

Prevalence of hepatitis C virus (HCV) genotypes in Balochistan

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Abstract A molecular study was conducted to investigate the prevalence of Hepatitis C virus genotypes in HCV infected population of Balochistan. Forty HCV seropositive samples belonging to seven different locations of Balochistan were collected from different health care centres. Qualitative analysis of these samples using PCR resulted in 28 positive samples. The PCR positive samples were subjected to genotyping using the method described by Ohno et al (J Clin Microbiol 35:201–202, 1997) with minor modifications. Genotyping of 28 samples revealed three different genotypes including 3a, 3b and 1a. The most prevalent genotype was 3a with rate of 50% followed by genotype 3b and 1a, respectively. Nine samples remained untyped, suggesting the need of further investigation of genotypes in this region. It has been proposed that sequencing of these samples may be helpful to unveil these genotypes and further epidemiology of HCV genotypes. Further more, extensive and large scale studies are needed to understand the epidemiology of HCV genotypes, as no such study has been carried in this province.

Keywords Prevalence · Hepatitis C Virus · Genotypes · Balochistan

Introduction

Hepatitis is one of the major causes of morbidity and mortality developing countries including Pakistan. In Pakistani population its prevalence has been estimated to be 8% and is increasing day-by-day due to the lack of basic health care safety measures, community awareness and/or resources.

Acute hepatitis C is mild and often asymptomatic while chronic hepatitis C is an indolent course but may progress to cirrhosis and HCC [1]. It is a slowly progressive infection spread primarily through intravenous drug users, can also spread by sharing of tooth brushes, razors and contaminated needles, sexual relations, from mother to child, etc. HCV RNA has not been detected in semen, urine, stool or vaginal secretion and whether it is present in saliva remains controversial [2]. An estimate of 53000 deaths per year caused due to HCV in world. Most HCV infected people remained unidentified until the development of late symptoms, while some remained carrier through their life and do not develop any complication.

HCV identified in 1989 belongs to family Flaviviridae. Its whole genome has been sequenced and identified. The viral particle consists of an envelope derived from host membranes into which are inserted the virally encoded glycoproteins (E1 and E2) surrounding a nucleocapsid and a positive sense, single stranded RNA genome which has been identified and sequenced [1]. The whole genome of 9,500 nucleotides contains highly conserved untranslated regions (UTR) at both the 5' and 3' termini, which flank a large translational open reading frame encoding a poly-protein of 3,000 amino acids. This is processed by both cellular and viral proteases to produce the specific viral gene products. The structural proteins (core, E1 and E2) are located in the N-terminal quarter while non-structural (NS)

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proteins (NS2, NS3, NS4A, NS4B, NS5A, NS5B) in the remaining portion of the polyprotein.

Early analyzes of the 3' UTR resulted in conflicting data regarding its exact sequence content. Most studies indicated that the genome terminated with a poly (U) tract, while one group reported a poly (A). These differences in the 3' UTR were considered unusual given the importance of these generally well-conserved untranslated regions in the RNA replication of other positive-stranded viruses. The 5' UTR is the most conserved portion of the HCV genome, although nucleotide variations characteristic of different HCV types exist that have been used in polymerase chain reaction based genotyping assays [3].

HCV has population specific genotype [4]. A recent proposal for HCV nomenclature defined six major genotypes [1–6] based upon phylogenetic analyzes of the core E1 and NS5 regions with further divisions into subtypes (1a, 1b, 2c, etc.). The genetic variability is due to high mutation rate in the envelope gene coupled with the absence of a proofreading function in the virion-encoded by RNA polymerase [4]. Researchers have not yet agreed whether or not five different viruses, three found only in Vietnamese individuals, the remaining two seen in Indonesians, are actually subtypes of genotype 6 or ought to be designated as genotypes 7 through 11.

HCV genotyping provides valuable epidemiological and therapeutic information. Current therapy, which consists of a combination of pegylated interferon and ribavirin, gives a response rate of between 48% (genotypes 1, 4, 5 and 6) and 88% for genotypes 2 and 3 [5]. The duration of therapy is variable as well for different genotypes. These findings indicate the importance of genotype knowledge before therapy. In this connection our studies can be very valuable for the health care providers and clinicians in designing the therapeutic strategies to cope this manic disease in this poorly developed area of Pakistan. As such kind of study has not yet been performed in this province.

Material and methods

Present study was conducted at Molecular Diagnostic and Research Laboratory, Faculty of Biotechnology and Informatics, BUITMS, Quetta. In total 40 serum samples were collected from patients visiting different health care centers (Yarkan Centre, Bolan Medical College Quetta and Akram Hospital Quetta). All patients were seropositive for anti-HCV by second generation enzyme immunoassay. Of the 40 patients, 33 were from Quetta, two from Dera Bugti and one each from Muslim Bagh, Loralai, Khuzdar, Musakhel and Barkhan.

HCV RNA was extracted from serum samples by using AcuPure DNA/RNA extraction kit. Four μ l of viral RNA

Table 1 HCV Distribution according to locality

Sample no.	PCR HCV RNA	Genotype	Location
1	Positive	3b	Quetta
2	Positive	1a	Quetta
3	Positive	Unknown	Quetta
4	Positive	3b	Quetta
5	Positive	Unknown	Quetta
6	Positive	1a	Barkhan
7	Positive	3a	Quetta
8	Positive	Unknown	Quetta
9	Positive	3a	Quetta
10	Positive	3b	Quetta
11	Positive	Unknown	Quetta
12	Positive	3a	Musakhel
13	Positive	3a	Quetta
14	Positive	Unknown	Quetta
15	Negative		Quetta
16	Negative		Quetta
17	Positive	3a	Quetta
18	Negative		Dera Bughti
19	Negative		Dera Bughti
20	Negative		Quetta
21	Negative		Quetta
22	Positive	Unknown	Quetta
23	Positive	3a	Quetta
24	Positive		Quetta
25	Positive	3a	Quetta
26	Negative		Khuzdar
27	Positive	3a	Muslim Bagh
28	Negative		Quetta
29	Negative		Quetta
30	Negative		Quetta
31	Positive	3a	Quetta
32	Positive	3a	Quetta
33	Negative		Loralai
34	Positive	3a	Quetta
35	Negative		Quetta
36	Positive	3a	Quetta
37	Positive	Unknown	Quetta
38	Positive	3a	Quetta
39	Positive	Unknown	Quetta
40	Positive	3a	Quetta

was used to reverse transcribe the 5' non-coding region of the viral RNA using Moloney murine leukemia virus reverse transcriptase (M-MLV RTase) (GIBCO-BRL) in a reaction volume of 10 μ l for 50 min at 37°C.

The amplified cDNA was used for qualitative analysis of HCV. The PCR reaction was carried out by using an outer sense and antisense primers. Nested PCR was performed

Table 2 Prevalence of HCV genotypes in HCV-PCR positive samples

No.	Genotype	Total no.	% Age
1	1a	02	7.14
2	3a	14	50
3	3b	03	10.72
4	Unidentified	9	32.14

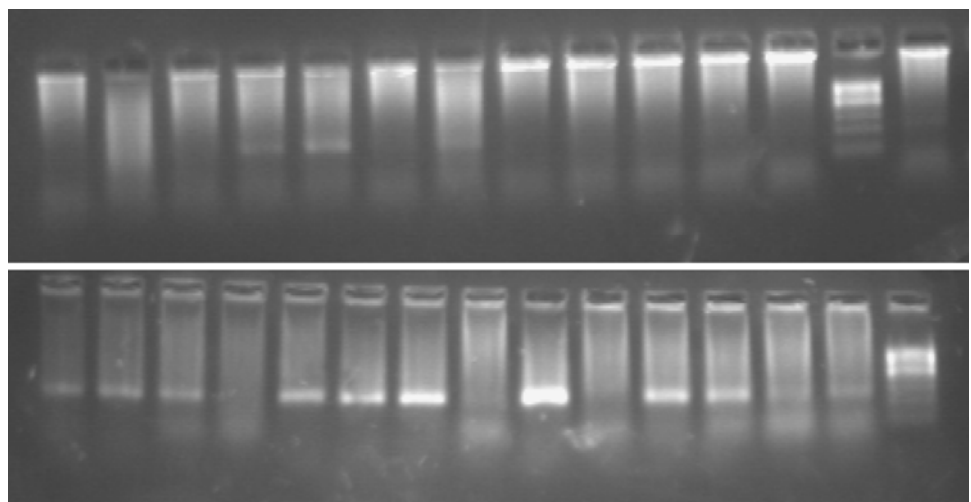
by using inner sense and antisense primers. Second round PCR (nested PCR) was performed with volume of 0.5 μ l of the first round PCR product for 35 cycles. Amplified DNA fragments were run on 1.5% agarose gel electrophoresis and visualized under UV-transilluminator. Out of 40 HCV ELISA positive samples, 28 samples were PCR positive.

The PCR positive samples were screened for the nine different genotypes following the genotyping method of Ohno et al. [4].

Ten μ l of extracted HCV RNA was transcribed into cDNA with Moloney murine leukemia Virus Transcriptase (M-MLV RTase) for genotyping. Amplification of HCV cDNA for genotyping by regular PCR (first round PCR) was carried out. The reaction was performed with Taq DNA polymerase. The amplification was performed with 5.0 μ l of cDNA 5 μ l of the PCR products were electrophoresed on 1% agarose gel along with 100 bp DNA as size marker and visualized on UV-transilluminator and genotype specific bands were marked.

Results

PCR analysis of 40 HCV-ELISA positive samples belonging to seven different locations of Balochistan province end up with 28 positive samples (Table 1). So the net percentage for PCR positive samples is 70%.

Fig. 1 Showing genotype 3a specific bands

Genotyping of 28 HCV-PCR positive samples revealed three different genotypes (Table 2). Genotype 3a is found to be more prevalent (50%), as 14 samples are found to be positive for this genotype, followed by genotype 3b and 1a with positive samples of three and two, respectively. Nine samples could not be typed by using the method of Ohno et al. 1997. All untyped samples are from the Quetta city. So they seem to belong to genotype not identified by this method or to some new genotype.

Discussion

HCV is known to have marked genetic heterogeneity with nucleotide substitution rate of 1.44×10^{-3} and 1.92×10^{-3} per site per year [6, 7]. Accumulation of nucleotide substitutions in the HCV genome results in diversification and evolution into different genotypes. Presently, HCV can be classified into at least six major and a series of subtypes [8, 9]. There is increasing evidence that patients infected with different genotypes may have different clinical profiles, severity of liver diseases, and response to alpha interferon therapy [10–14]. Hence, a convenient and reliable HCV genotyping system is essential for large epidemiological and clinical studies. In this context, a genotyping method, based on genotype specific primers for PCR of the core gene, by which HCV isolates can be classified into genotypes was described [4]. Our study indicates that genotype 3 seems to be the most prevalent type in Balochistan concordant with the finding from other regions of the country, Northern and Southern India, Bangladesh and Nepal.

Multiple studies confirm that type 3 is the predominant HCV genotype in Pakistan, with prevalence of 75–90% [15, 16]. The rest are mostly type 1 and occasionally type 2, with no evidence for others genotypes. Amongst the type

3 infections, subtype 3a is the commonest followed by 3b. Studies document a high prevalence of genotype 3 and very low prevalence of genotype 2. Genotype 3a (Fig. 1) has been found to be the highest (50%) with the decreased frequency of approximately 25% in the case of 3b, approximately 14% in 1b and approximately 10% in 1a [17].

Presence of nine untyped samples indicates that either the genotyping method of Ohno [4] is not detecting these genotypes or some noval genotypes are present in this region. In this connection, sequencing of these samples can be very helpful.

Our findings suggest that further studies are needed in this connection to fully unravel the mystery of the prevalence of HCV genotypes in Balochistan, as no such study has been carried out so far in this province.

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