# FREQUENCY, RISK FACTORS, AND GENOTYPES FOR HEPATITIS C VIRUS IN HEALTHY MALE INDIVIDUALS FROM OKARA GARRISON

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## ABSTRACT

***Objective:*** To determine the frequency, risk factors, and genotypes for hepatitis C virus (HCV) in healthy male individuals from Okara garrison

***Study Design:*** Descriptive cross-sectional study.

***Place and Duration of Study:*** The study was conducted in Departments of internal medicine and pathology, Combined Military Hospital Okara and Armed Forces Institute of Pathology, Rawalpindi, from Oct 2013 to Mar 2014.

***Material and Methods:*** A total of 6500 healthy individual from Okara garrison and the surrounding areas, coming to Combined Military Hospital, Okara, without pervious history of HCV infection were sampled through non-probability consecutive sampling. Blood samples were subjected to rapid screening of HCV infection using Intec immune chromatographic kits. All positive cases were confirmed by 4th generation Monolisa HCV Ag-Ab ULTRA enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) for HCV RNA using smart cycler automated real-time PCR system. The genotyping of HCV RNA was done with COBAS AMPLICOR HCV MONITOR Test, version 2.0. All positive cases were asked to fill a questionnaire in Urdu regarding different risk factors for HCV infection. The data were analysed using SPSS version 20.

***Results:*** A total of 6235 individuals were tested for anti HCV antibodies. Out of 6235, 270 (4.3%) participants were positive on immunochromatography and ELISA. among 270 participants (mean age: 31 ± 7 years), 162 (60%) were positive for HCV by PCR, whereas, 108 (40%) were negative. Genotyping for only 211 personnel could be made available. The genotype 3 was the commonest (68.7%, n=145) genotype. The most common risk factor was dental treatment in the past (26.3%) followed by previous surgery (23.7%).

***Conclusion:*** A frequency of 4.3% for HCV seropositivity was observed in our cohort of male individuals from Okara garrison and the surrounding areas. Genotype 3 was the commonest (68.7%) genotype observed for HCV. Previous dental procedures and surgeries were the commonest risk factors found in HCV infected personnel.

**Keywords:** Frequency, Genotype, Hepatitis C virus, Risk factors.



## INTRODUCTION

Hepatitis C virus (HCV) was discovered in 1989 as a major causative agent for chronic hepatitis[1]. Around eighty million people are infected with HCV worldwide[2]. Pakistan is among the countries worst affected by HCV infection. The prevalence of HCV is different in different regions of Pakistan and even various groups of the same community show dissimilar frequency rates[3]. Studies on HCV have revealed prevalence rates ranging from 2.45% to 25.7% from various regions of Pakistan[3-6]. HCV genotype 3a continues to be the most prevalent subtype infecting people in Pakistan[3-5,7-11]. A detailed study on 3351 HCV polymerase chain reaction (PCR) positive cases in Pakistan for genotype detection showed that genotype 3 (3a and 3b) was the most prevalent (67.3%)[12]. Identified risk factors for HCV infection included exposure to infected blood and blood products, injectable drugs abuse, high risk sexual activity (multiple sexual partners), tattooing, ear/nose piercing, unsafe injections, sharing of tooth brushes, used shaving blades, acupuncture, and needle stick injury[3,6,11-13]. Infection acquired through surgery is also a well-recognised risk

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factor for HCV infection in Pakistan[3,11-13]. However, in many HCV positive individuals, no risk factor had been identified. As HCV infection requires costly and long-term treatment. Presently hepatitis B virus (HBV) and HCV screening is compulsory before induction and the positive individuals are rejected on medical grounds. Considering this, it was assumed that HCV infection in persons residing in Cantonment was much less common than the general Pakistani population. To scrutinize this assumption, we planned a study to estimate the frequency of HCV in a cohort of individuals belonging to Okara garrison and the surrounding areas. The estimation of associated risk factors and the genotype of HCV were secondary goals of the study. Till now, only frequency of HBV and HCV infection has been estimated in Pakistani recruits[14-16]. Our study would be one of the pioneer studies to estimate risk factors for HCV infection and the genotypes of HCV in apparently healthy personnel of Okara Garrison.

## PATIENTS AND METHODS

This cross-sectional study was carried out in the departments of internal medicine and pathology, Combined Military Hospital Okara and Armed Forces Institute of Pathology, Rawalpindi, from October 2013 to March 2014. A sample size of 2056 was estimated via Epi Tools Epidemiological Calculator[17] while keeping confidence level 99%, estimated true proportion 3.2%[14] and 1% of absolute precision. We sampled 6500 male individuals in the age range of 18 to 57 years through non-probability consecutive sampling, serving in Okara garrison and the surrounding areas. Two hundred and sixty-five individuals suffering from HBV or HCV infection or had already taken antiviral treatment for HBV or HCV infection were excluded from the study. Informed written consent was taken from each included patient and permission from the hospital ethical committee was also sought. Those agreeing to participate in the study were tested for anti-HCV antibodies. A blood sample of 3 mL was taken from all participants in disposable syringes and was subjected to rapid screening using Intec immunochromatographic kits (In Tec ProductsInc., Xiamen, Fujian, China) with sensitivity and specificity of 99.8% and 95% respectively. All the positive cases were confirmed by 4th generation enzyme-linked immunosorbent assay (ELISA) and PCR for HCV RNA.The ELISA was carried outwith the G4 Monolisa HCV Ag-Ab ULTRA ELISA assay (Bio-Rad., Marnes-la-Coquette, Paris, France) and PCR by smart cycler automated real-time PCR system (Cepheid, Sunnyvale, CA, USA). The ELISA positive cases irrespective of their PCR result were considered infected. The HCV infected individuals were given a questionnaire in Urduto

**Table-I: Descriptive properties of the whole sample (n=6235).**

| Variables | n (%) |
|---|---|
| **Marital status** | |
| Married | 4440 (71.2) |
| Single | 1795 (28.8) |
| **Ethnicity based on provinces** | |
| Punjab | 4010 (64.3) |
| Sindh | 1143 (18.3) |
| Khyber Pakhtunkhwa | 630 (10.1) |
| Balochistan | 108 (1.7) |
| Azad Jammu and Kashmir | 280 (4.5) |
| Gilgit Baltistan | 64 (1) |
| **Anti HCV* antibodies through ELISA**** | |
| Positive | 270 (4.3) |
| Negative | 5965 (95.7) |

*Hepatitis C virus, **Enzyme-linked immunosorbent assay

fill. The questionnaire included questions on age, marital status, history of unsafe injection use, admission in the hospital, blood transfusion, surgery or dental treatment in the last one year, sharing of razors, tattooing, spouse infected with HCV, extramarital relations, and close contact with HCV positive person inthe past. The genotyping of HCV RNA was done with COBAS AMPLICOR HCV MONITOR Test, version 2.0. (Roche Diagnostics., Mannheim, Baden-Württemberg, Germany). All the data were analysed using statistical package for social sciences version 20 (IBM Corp., Armonk, NY, USA). The variables were defined qualitatively by frequencies and percentages and quantita-tively by means and standard deviations.

## RESULTS

There were a total of 6235 male individuals with a mean age of 30 ± 6.8 years and in a range of 17 to 57 years. Most (n=4440, 71.2%) were married with an average of 3 ± 2 (range 1-11) children. The majority (n=4010, 64.3%) hailed from the Punjab province. Following evaluation by ELISA, n=270 (4.3%) personnel were found positive for anti HCV antibodies (table-I). The mean age for ELISA positive patients was 31 ± 7 years (range: 19-55 years). Majority of them were married (n= 214, 79.3%) and had an average of 3 ± 2 children. Of 270 patients, n=207 (76.7%) had a positive PCR for HCV RNA. The commonest risk factor was found to be previous dental treatment (26.3%). Genotyping for only 211 personnel could be made available. The commonest genotype was 3 (68.7%, n=145) followed by non-typeable (16.1%, n=34) HCV RNA (table-II).

**Table-II: Descriptive statistics of HCV* positive patients (n=270).**

| Variables | n (%) |
|---|---|
| **Marital status** | |
| Married | 214 (79.2) |
| Single | 56 (20.8) |
| **HCV RNA detection by PCR**** | |
| Detected | 207 (76.8) |
| Not detected | 63 (23.3) |
| **Risk factors** | |
| Dental treatment | 71 (26.3) |
| Past surgery | 64 (23.7) |
| Unsafe injection use | 27 (10) |
| Blood transfusion | 14 (5.1) |
| Razor sharing | 23 (8.5) |
| Spouse with HCV infection | 11 (4) |
| Tattooing | 22 (8.1) |
| Extra-marital relation | 2 (0.7) |
| Close contact (other than sexual) with HCV positive people | 10 (3.7) |
| No risk factor identified | 26 (9.6) |
| **Genotype of HCV RNA N=211 (78.15%)** | |
| Type 1 | 14 (6.6***) |
| Type 2 | 6 (2.8***) |
| Type 3 | 145 (68.7***) |
| Type 4 | 5 (2.4***) |
| Type 1,2,3 Mix | 1 (0.5***) |
| Type 3,4 Mix | 3 (1.4***) |
| Type 3,6 Mix | 2 (1***) |
| Type 3,4,6 Mix | 1 (0.5***) |

*Hepatitis C virus, **Polymerase chain reaction
***The percentages are from 211 individuals

## DISCUSSION

A frequency of 4.3% for HCV sero positivity was found in our study, which is a little less than the latest HCV prevalence in Pakistan. But comparing with the studies on other armies of the world, it seems a little higher. An American study found an overall prevalence of 0.48% among 10,000 active-duty personnel[18]. A Brazilian study[19] found a prevalence of 0.7% in 433 male individuals while an Ethiopian study[20] found a prevalence of 0.2% in 403 individuals. In our study, a lower frequency in the inidividuals was also expected because there is a strict pre-induction screening for HCV in Pakistan Army, which filters out all positive cases. This surprisingly higher prevalence is probably due to certain perils more prevalent in the military environment. Individuals are inclined to injuries and subsequent surgeries. There is a high incidence of dental treatment/ extraction in soldiers[19,21]. They also live together in large numbers in camps where there are chances of sharing hair-brushes, combs, razors, and tooth brushes due to poor knowledge about transmission of HCV[20,22]. More than eight percent of HCV positive individuals in our study had positive history of razor sharing. A study[15] carried out on 200 HBV or HCV positive recruits in 2005 revealed that sharing of tooth brushes and razors amongst family members was present in 154 (77%) cases. The barbers in army are usually screened for HBV and HCV infection but they are, in general, unaware of modes of spread of these viruses[23]. As soldiers are sent on leave after months, they may get involved in extra marital relations[20]. There is a high incidence of caesarean sections in the tertiary care hospitals[24,25], predisposing the spouses to this virus and subsequent acquisition of HCV infection in soldiers through sexual transmission. Dental treatment either in setup or outside was

the commonest risk factor for HCV acquisition. Its frequency was 26.3% among HCV positive individuals which is quite alarming when we compare it with other studies in Pakistan[26,27]. This may be related to poor sterilization techniques of dental instruments in the tertiary care hospitals or seeking dental treatment frequently from the civil sector and quacks. Approximately, 24% of HCV positive soldiers had past history of minor or major surgeries which is quite a high percentage when we compare it with other studies from Pakistan. A study from Peshawar on 161 patients concluded that surgical procedure was the possible cause of HCV infection among 8% of the patients[26]. In a review, past surgery was claimed in 6.92%, 16.6%, 11.66%, and 11.26% of HCV positive patients[28]. Possible explanation of high transmission rate of HCV during surgery may be the improper sterilization of operation theatre equipment. This is due to the fact that surgical causalities are frequently received in bulk due to on-going terrorist activities and proper sterilization equipment is unavailable in the forward military deployment areas where surgeries are performed as an emergency procedure. Also, in Pakistan, people are in habit of using injectable therapies unnecessarily. According to a study, estimated injections per person per year in Pakistan were 8.2 to 13.6%, out of which 94.2% were unnecessary[29]. As most of our soldiers come from rural areas with low socioeconomic back ground and possess meagre knowledge about spread of HCV virus, so it is not surprising that they can also use injectable treatment for minor ailments and can acquire the disease. In a study by Khan *et al*., out of 135 habitual injection users for minor ailments, HCV infection was positive in 44% of patients[30]. In our study, 10% HCV positive soldiers gave history of routine use of injections. In our study, a substantial majority of HCV infected soldiers was married. Hajiani *et al*. in Southern Iran, found increased HCV antibody positivity among spouses of HCV positive patients than other family members and the infection rate increased with duration of marriage[31]. The HCV transmission via sexual route is rare[32] but marital relations also include other kinds of body contacts and risks (e.g. sharing tooth brushes, razors, dental appliances, etc). From 1990 onwards, in Pakistan, after introduction of donor screening, the transmission of HCV by blood transfusion is nearly eliminated. In our study we found that the frequency of HCV infection among soldiers who received blood transfusion was 5.1%, which seems quite higher as compared to standard risk of one case per million blood transfusions per person[33].

In our study, 8.1% of HCV positive soldiers had tattoos on their skin. The risk of HCV acquisition in soldiers having tattoos is 3 times higher than normal population[34]. A hospital-based study inthe Punjabi population, in which 281 HCV positive cases were included, showed that tattooing was reported by 11.4% of cases[35]. The province wise frequency of HCV in our study showed maximum cases from Sindh (5.8%) followed by Punjab (4.4%), Khyber Pakhtunkhwa (2.8%), Azad Jammu and Kashmir (2.8%) and Balochistan (0.9%). No case was detected from Gilgit Baltistan. A similar study was conducted on recruits from all provinces intheyear 2005 by Azam *et al*[14] and its results closely matched with our study i.e. Sindh 6.4%, Punjab 4%, Khyber Pakhtunkhwa 1.4%, and Balochistan and Northern areas 0%.

Discovery of a high prevalence of HCV positive personnel in Pakistan Army approaching the general Pakistani population despite pre-induction screening of HCV infection is an alarming situation. Strict measures are required to be taken for sterilization of surgical and dental instruments. Furthermore, education of soldiers through healthcare professionals about the importance of infection control measures should be taken into practice.

Our study showed that genotype 3 was the commonest (68.7%) genotypefollowed by non-typeable (16.1%), genotype 1 (6.6%), mixed (3.3%), genotype 2 (2.8%), and genotype 4 (2.4%). A meta-analysis of 34 studies in Pakistan showed

following pattern of genotype prevalence: 7.03% cases of genotype 1; 3.81% cases of genotype 2;78.96% cases of genotype 3, 1.59% cases of genotype 4, 0.1% cases of genotype 5; 0.13% cases of genotype 6, 5.03% cases of mixed genotypes, and 3.3% cases of non-typeable genotypes[36]. The difference in the results might be due to use of different polygenetic analytic facilities in our study.

## LIMITATION OF STUDY

Our study had few limitations. Our study was exclusive for the male gender. Thus, the results can not be applied to the general population. Secondly, already known HCV infected cases and previously treated chronic HCV cases were not included in our study thus study results actually underestimated the burden of HCV infection in Pakistan army. Thirdly, the risk factors were not verified through statistical analysis, thus significance of described risk factors is questionable.

## CONCLUSION

A frequency of 4.3% for HCV seropositivity was observed in our cohort of male individuals from Okara garrison and the surrounding areas. Genotype 3a was the commonest (68.7%) genotype observed for HCV. Previous dental procedures and surgeries were the commonest risk factors found in HCV infected personnel.

## CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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