

Full Length Research Paper

Distribution of hepatitis C virus (HCV) genotypes in different remote cities of Pakistan

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Accepted 21 March, 2012

Hepatitis C virus (HCV) infection is a serious health problem that affects millions of individuals worldwide. About 85% of the HCV patients develop persistent HCV infection that leads to liver cirrhosis with chronic Hepatitis C. HCV genotypes have a diversified pattern of frequency and distribution throughout the world and are critically important in understanding the epidemiological problems. Hepatitis C genotype pattern was studied in different remote cities from all five major geographical divisions of Pakistan. A total of 1000 samples were analyzed for genotype. Alanine aminotransferase (ALT) and aspartate-aminotransferase (AST) measurement is higher for genotype 1a and 1b; mean value of ALT and AST for genotypes were between 67 to 129 IU/L and 56 to 99 IU/L, respectively. Real time quantitation showed that there was high grade infection in most of the HCV screened patients. The most prevalent of genotype is 3a, followed by 3b, 1a, 2a and 1b in Pakistan but Kandiaro, Quetta and Loralai showed different pattern of genotype 1a as 20.9, 21.6 and 20.2%, respectively.

Key words: Hepatitis C virus infection, genotype, alanine aminotransferase (ALT), gender ratio, Pakistan.

INTRODUCTION

Hepatitis C Virus (HCV) is rapidly emerging as a major health problem with approximately 170 to 200 million infected patients worldwide. According to an estimate, almost 3 to 4 million people become infected with HCV every year (Idrees and Riazuddin, 2008a). Currently, approximately 10% people in Pakistan are suffering from Hepatitis C infection (Idrees et al., 2008b). Which is more than the rate of HCV in USA and Europe like developed regions of the world (Higuchi et al., 2002). Hepatitis C infection is also a major cause of liver cirrhosis and hepatocellular carcinoma in developed as well as developing countries, including Pakistan. Chronic HCV is predicted to be more than 20% in major Asian countries and 30% in Pakistan (Khan et al., 2000). Pakistan is a country with more than 800,000 km² area, and twice the size of the state of California in the USA and 170 million

populations. Its capital is Islamabad, and Pakistan comprises of 4 provinces (that is, Punjab, Sindh, Khaiber Pakhtoonkhwa, and Balochistan) as well as federally administered areas including Federally Administered Tribal Areas (FATAs), and Azad Jammu and Kashmir (<http://lcweb2.loc.gov/frd/cs/profiles/Pakistan.pdf>). HCV is the member of genus hepacivirus of family flaviviridae. The targets of HCV are hepatocytes and B lymphocytes. HCV has a positive, single stranded ribonucleic acid (RNA) genome of 9400 nucleotides approximately and encodes a single, large polyprotein of approximately 3010 to 3033 amino acids, varying between different genotypes and strains (Bukh et al., 1994). It has a single open reading frame flanked by 5' and 3' UTR's. The polyprotein precursor encoded by the open reading frame is cleaved by viral and host proteases (Moriya et al., 1998). HCV has been classified into at least six major genotypes with different frequency worldwide based on geographical location (Ohba et al., 1995). The epidemiology rate of sustainable virological response

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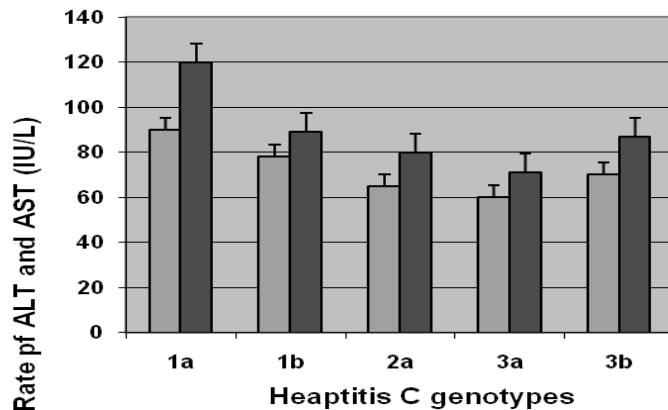


Figure 1. Comparison of HCV genotype and rate of ALT and AST (IU/L).

(SVR) and prediction value of response to antiviral therapy greatly depends upon the knowledge about the genotypes. Various studies have been made to establish the relationship between genotype geographical distribution pattern and prevalence because such studies are helpful in understanding epidemiological problems and taking therapeutic decisions (Idrees et al., 2009). Genotype 1a and 1b are common in Western Europe, genotype 5 in South Africa and genotype 4 in Middle East (Ruggieri et al., 1996). However, Genotype 3 is most frequent in the South East Asia including Pakistan, India, Nepal and Iran (Umar et al., 2010). Several research groups have shown that Hepatitis C infection genotype-3a is most prevalent in Pakistan, followed by genotypes 3b and 1a (Shah et al., 1997; Sherif and Tariq., 2006; Ahmad et al., 2007; Ijaz et al., 2007). None of the reports published up till now has investigated the rate of HCV in remote areas of Pakistan. These areas are underprivileged with no medical facilities and pitiable health and hygienic conditions are playing havoc and there frequency of HCV infection is expected to be extremely high.

MATERIALS AND METHODS

Sample collection

Suspected HCV infected patients were collected from different remote cities of Pakistan from January, 2006 to December, 2010, some 1000 sample for representative samples were selected for this study. These samples from HCV infected patients were analyzed through, ALT and AST measurement, ELISA, real time PCR and were also genotyped.

Serum samples

The blood samples were taken from Hepatitis C patients in

vacutainer (BD Becton, Dickinson and Company, USA). Samples were centrifuged and serum was isolated, packaged and stored at -80°C before analysis to avoid RNA degradation. The present study was approved by the Ethical Committee of CitiLab and Research Centre Lahore and the informed consent was signed.

Enzyme-linked immunosorbent assay (ELISA) for anti-HCV

Test of the anti-HCV antibody were performed by ELISA using commercial kit (Micro LISA HCV Ab. Amgenix, USA). 96-well plates were coated with antigen. Patient's blood serum was isolated and added into wells before incubation. The plates were subsequently washed 5 times with PBST, and then the horseradish peroxidase labeled mono-antibody was added. After incubation, plates were washed and developed colorant was used to determine the results with absorbance reader (microplate reader RT-6000 Rayto company, Germany) at 450 nm, following the manufacturer's instructions.

Alanine-aminotransferase (ALT) and Aspartate-aminotransferase (AST)

To measure the ALT and AST level in blood serum (human, Germany) an automatic biochemistry analyzer was used (Micro Lab 300, Merk Germany).

HCV RNA Isolation and cDNA synthesis

RNA from donor's blood sample was prepared according to the manufacturer's protocol with minor alterations (Trizol. Invitrogen, USA). 300 μl isolated serum was mixed with 500 μl TRIzol reagent and extracted with chloroform and alcohol. After quantification, reverse transcription into cDNA using antisense primers was performed (Qaigen, Hilden Germany).

HCV qualitative, viral load and genotype detection

Qualitative PCR of the 5' UTR region with appropriate primers was performed.

The real time PCR was used to measure HCV viral load with standard controls using AJROBOSCREEN kit and PCR analyzer (Mini Optic. Bio Rad, USA). The viral load was taken in IU/ml. Genotyping was accomplished according to reported methods Ohno et al. (1997).

RESULTS

100 samples from different remote cities of Pakistan were selected. Genotype distribution with reference to viral load categories, alanine aminotransferase (ALT) and aspartate-aminotransferase (AST) measurements, age and gender are shown in Figure 1. Genotype 1a and 1b showed high value for ALT and AST measurement. The mean values for genotypes were between 67 to 129 IU/L and 56 to 99 IU/L for ALT and AST, respectively (Table 1). HCV viral load and its correlation with genotypes were studied with the representative samples (100) and each genotype were performed by polymerase chain reaction (PCR). Most of the samples from all genotypes belong to particular range. 33% of the samples have an average viral load of 1×10^5 IU/ml, while 23% of the samples exhibited an average viral of 1×10^6 IU/ml. There was high

Table 1. Genotype distribution with reference to viral load categories, ALT and AST measurements, age and gender.

Hepatitis C virus genotypes	No. Samples with mean viral load (IU/ml)					ALT	AST	Age	Gender ratio
	10 ³	10 ⁴	10 ⁵	10 ⁶	10 ⁷				
1a	11	17	32	28	12	120±9	90±9	32±9	1.8
1b	16	15	35	25	9	89±8	78±8	30±10	1.1
2a	28	21	32	16	3	80±5	65±5	32±8	0.8
3a	17	15	38	23	7	71±4	60±4	28±10	1.7
3b	18	19	32	25	6	87 ±8	70±8	30±10	1.2

Gender ratio was counted on the number of male to that of female.

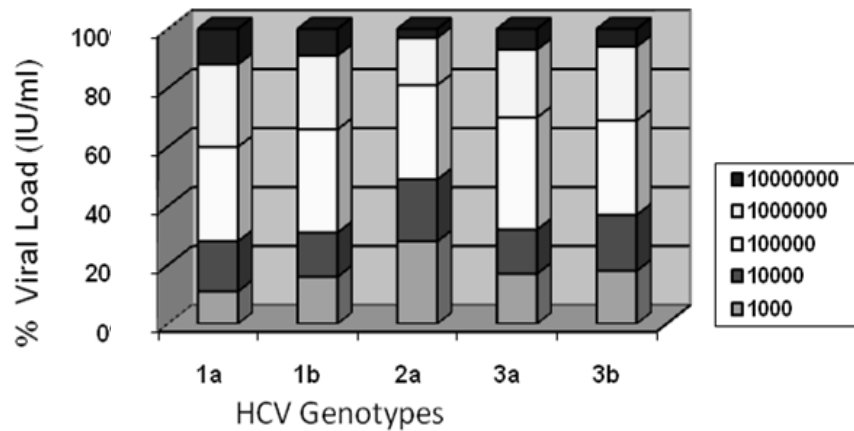


Figure 2. Correlation between Hepatitis C Virus Genotypes and viral load (IU/ml).

Table 2. Prevalence of HCV genotypes in selected cities from five major geographical divisions of Pakistan.

Major geographical distribution of Pakistan	Selected cities	Frequency of HCV genotypes (%)				
		1a	1b	2a	3a	3b
Punjab	Gojra	10.9	2.8	0.4	61.1	24.8
	Shakargarh	11.9	2.7	0.3	59.2	25.9
Sindh	Kashmore	9.2	2.9	0.9	64.2	22.8
	Kandiaro	20.9	2.6	0.7	59.8	16
Balochistan	Loralai	21.6	2.3	0.4	57.3	18.4
	Quetta	20.2	2.9	0.9	56.9	19.1
Khaibar Pakhtoon Khaw	Haripur	12.2	2.8	0.7	59.4	24.8
	Hangu	11.9	2.7	0.9	56.9	27.6
Azad Kashmir	Kotli	10.1	1.4	0.4	66.2	27.9
	Bagh	9.8	1.9	0.3	68.3	19.7

grade infection in most of the HCV screened patients (Figure 2). Moreover, neither age group nor sex has any correlation with HCV infection (Ali et al., 2003). The

prevalence of HCV Genotypes in selected cities from five major geographical Divisions of Pakistan were also studied (Table 2). In Punjab, the largest and most

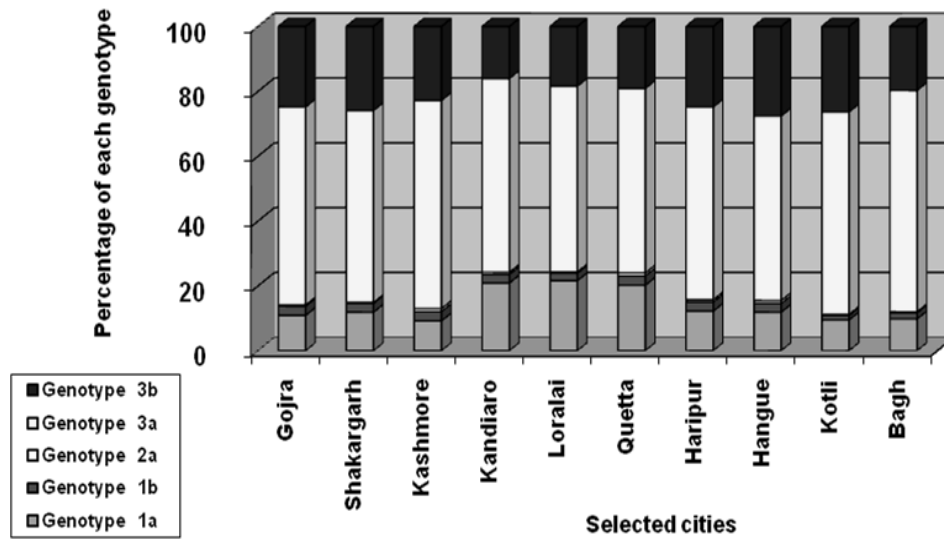


Figure 3. Pattern of HCV genotypes distribution in selected cities of Pakistan.

province of Pakistan cities chosen were Gojra and Shakargarh. These two cities have 3a genotype most prevalent (59.2 and 61.1%), followed by genotype 3b with (24.8 and 25.9%). Kashmore (Sindh Province), the prevalence results for genotype were similar with 3a (59.8%) and 3b (22.8%). On the other hand from the same province, the city of Kandiaro showed different results. This city is located near province Balochistan and shows a contrast pattern of HCV genotype distribution which is similar to the cities selected from Balochistan, (Quetta and Loralai). At Kandiaro, Quetta and Loralai, genotype 3a is most prevalent (59.8, 57.3 and 56.9%, respectively). However, genotype 1a is (20.9, 21.6 and 20.2%, respectively) is more prevalent than genotype 3b, (16, 18.4 and 19.1%, respectively) (Figure 3). Such pattern of genotype prevalence varies from the genotype prevalence pattern in rest of the Pakistan. Cities under study from Khaibar Pktoonkhwa and Azad Kashmir region have shown similar HCV genotype distribution pattern as rest of the Pakistan. According to this study, Khaibar Pktoonkhwa predominant genotype was 3a at Haripur 59.4% and Hangu 56.9% was 3b is 24.8%, 27.6% and genotype 1a is 12.2%, 11.9%, respectively. Federally administered areas of Azad Kashmir, Kotli and Bagh, were selected for this study where genotype 3a was 66.2, and 68.3%, respectively, followed by genotype 3b 27.9, 19.7%, respectively whereas genotype 1a was 10.1%, 9.8% in both cities, respectively.

DISCUSSION

In this study, blood samples were taken from HCV

suspected patients from different remote cities of Pakistan in last five years. One thousand representative samples were selected for the study on the basis of geographical distribution, 59.6% of the selected patients were males and 40.94% were females. Most of the cases reported were from selected cities (Gojra and Sharkargarh) of Punjab and Sindh (Kashmore and Kandiaro) region. Least number of Hepatitis C infection cases was reported from Quetta and Loralai Balochistan, 7 and 4%, respectively. This by no means represents that the prevalence of HCV is less in Quetta and Loralai. But the fact that rate of literacy, militancy and poor health facilities are the main factors. This scenario is alarming especially in an under developed country like Pakistan with such an unstable social, economic and political situation. This present study indicates that HCV genotype 3 is most common in Pakistan which concurs with the previous studies on HCV in Pakistan. On the other hand, the pattern of genotype distribution in Balochistan (Quetta and Loralai) and Sindh (Kandiaro) is in contrast to rest of the Pakistan. The reason behind this may be that Balochistan shares a long border with Iran, where predominant genotype is genotype 1 (Sayyed et al., 2010). There are also similarities between the HCV genotype in Pakistan and in neighboring countries like Iran and India. Accordingly, genotype 3 is reported to be most prevalent in Iran and India and genotype 2 is rare in both of these countries similar to our study (Bouvier-Alias et al., 2002; Qiao-hong et al., 2010). On other hand, there is discordance in case of China where genotype 1 is most prevalent. The reason of similarity with Iran and India may be the more relatedness between socio cultural environment and rate of migration. It is essential to study

HCV genotype distribution to understand the epidemiology and clinical management of the disease.

The most prevalent genotype in Pakistan is 3a followed by 3b,1a, 2a and 1b but 1a has different prevalence patterns as on average, this genotype is 20% in Kandiaro, Quetta and Loralai which is about 10% more prevalence than in other selected remote cities and is an indication of link of this area with Iran. Further studies are required in to fully unravel the mystery of the prevalence of HCV genotypes in Pakistan on the basis of demographic patterns.

ACKNOWLEDGEMENTS

This study was supported by Higher Education Commission of Pakistan.

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