DISTRIBUTION FREQUENCY OF HEPATITIS C VIRUS GENOTYPES IN PATIENTS ATTENDING LIAQUAT UNIVERSITY HOSPITAL JAMSHORO/HYDERABAD

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Date Received:
March 05, 2014
Date Revised:
November 07, 2014
Date Accepted:
December 21, 2014

ABSTRACT

Objective: To assess the frequency of different genotypes of Hepatitis C virus in patients attending Liaquat University Hospital (LUH) Jamshoro Sindh.

Methodology: This descriptive cross sectional study was carried out at Department of Medicine at Liaquat University hospital Jamshoro from June to December 2012. A total of 107 patients with Hepatitis C fulfilling the selection criteria and with informed consent were included in the study. Blood samples were taken from patients for HCV genotyping. A qualified pathologist performed HCV genotyping.

Results: Total 107 patients of Hepatitis C were selected by non probability consecutive sampling. Mean age was 36.58 ± 10.5 years. Fifty six (52.3%) were male and fifty one (47.7%) were female. The commonest genotype was genotype 3 (72.9%) followed by genotype 2 (18.7%), genotype 1 (7.5%) and genotype 4 (0.9%). The commonest HCV genotype 3 subtype was subtype a (73.8%) followed by subtype b (25.2%) and subtype c (0.9%).

Conclusion: HCV genotype 3 with distribution frequency of 72.9% and subtype a with distribution frequency of 73.8% were the commonest in our study population.

Key Words: Hepatitis Virus, Genotypes, Subtypes

This article may be cited as: **Baig S, Masood N, Shaikh IA. Distribution frequency of hepatitis C virus genotypes in patients attending Liaquat University Hospital Jamshoro/Hyderabad. J Post Med Inst 2014; 28(4): 367-71.**

INTRODUCTION

Hepatitis C has been recognized as a major public health problem all over the world, including Pakistan. Hepatitis C virus (HCV) is a human blood-borne pathogen that has infected almost 170 million people worldwide[1]. According to World Health Organization (WHO) Pakistan with a prevalence rate of 4.8%, have almost 10 million Hepatitis C patients[2]. Infection with Hepatitis C virus becomes chronic in about 85% of patients and of these 65% patients develop liver cirrhosis[3]. After 20 years of infection, 6-8% of patients with chronic Hepatitis C develop hepatocellular carcinoma[4]. Hepatitis C is estimated to result in 366000 deaths annually[5].

The rate of disease progression is variable and several factors have been identified as important in predicting the outcome of progression. These include age at infection, gender, genotype/subtype, viral load, and mode of infection[6]. Hepatitis C virus (HCV), a member of the Flaviviridae family of RNA viruses, is characterized by genetic heterogeneity. At least 6 major Hepatitis C virus genotypes are identified[7]. Genotypes 1, 2, and 3 are common throughout United States and Europe[8]. Genotype 3 is highly prevalent in Australia and south Asia[9]. HCV genotype 4 is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections[10]. In Pakistan Genotype 3 is predominant[11].

A study was conducted in Bahawalpur that found that the most frequent genotype for HCV infection was genotype a[12]. Another study was undertaken in Baluchistan in 2008. It included 40 HCV positive patients diagnosed by PCR. It determined that the most prevalent HCV genotype in Baluchistan is genotype a[13].

Another study carried out in Khyber Pakhtunkhwa concluded genotype a was most prevalent cause of Hepatitis C[14].

A Study carried out in Baluchistan showed most of the Hepatitis C infection was caused by HCV genotype 3[13].

Similar study conducted in Karachi showed the most prevalent HCV genotype to be 3[15].

Hepatitis C virus genotype is recognized as a major independent predictor of response to anti-HCV therapy[17]. Patients with different Hepatitis C virus genotypes respond differently to interferon therapy. Patients with genotype 2 and 3 responds well to peg interferon therapy with duration of 24 weeks of treatment whereas patients with genotype 1 and 4 respond poorly to peg interferon therapy with duration of 48 weeks of treatment[18].

As proper identification of Hepatitis C virus genotype before therapy would lead to effective treatment for optimal duration, so this study was initiated to find out the distribution of various Hepatitis C virus genotypes present across Hyderabad where genotype determination prior to therapy is not a common practice.

METHODOLOGY

A total of 107 patients with Hepatitis C fulfilling the selection criteria of either gender above 12 years of age diagnosed by HCV RNA by PCR with informed consent were included in the study. The sampling technique was non probability consecutive .

The Patients with evidence of concurrent Hepatitis B or HIV infection were excluded from this study.

After consent, blood samples were taken from patients for HCV genotyping. A qualified pathologist performed HCV genotyping. The pathologist reverse transcribed HCV-RNA to cDNA using 100 U of M-MLV reverse transcriptase at 37°C for 50 minutes.

Two µl of synthesized cDNA were used by pathologist for subsequent PCR amplification of 470-bp region from HCV 5′ non coding region plus core region by first round PCR. The second round nested PCR was performed by pathologist using Type-specific primers for genotypes.

The final PCR product underwent electrophoresis on a 2% agarose gel to separate type-specific amplified fragments. A 100-bp DNA ladder was run in each gel as DNA size marker and the HCV genotype for each sample was determined by identifying the HCV genotype-specific PCR RNA sequence band as described in operational definitions.

DATA ANALYSIS

Variables studied included age, gender, HCV RNA, genotype and subtype.

Data was described in the form of frequencies, percentages and Mean ± SD. Frequencies and percentages were calculated for qualitative data such as gender, HCV RNA, genotype and subtype.

Mean ± SD was calculated for numerical variable such as age (in years).

The relevant data was documented and results were compiled using statistical program SPSS® version 16.

Statistical test was applied for stratification such as age and gender to see the impact of these on outcome variable through Chi Square test. P value of ≤ 0.05 was taken as significant.

RESULTS

Our sample was of 107 patients with Hepatitis C. Mean age was 36.58 ± 10.5 years. Amongst the total patients 46.73% patients were between 15-34 years of age, 46.73% were between 35-54 years of age and 6.54% above 55 years of age. All patients (100%) were HCV RNA positive by PCR. Fifty six (52.3%) were male and fifty one (47.7%) were female.

Most common HCV genotype was genotype 3 (72.9%) followed by genotype 2 (18.7%), genotype 1 (7.5%) and genotype 4 (0.9%). The most common HCV subtype is subtype a (73.8%) followed by subtype b (25.2%) and subtype c (0.9%).

Chi square test was applied for stratification and no significant association was found between distribution of HCV genotypes with age and gender ($p < 0.8$).

DISCUSSION

It is clear that HCV infection varies considerably re-

Table 1: Distribution of patients by Genotype (η=107)

Genotype	η	%
Genotype 1	8	7.5
Genotype 2	20	18.7
Genotype 3	78	72.9
Genotype 4	1	0.9
Total	**107**	**100.0**

Table 2: Distribution of patients by Subtype (n=107)

Subtype	n	%
Subtype a	79	73.8
Subtype b	27	25.2
Subtype c	1	0.9
Total	**107**	**100.0**

Table 3. Distribution of HCV Genotype in Association of Gender (n=107)

Gender		**Genotype**				**Total**	
		1	**2**	**3**	**4**		P value
Female	Count Expected Count % within Gender	4 3.8 7.8%	9 9.5 17.6%	38 37.2 74.5%	0 0.5 0%	51 51.0 100.0%	<0.79
Male	Count Expected Count % within Gender	4 4.2 7.1%	11 10.5 19.6%	40 40.8 71.4%	1 0.5 1.8%	56 56.0 100.0%	<0.8
Total	Count Expected Count % within Gender	8 8.3 7.5%	20 20.0 17.6%	78 78.0 74.5%	1 1.5 0%	107 107.0 100.0%	

Table 4 Distribution of HCV Genotype in association of Age (n=107)

Age (Years)		**Genotype**				**Total**	
		1	**2**	**3**	**4**		P value
15 - 34	Count Expected Count % within Gender	6 3.7 12.0%	8 9.3 16.0%	36 36.4 72.0%	0 0.5 0%	50 50.0 100.0%	<0.8
35 - 54	Count Expected Count % within Gender	2 3.7 4.0%	10 93 20.0%	37 36.4 74.0%	1 0.5 2.0%	50 50.0 100.0%	<0.85
55+	Count Expected Count % within Gender	0 .5 0%	21 1.3 28.6%	5 5.1 71.4%	0 0.1 0%	7 7.0 100.0%	<0.79
Total	Count Expected Count % within Gender	8 8.0 7.5%	20 20.0 18.7%	78 78.0 72.9%	1 1.0 0.9%	107 107.0 100.0%	

garding severity, disease progression and efficacy of treatment according to genotypes[25].

A study was undertaken in Iran to find out the distribution of various HCV genotypes in Isfahan province in 2010. This study included 146 patients. 97 patients were HCV RNA positive (66.43%). The mean age of patients was 35 years. HCV positive patients were male 95 (97.9%) and 2 patients were female (2.1%). These patients were genotyped by Type specific PCR. The most prevalent genotype detected was genotype a (61.2%) followed by genotype a (29.5%) and b (5.1%). No significant correlation was found between HCV genotype distribution with age ($p<0.44$) or gender ($p< 0.51$). This study concluded that the most common HCV genotype in Iran is genotype a[26]. This study is quite matchable to our study except gender.

Another study conducted in Bangladesh has shown, the most prevalent genotype detected to be genotype b (45.6%). No relationship was detected between age and HCV genotype incidence ($p< 0.83$)[27].

All result of above study were almost same to our results in which most common genotype was 3 and subtype was a.

In another study conducted in Baluchistan that included 40 patients who were HCV PCR positive, found that 50% patients had HCV subtype a followed by subtype b (22%). There was no positive association of age

($p<0.29$) and gender ($p<0.55$) with HCV genotypes. In our study correlation of genotype to age and gender was not significant $p<0.8$.

Idrees and Riazuddin conducted the most comprehensive study that included 3351 patients from different areas of Pakistan. The results showed that the predominant HCV genotype in Pakistan is genotype 3 (49.05%) followed by genotype 2 (17.66%) and genotype 1 (8.35%). Both male and female were equally affected ($p<0.15$), no particular age group ($p<0.29$) was affected by any particular genotype[28].

CONCLUSION

The majority of the cases of Hepatitis C infection resulted from HCV genotype 3 with distribution frequency of 72.9% and subtype a with distribution frequency of 73.8%. We recommend further studies with large sample and multiple settings to assess changing frequency of HCV genotype.

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CONTRIBUTORS

NM conceived the idea and helped in manuscript writing. MS did data collection. IAS did the statistical analysis and planning. All the authors contributed significantly to the final manuscript.