. The high percentage in illiterate populations may be because they have very little or almost no information about disease transmission and associated risk factors. Akhter *et al.* (2013) conducted a study to determine HCV prevalence among blood donors in Lahore and concluded occupational status to be associated significantly with infection. These finding coincide with our results where p-value was significant for occupational status of patients. Akhter *et al.* (2013) also reported gender, surgical procedure, dental extraction at home and shaving at barber shop non-significant with HCV infection that coincide with our findings except dental treatment that contradicts with our findings. The contradiction in various factors may be due to individual health care status and standards. As most of the risk factors were associated with hospitals based and it may be because different hospitals have different safety practices and protocols.

CONCLUSION

The finding of current study shows high prevalence of HCV infection in Kohat division. Patients with age between 15-40 years are mostly affected. Male population is more affected as compared to female population. Multiple risk factors were associated with infection such as blood transfusion, dental treatment and diagnosis in family member. Educational and occupational status is significantly associated with infection. Poor health care facility and avoiding standard health protocol contribute a lot in spreading of disease. ELISA is more sensitive serological technique compared to ICT method. Awareness of infection in general population is also very important as most of the infected individuals are illiterate. Scientific seminars and conferences should be held frequently in different parts of country to educate the common population about the infection.

Statement of conflict of interest

Authors have declared no conflict of interest.

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