

DEMOGRAPHIC FEATURES OF HEPATITIS C IN PAKISTANI POPULATION

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ABSTRACT

Objectives: To know the demographic features of Hepatitis C in Pakistani population

Material & Methods: A specifically designed data sheet for patients visiting OPD of KRL Hospital Islamabad was used which enrolled their demographic characteristics. This study conducted in the department of medicine KRL Hospital Islamabad Pakistan from January 2016 to December 2016. A total of 103 patients were included in the study having HCV antibodies positive by ELISA. Quantitative PCR for HCV-RNA was done using Qiagenartus®. For HCV genotyping, viral RNA was extracted using QIAamp Viral RNA Mini kit. Allele-specific PCR was used for the genotyping of IL28B. Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis.

Results: Out of 103 patients 38 (36.9%) were males and 65 (63.1%) were females. Mean age was 48.67 years. Mean HCV-RNA viral load was 906661.42 IU/ml. 35 (33.9%) patients were graduates, 43 (41.7%) were matric while 25 (24.3%) patients were below matric or uneducated. 28 (27.2%) patients had positive family history. Risk factors for acquiring HCV was present in 66 (64.1%). 26.2% patients reported having dental procedure in the past. 6.8% patients had history of surgical procedures. 3.9% patients had history of shaving by barbers. 1.9% patients had history of needle prick injury. 1.9% patients reported of having blood transfusions in the past. 23.3% had multiple exposure. No risk factor was reported in 35.9% patients. 41 (39.8%) patients had a comorbid condition. Genotype 3a was the most common genotype accounting for 101 (98.1%) patients while only 02 (1.9%) patients had genotype 3b. Total of 73 patients were tested for IL28B polymorphism. 29 (39.7%) patients had CC genotype. 36 (49.3%) patients had CT genotype and 08 (11%) patients had TT genotype. 73 (70.9%) patients had not received treatment in the past, 21 (20.4%) patients were relapsers while 09 (8.7%) patients were non-responders.

Conclusion: Dental procedure was by far the most common identifiable risk factor in our study. Patients with relatively low educational background had high incidence of hepatitis C in this study. Genotype 3a was the most prevalent hepatitis C genotype. Interleukin 28-B (IL28B) polymorphism reveals a different model of distribution than previously reported showing high CT genotype as compared to CC genotype.

Key Words: Demography; Hepatitis C; HCV; Interleukin 28 B; IL28B; Genotype 3a; Risk factors

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INTRODUCTION

An estimated 185 million people are infected with Hepatitis C virus (HCV) worldwide. In Pakistan more than 10 million people are living with HCV, with high morbidity and mortality. Risk factors for having hepatitis C varies among different regions. There is a need for identifying these risk factors so that they can

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be combated. Interleukin 28-B (IL28B) polymorphism should also be estimated in our population as long as Interferon based regimens are used. Hepatitis C virus (HCV) is an enveloped, single-stranded, RNA virus that belongs to Flaviviridae family¹. An estimated 185 million people are infected with HCV worldwide (prevalence of 2.8%). Central, East Asia and North Africa/Middle East are estimated to have high prevalence (>3.5%); South and Southeast Asia, sub-Saharan Africa, Andean, Central, and Southern Latin America, Caribbean, Oceania, Australasia, and Central, Eastern, and Western Europe have moderate prevalence (1.5%-3.5%); whereas Asia Pacific, Tropical Latin America, and North America have low prevalence (<1.5%)². Approximately 700,000 people die each year from hepatitis C-related

liver diseases.³ HCV infection leads to chronic hepatitis in 50% to 80% of individuals.⁴ In Pakistan more than 10 million people are living with Hepatitis C virus (HCV), with high morbidity and mortality.⁵ HCV is classified into eleven different genotypes, of which six are the major genotypes and these genotypes are further classified into many subtypes.⁶ Genotype 3a is the most common genotype of HCV infections in Pakistan.^{7,8} In a study it was found that genotype 3a is the most prevalent HCV genotype in Pakistan. Major risk factors include Injection Drug Users (IDUs), blood transfusions, reuse of syringes, facial and armpit shaving by barbers and unprotected sex.⁵ In another study it was concluded that the most common HCV genotype in Pakistan is type 3a. Receiving multiple injections in the past was found to be the major risk factor HCV spread.⁹ In a study conducted in Spain hospitalization was believed to account for 67 % of acute HCV cases documented in 18 Spanish hospitals, which accounted for more cases than intravenous drug use (8 %), needle stick injury (6 %), and sexual contact (5 %).¹⁰ In a systematic review, the mean prevalence of anti-HCV reactivity after one year of injection drug use was 32 percent in resource-rich settings and 59 percent in resource-limited settings.¹¹ Blood transfusion was a major risk factor for acute infection in the past, with more than 10 percent of transfusion recipients acquiring infection in some studies.¹² The subsequent initiation of donor screening for anti-HCV antibodies in 1990 has nearly eliminated the risk of posttransfusion acute HCV infection. The estimated risk is now less than one in a million per unit transfused.¹³ The efficiency of HCV transmission by sexual or household contact is low. The majority of the data does not support transmission to nonsexual partners.¹⁴ In a study conducted in Dera Ismail Khan, the main identifiable risk factor was the use of un-sterilized injections.¹⁵ In another study done in Sargodha region, 75% patients had history of (H/O) I/V injection/drip and nail cutter sharing, 70% had H/O shaving from barbers while 33% had H/O razor sharing, needle stick accident and extraction dental procedure and only 8% had H/O sexual promiscuity or ear/nose piercing.¹⁶ No identifiable risk factors for HCV infection during the previous six months can be identified in up to 44 percent of cases with new infections.¹⁷ The IL28B gene is involved in the immune response to certain viruses, including hepatitis C. There are three IL28B subtypes (called genotypes): CC, CT, and TT. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes (called non-CC genotypes). South Asians have 65-98% likelihood of having CC genotype.¹⁸ There is a need to study the demographic features of hepatitis C in Pakistani population including age, gender, HCV RNA viral load, risk factors, comorbid conditions, Interleukin

28-B (IL28B) polymorphism and therapy status. This may guide us in better understanding of the disease process, its distribution and role of IL28B polymorphism in treatment strategies.

MATERIAL AND METHODS

This study conducted in the department of medicine KRL Hospital Islamabad Pakistan from January 2016 to December 2016. Total of 103 serum samples were collected from HCV infected patients for genotyping and Interleukin 28-B (IL28B) polymorphism along with specifically designed data sheets from patients visiting OPD of KRL Hospital Islamabad. Informed consent was taken from each participated patient including, demographic characteristic, age, comorbid conditions, family history, risk factors and therapy status with complete address and phone number of the patients.

Quantitative PCR for HCV-RNA was done using Qiagenartus® HCV RG RTPCR kit for quantification. For HCV genotyping, viral RNA was extracted using QIAamp Viral RNA Mini kit following the manufacturer's instructions. Then HCV genotype was determined by using RNA UltraSense® One-Step Quantitative RT-PCR system and in-house designed primers (Ohno et al., 1997).²⁰

Allele-specific PCR was used for the genotyping. PCR products were visualized on 2% agarose gel. For the genotyping of rs12979860 and rs8099917, specific SNP genotyping assay based on TaqMan® allelic discrimination was used (Applied Biosystems; Part no. 4331349, 4351379). Real-time PCR reactions were performed on an ABI prism 7000 Sequence Detection system (Applied Biosystems)

Statistical Package for Social Sciences (SPSS) version 20 was used for data analysis. The clinical data of the study patients were stated as number of patients and percentages. The difference between two groups were examined by t-test or ANOVA for continuous variables and by chi-square test for categorical variables.

RESULTS

A total of 103 patients were included, belonging to Islamabad/Rawalpindi region and were recruited from Medical OPD of KRL Hospital Islamabad. Out of 103 patients 38 (36.9%) were males and 65 (63.1%) were females. Mean age was 48.67 years. Mean HCV-RNA viral load was 906661.42 IU/ml. Educational level was divided into graduate level, matriculation level and others, who included below matric and uneducated. 35 (33.9%) patients were graduates, 43 (41.7%) had done matric while 25 (24.3%) patients belonged to last category. Family history for hepatitis C (HCV) was asked and 28 (27.2%) patients replied yes while in 75 (72.8%) patients

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family history was absent. Risk factors for acquiring HCV was present in 66 (64.1%) patients while 37 (35.9%) patients had no risk factor. 41 (39.8%) patients had a comorbid condition present in past medical history while 62 (60.2%) patients had no comorbid condition in the history. Genotype 3a was the most common genotype accounting for 101 (98.1%) patients while only 02 (1.9%) patients had genotype 3b. 73 (70.9%) patients had not received treatment in the past, 21 (20.4%) patients were relapsers while 09 (8.7%) patients were non-responders. No risk factor was reported in 35.9% patients. 26.2% patients reported having dental procedure in the past. 6.8% patients had history of surgical procedures. 3.9% patients had history of shaving by barbers. 1.9% patients had history of needle prick injury. 1.9% patients reported of having blood transfusions in the past. 23.3% had multiple exposures in the past.

Table 1: Comorbid conditions in patients along with their numbers and percentages.

Comorbid conditions	
Disease	No. of patients & percentage
None	69 66.9%
Diabetes Mellitus	11 10.6%
Hypertension	10 9.7%
Dyslipidemia	01 0.9%
Multiple	12 11.6%

Details of comorbid conditions reported by HCV patients are shown in Table 1. The IL28B gene is involved in the immune response to certain viruses, including hepatitis C. There are three IL28B subtypes (called genotypes): CC, CT, and TT. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes (called non-CC genotypes). Total of 73 patients were tested for IL28B polymorphism. 29 (39.7%) patients had CC genotype. 36 (49.3%) patients had CT genotype and 08 (11%) patients had TT genotype. As far as patients tested for IL28B polymorphism, 26 (35.6%) were males and 47 (64.4%) were females. Mean age was 47.89 years. Mean HCV-RNA viral load was 792983.84 IU/ml. Family history for hepatitis C (HCV) was positive in 20 (27.4%) patients while in 53 (72.6%) patients family history was Negative. Risk factors for developing HCV was present in 46 (63.1%) patients while 27 (36.9%) patients had no risk factor. 23 (31.5%) patients had a comorbid while 50 (68.5%) patients had no comorbid condition in the history. Genotype 3a was the most common genotype accounting for 71 (97.3%) patients while only 02 (2.7%) patients had genotype 3b. 52 (71.2%) patients had not received treatment in the past, 14 (19.2%) patients were relapsers while 07 (9.6%) patients were non-responders are shown in Table 2.

DISCUSSION

The word Demography comprises of two parts, Demo meaning "people" and graphy meaning "writing,

Table 2: Characteristics of IL28B tested patients.

IL28B tested group				
		No. of patients with Percentage	Mean	Standard Deviation
Number of Patients		73		
Age (Years)			47.89	12.69
HCV RNA Viral load			792983.84	1730047.74
Gender	Male	26 (35.6%)		
	Female	47 (64.4%)		
Family History of HCV	Present	20 (27.4%)		
	Absent	53 (72.6%)		
Risk Factors	Present	46 (63.1%)		
	Absent	27 (36.9%)		
Comorbid condition	Present	23 (31.5%)		
	Absent	50 (68.5%)		
HCV genotype	3a	71 (97.3%)		
	3b	02 (2.7%)		
Therapy status	Naïve	52 (71.2%)		
	Relapser	14 (19.2%)		
	Non-Responder	07 (9.6%)		

description or measurement". It is the statistical study of populations, especially human beings. In present study we have measured different demographic aspects of Hepatitis C patients. We have come to know that HCV is present in almost all age groups, minimum age was 16 years while maximum age was 80 years. Average age was 48.67 ± 12.90 years. These findings suggest that majority of the patients suffering from HCV are young. There is a need of early screening in all the suspected individuals so that we can detect HCV early and thereby initiate early treatment leading to high cure rates. In current study out of 103 patients 65 (63.1%) were females while 38 (36.9%) patients were males. Proper health education should be given to masses so that they may become aware of the risks associated with different procedures.

In a study conducted in China it was found that Family clustering was an important risk factor in HCV epidemiology. They confirmed that contact with an HCV carrier/patient in the family was a risk factor for infection. Intra-familial transmission of HCV infection was mainly through sexual activity between spouses.¹⁹ Recently, there have been reports of the presence of the hepatitis C virus RNA was found in the semen of up to 1/3 of HCV viremic men²¹. In present study family history of HCV was positive in 27.2% patients. As soon as patients are diagnosed with HCV, proper counselling should be undertaken and they be informed of the threat they pose to other family members especially their spouse as long they have high level of viremia.

In a study done in Denmark, educational status was not associated with survival in HCV patients²². In our study patients were asked about their educational status. 33.9% patients were graduates, 41.7% patients were matric and 24.3% were below matric or uneducated.

No identifiable risk factors for HCV infection during the previous six months can be identified in up to 44 percent of cases with new infections.¹⁷ In a study done in Sargodha region, 75% patients had history of I/V injection/drip and nail cutter sharing, 70% had H/O shaving from barbers while 33% had H/O razor sharing, needle stick accident and extraction dental procedure and only 8% had H/O sexual promiscuity or ear/nose piercing.¹⁶ In another study it was concluded that Injection Drug Users (IDUs), blood transfusions, reuse of syringes, facial and armpit shaving by barbers and unprotected sex were the major risk factors for HCV transmission in Pakistani population.⁵ In our study 35.9% patients had no identifiable risk factor. 26.2% patients had dental procedures in past. History of other surgical procedure was positive in 6.8% patients. 3.9% patients had history of shaving from barbers. 1.9% patients gave history of

needle prick injury. 1.9% patients reported of having blood transfusions in the past. 23.3% reported multiple exposures in the past. These findings depict that dental procedures are a major risk factor for HCV transmission. Measures should be taken regarding proper sterilization of instruments used in dental procedures. Screening for HCV should be made a pre-requisite for any dental procedure. No comorbid condition was reported in 66.9% patients. 10.6% patients gave history of having Diabetes Mellitus. 9.7% had Hypertension.

Hepatitis C virus (HCV) is classified into eleven different genotypes, of which six are the major genotypes and these genotypes are further classified into many subtypes.⁶ Genotype 3a is the most common genotype of HCV infections in Pakistan.^{7,8} In present study, genotype 3a was the most prevalent genotype accounting for (98.1%) patients while only (1.9%) patients had genotype 3b. Average viral load of HCV RNA was 906661.42 IU/ml.

The IL28B gene is involved in the immune response to certain viruses, including hepatitis C. There are three IL28B subtypes (called genotypes): CC, CT, and TT. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes (called non-CC genotypes). South Asians have 65-98% likelihood of having CC genotype.¹⁸ In current study a total of 73 patients were tested for IL28B polymorphism. (39.7%) patients had CC genotype. (49.3%) patients had CT genotype and 08 (11%) patients had TT genotype. This is in contrast to the data available in international literature. CT genotype is the most common genotype in our study followed by CC and TT. As CT is a non-favorable genotype, there are chances that these patients may not benefit from PEGylated Interferon therapy¹⁹⁻²¹. So there is a need that newer Direct Acting Antivirals (DAAs) be made available and be included in all treatment regimens for favorable outcomes.

CONCLUSION

Dental procedure was by far the most common identifiable risk factor. Patients with relatively low educational background had high incidence of hepatitis C. Genotype 3a was the most prevalent hepatitis C genotype. Interleukin 28-B (IL28B) polymorphism reveals a different model of distribution than previously reported showing high CT genotype as compared to CC genotype.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Ali A: Main idea, concept
Abbasi AS: Literature review
Shaikh JI: Data collection

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.