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HCV Genotypes Distribution among HCV Patients with Biochemical markers in District Mardan

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Abstract: *Hepatitis C causes chronic liver disease, cirrhosis, and hepatocellular cancer, and its genotypes vary widely among countries. This study aimed to determine HCV patients' genotype distribution and biochemical indicators. Blood samples from enrolled HCV-positive cases were processed and viral RNA was extracted using the "RNA extraction Qiagene kit" to genotype and quantify viral load using Real Time polymerase chain reaction (Rotor Gene Q-PCR equipment). Analysis was performed by SPSS with p-value < 0.05. Results showed that 198 (91.66%) of 216 patients were 3a genotyped and had higher Alanine aminotransferase (ALT; 39-85%). Genotype 3a dominates in Mardan and nearby areas. Infected patients with genotype-3a had a greater pre-treatment HCV viral load than those with genotypes 1a, 1b, and 5a, although other genotypes had higher biochemical markers (P<0.05).*

Key Words: Biochemical Markers, Genotype, Hepatitis C Virus, Prevalence, Viral Load

Introduction

Viral infection of the liver is a major cause of mortality worldwide. Hepatitis C virus (HCV) commonly infects humans and is the main contributing agent of chronic liver disease. (Bhattacharjee, 2015; Ali et al., 2010). HCV belongs to the C group of species and Flaviviridae family. It is a 55 to 65 nm small, enveloped and single positive stranded RNA virus. The size of HCV genome as a whole is 9.6 Kb. A single polypeptide chain of 300 amino acids is encoded by a single open reading frame in its genome. (Kumthip & Maneekarn, 2015; Vassilaki et al., 2008)

On the basis of its present heterogeneity at genome level, HCV is classified in to seven different genotypes (Bhattacharjee, 2015 ; Bollati et al., 2010) and has 67-70 different subtypes based on diversity analysis and sequencing assessment of entire virus genomes. The main genotypes include 1b, 3a, 2a, 1c, 4, 5a, and 6. (Kumthip & Maneekarn, 2015; Bukh, 2016; Smith et al., 2013b)

HCV genotype 1 is another very often occurring infection worldwide which accounts for around 83.4 million patients which is about 46.2% of the HCV infected population (8). Approximately one-third of the HCV-1 genotype infections occur in the East Asian regions. HCV genotype3 is the next most commonly occurring infection globally which is estimated around 54.3 million cases and 30.1% of the entire HCV infections. Similarly, genotypes 2, 4 and 6 are

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accountable for an entire 22.8% of all hepatitis C incidents although genotype 5 is the least occurring hepatitis C infection which comprises of only <1% of all cases worldwide. Looking at the prevalence of genotype distribution, genotypes 1 and 3 are most commonly occurring HCV infections in most of the nation regardless of their financial status, while the highest prevalence of genotype 4 and genotype 5 are in the lower socioeconomic nations. (Bukh, 2016; Messina et al., 2015.

Different known molecular and biochemical markers have been introduced these days to diagnose, screen and monitor HCV infection and the efficacy of treatment with antiviral therapy. (Coppola, 2015; Ali et al., 2011a). Viral load and genotype have some clinical relevance and has importance in deciding the required treatment options and duration of the therapy 21. In patients having chronic HCV, an elevated viral load and serum alanine amino-transferase (ALT) levels are very important parameters and have clinical relevance to the disease outcome. (Akkaya, 2007; Riaz et al., 2016; Hall & Cash 2012)

Rationale

The diagnosis and prognosis of HCV is strongly correlated with the viral burden and the genotype of HCV virus.

Materials and Methods

This experimental study was conducted at laboratories of the Mardan Medical Complex (MMC) with duly approved certificate from the ethical committee vide letter no. 191 dated 10-3-2022 from June 1, 2019 to September 30, 2019 for a period of 4 months.

Non-probability convenience sampling technique was utilized in the current research for data collection. Sample collection and Lab procedures were performed in the MMC lab. The study sample included both men and women HCV positive participants who met the acceptance and rejection requirements and introduced to the out-patient (OPD). Written agreement was obtained from the guardians of all participants. The total sample was 216, as calculated by the World Health Organization sample size calculator base on the prevalence of the disease.

$$\frac{n = z^2(p \times q)}{e^2} = 216$$

Whereas, n=sample size, Z=Z score at 95% CI, P=Infection occurrence from literature (83%), q=1-p, e =Margin of error (5%=5/100=0.05). n=e $(1.96)^2(0.83 \times 0.17) / (0.05)^2$.

Sample selection was done on the presence and elimination criteria set for sampling and were strictly followed throughout the sample collection. All newly diagnosed adult patients with age range from 35 - 75 years were including (males and females) and patients diagnosed as HCV positive by ELISA. Those patients already taking antiviral, or any other immunosuppressive therapy were excluded from the study and those who had associated HBV infection and HIV infection were excepted from the study.

Aspartate aminotransferase (AST), ALT, Total serum bilirubin, Alkaline phosphatase (ALP) biochemical tests were performed for all the patients enrolled in this study. All tests were performed by using ARCHITECT MACHINE (Abbott Core Lab USA).

Patient Plasma or serum was extracted from the stored blood by centrifugation and HCV RNA was removed which was further used for detection of viral burden by QIAGEN RT-PCR (Qiagene, Germany) and genotyping by Amplisens kit (Qiagene, Germany) following the manufacturer instructions.

Statistical analysis was made using SPSS (version 23). Descriptive statistics were applied to measure the percentage, mean and standard deviation. Chi square test was applied for the association of biochemical markers. P value<0.05 was taken significant.

Results

Demographic data of all the study subjects was collected on previously mentioned structured proforma. Out of total 216 HCV positive patients,

110 were men and 116 patients were women. The commonest hepatitis C genotype was 3a in both males (93.39%) and females (93.63%). Patients were grouped age-wise from birth to 20 years, 21-40 years, 41-60 years and more than 60 years. The patients in these groups were 12, 85, 97 and 19 respectively. The prevalent genotype in all age groups were 3a shown in table I.

Among 216 patients, 174 were from tehsil Mardan, 24 patients were from tehsil Takhat Bahi, while 18 patients were from tehsil Katlang of district Mardan. In all tehsils, genotype 3a was the most common.

Table 1. Demographic data of all the study subjects (n=216)

Variables	Genotypes/ subtypes								
	1 a	1 b	3 a	2	4	5 a	6	untypable	n(%)
Gender									
Men	2 (1.88)	1 (0.94)	99 (93.39)	1 (0.94)	1 (0.94)	0	0	3 (2.83)	107 (49.53)
Women	1 (0.91)	1(0.91)	103 (93.63)	3 (2.73)	0	0	0	2 (1.82)	110 (50.92)
p-value = 0.0015(<0.05) significant									
Age Group in Years									
20	0	0	12	0	0	0	0	0	12 (5.5)
21-40	3 (3.52)	0	78 (91.76)	1 (1.17)	1 (1.17)	0	0	2 (2.35)	85 (39.35)
41-60	0	1 (1.03)	90 (92.78)	3 (3.09)	0	0	0	3 (3.09)	97 (44.90)
61	0	1 (5.2)	18 (94.7)	0	0	0	0	0	19 (8.7)
p-value = 0.0015(<0.05) significant									
Mardan	3 (1.72)	2 (1.15)	161 (92.52)	4 (2.29)	0	0	0	4 (2.29)	174 (80.55)
Katlang	0	0	18 (100)	0	0	0	0	0	18 (8.33)
Takhtbhai	0	0	22 (91.66)	0	1 (4.16)	0	0	1 (4.16)	24 (11.11)
p-value = 00.1637(>0.05) non-significant									

The relationship among different genotypes of hepatitis C virus and patient s serum biochemical markers was also done. The viral load was highest in case of untypable genotypes while it was higher in genotype 1a, 1b and 3a as likened to the additional genotypes initiate in the present research. There was a substantial variation in the levels of ALT between many infections associated with the genotypes (1a, 1b, and 3a), and huge disparity between genotypes in the rate of other biological parameters was identified, as demonstrated in table II, extensive review of the connection between HCV genotypes and biochemical markers like ALT, AST, albumin, total serum proteins, and ALP.

Table 2. Association among genotypes and Biochemical markers (n=216)

Bio-chemical Markers	Genotypes/ Subtypes								
	1 a n= 3	1 b n= 2	3 a n= 198	2 n= 4	4 n= 1	5 0	6 0	Untypable n=5	P-value
ALT	120.3 11.2	90.3 9.03	69.02 5.7	122.3 10.9	128.3 11.2	0	0	121 10.3	0.001
AST	92.3 9.01	80.2 8	62.2 4.8	81.1 5.06	85.3 81.2	0	0	63.4 83.4	0.0264
Albumin	3.26 0.28	3.71 0.33	3.53 0.41	3.56 0.52	3.01 0.45	0	0	3.51 0.41	0.0145
Total proteins	9.03	8.95	8.601	8.61	7.92	0	0	8.50	0.321

Bio-chemical Markers	Genotypes/ Subtypes								
	1 a n= 3	1 b n= 2	3 a n= 198	2 n= 4	4 n= 1	5 0	6 0	Untypable n=5	P-value
ALP	1.01 483.2 11.3	0.61 442.1 10.5	0.32 391.2 7.2	0.46 390.8 8.5	30.1 4769.02 6.3	0	0	0.51 487 021	0.001
p-value= 0.005									

Discussion

Hepatitis C is the commonest viral infection in the developing world including Pakistan. (Akhtar et al., 2020; Khan et al., 2003)HCV genotype dispersal pattern vary widely in different areas of the world and from continent to the continent based mostly on environmental factors. As we know that hepatitis C is classified in to seven genotypes and almost 70 subtypes on the basis of their viral genetic heterogeneity, (Bukh et al., 1995) these genotypes have great importance when drug regimens are decided by the treating physicians and they also have great important in acute and chronic problems of HCV infection. Biochemical markers and viral load of HCV infection are also different in different genotypes and all these parameters contribute to the efficacy of a certain antiviral treatment regimens.

Among all seven genotypes of HCV, the most prevalent one is genotype 1 throughout the world. Its prevalence is 46.2% of all HCV patients, which constitute around 83.4 million cases of which 1/3rd patients belong to East Asia. HCV genotype 3 is the second commonest genotype worldwide which accounts for 54.3 million patients. (Sanjuan et al., 2010) Population based data regarding HCV genotypes and subtypes are of great clinical importance for deciding the treatment regimens and future outcome of the disease. (Hoofnagle, 2002; Valutite et al., 2022). The current project was accompanied for the purpose to find the HCV genotypes in different tehsils of district Mardan Khyber Pakhtunkhwa, Pakistan. The data was obtained after experimentation on 216 ELISA positive patients which were further confirmed by PCR. Analysis of all patients showed that the most prevalent type of genotype is the all tehsils of district Mardan, Khyber Pakhtunkhwa is 3a which was 93.51% of all HCV positive patients, followed by 1a and 1b respectively. These finding from our study are novel and the percentage is very high and this much percentages of HCV genotype 3a have not been reported from other cities of the countries. This could be because of our smaller sample size the study or due to an increase in the genotype 3a at local level. A similar previous study conducted by Umar M. et al. in 2016 showed that the frequency of HCV genotype 3a is 61.3% which is less than the percentage in our study. (Umer, 2016). Similarly a meta-analysis study on the current molecular information of genotypes of HCV from 34 published articles during 1996-2011 showed that HCV genotypes and subtypes distributions in four provinces of Pakistan showed that 3a genotypes is 58.01% in Pakistan (Attaullah et al., 2011). which is again less than our findings in the present study. Although the above information based on the recent literature does not support our findings but the meta-analysis was based on data from 1996-2011 which may have decreased prevalence at that time and now it has increased to more than 90%.

Similarly, the distribution of HCV genotype 3a is less compared to our study and the prevalence of genotype 1 is very high worldwide as shown by different studies. Han et al. (Han et al., 2019) indicated that the worldwide distribution of genotype 1 around 49.1%, while genotypes 3 is only 17.9% 98 which again demonstrates that the international prevalence is also less than the findings of our study. Similarly, other genotypes like 4a was also found in our study which is emerging in Pakistan but this study could not find any genotype 5 and 6 in our analyzed samples.

When we looked at the viral load and biochemical markers of hepatitis C virus, we found significant difference in the viral loads associated with 3a and the others genotypes. The viral load was very high in case of genotype 1a, 1b and 3a while it was low in other genotypes. However, the viral load was high in the untypable strains of HCV found in the present study which needs further analysis. Similarly, when we looked at the biochemical markers of the study population, the ALT and other biochemical markers were lower in case of genotype 3a as compared to the other genotypes. Results indicate that genotype 1a was linked with steadily higher serum levels of ALT, ALP than genotype 3a.

Conclusion

In conclusion based on the present study, HCV genotype 3a is the most predominant genotype in all tehsils of district Mardan Khyber Pakhtunkhwa, and its surrounding areas. Although genotype 1 is not much prevalent in our population at district Mardan, but it is associated with high viral load and higher levels of biochemical markers as compared to other genotypes including genotype 3a. The difference seen in the biochemical markers like ALT and AST among all genotypes is significant with a P value of .001.

Recommendations

Based on the present study, the following are my recommendations for researchers, treating physicians and general public, Hepatitis C virus genotyping is required in different areas of Khyber Pakhtunkhwa, Pakistan to know the exact overall situation at provincial and even country level, Data from all over Pakistan needs to be available online as a data bank which may help treating physicians in designing treatment strategies to overcome the problem at national level. Further studies based large sample size is also recommended to know the status of genotypes in people associated with different professions and different strata of the population. General public must be made aware of the different genotypes, their viral load and biochemical marker status and to educate them about the treatment of the disease in case of different genotypes to overcome its complications like cirrhosis liver and hepatocellular carcinoma associated with certain genotypes.

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