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# Hepatitis C Virus Genotype Distribution in Kermanshah Province, Western Iran

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**Abstract**

Six major hepatitis C virus genotypes have been characterized, which vary in their geographical distribution. Knowledge of the distribution of various genotypes is essential for successful future research, treatment and control strategies. In this study, the distribution of HCV genotypes and their association with possible risk factors in a group of HCV infected patients from Kermanshah province of Iran was investigated. HCV viral load test by Real time- PCR method was used for diagnosis of infected cases. The genotypes of cases were revealed using Nested- and Multiplex-PCR and with direct sequencing results were confirmed. Risk factors were also recorded and a multivariate analysis was performed. Among 180 infected people, 138 (76.6%) with 3a genotype, 35 (19.4%) with 1a genotype, 3 (1.7%) with 1b genotype and 4 (2.2%) with 3a and 1b were determined. HCV was transmitted by different routes such as intravenous drug abuse (IVDA), tattooing, sexual, blood transfusion and other risk factors. IVDA and sex are the main risk factors in the men and women, respectively. However, 3a is the predominant genotype in the all groups. This study revealed that 3a is the most prevalent genotypes in Kermanshah province.

**Keywords:** hepatitis C, Kermanshah, genotype, polymerase chain reaction

## 1. Introduction

Infection to Hepatitis C virus (HCV) is one of the critical health problems worldwide. HCV infection is one of the main causes of chronic viral hepatitis, hepatocellular carcinoma and also cirrhosis (Martins et al., 2011). HCV also is the leading reason for liver transplantation in the United States and it is controversial concepts in organ shortage (Zein, 2000). HCV infection has reached epidemic proportions. Annually, more than one million infected new cases are reported in world (Cooreman & Schoondermark Van, 1996). Even though, the incidence of new HCV infection is declining, at least in industrialized countries, yet HCV infection with an estimated prevalence of 3% in the world population is known as heavily burdens public health (Pol et al., 2012).

The HCV genome is an enveloped positive-sense, single-stranded RNA genome, with approximately 10 kb long. It has marked similarities to those of members of the genera Pestivirus and Flavivirus (Choo et al., 1991). In infected persons, HCV is a reservoir of related genetic variants, referred to as quasispecies (Duarte et al., 1994). The encoding genes for the envelope glycoproteins (E1 and E2), are the most heterogeneous, especially the 81 nucleotides encoding hypervariable region 1 (HVR1) of E2. HVR1 mutation occurs during the natural course of HCV infection in untreated immunocompetent persons (Higashi et al., 1993). The mutation rate has been estimated to be 0.1 to 0.2 nucleotide substitutions per genome site per year, and several amino acid changes occur over a period of 1 year or more (Kumar et al., 1993).

Up to now, many complete or partial sequences of HCV genome have been reported (Choo et al., 1991; Choo et al., 1989; Inchauspe et al., 1991; Kato et al., 1990; Okamoto et al., 1992) that disclosed marked genetic heterogeneity of the HCV genome (Ohno et al., 1997). HCV strains isolated in different part of the world were classified into 6 genotypes (genotypes 1-6) and numerous subtypes (e.g., subtypes 1a and 1b). Based on the identification of these genomic differences, HCV has been classified into different strains. Maybe genetic

heterogeneity of HCV has an important role for some of the differences in disease outcome and that genotypes are the strongest predictor of the virological response to treatment with interferon (IFN) (Zein, 2000; Scott & Gretch, 2007). Genotypes 1, 2, and 3 are widespread, whereas others are limited to certain geographical areas (Zein, 2000; Simmonds et al., 2005). The prevalence of hepatitis C virus (HCV) in an area is not constant, and depends on the changes in route of infection, which may change over time.

Kermanshah province, located in the west of Iran, has 1.95 million population which 69.7% reside in urban. While literacy of Kermanshah is 81.7%, this province has more drug abusers compared to neighbour provinces (http://www.amar.org.ir/Default.aspx?tabid=133). Furthermore, Kermanshah shares border with Iraq in the west. The aim of this study was to determine HCV genotype prevalence in Kermanshah Province, Iran. The distribution of HCV genotypes and their association with possible transmission routes (risk factors) in a group of HCV infected patients from Kermanshah province where located in western of Iran was investigated, as the data exclusively related to this area is limited.

## 2. Patients and Methods

### *2.1 Patients and Ethics*

In a cross-sectional study, a total of 300 people suspect to HCV infection by specialist physicians referred to the Central Medical Laboratory of Kermanshah from 2010 to 2013. Informed consent was signed by all participants. Data were stored in the database with no reference to the subjects' names. The study protocol and the consent forms were reviewed and approved by the Ethic and Research Committee of Kermanshah University of Medical Sciences. EDTA-anticoagulated specimens in less than 2 hours centrifuged and plasma separated.

### *2.2 Sampling*

Plasma samples were aliquoted and frozen in -70°C until RNA extraction. Total RNA was extracted by commercial QIAamp Viral RNA Mini kit (QIAGEN).

### *2.3 Determination of Viral Load by Quantitative Real-Time PCR*

The viral load was estimated in the peripheral blood specimens using a commercial artus HCV RG RT-PCR kit (QIAGEN) for the detection of HCV RNA using Real-time PCR on ABI Prism 7500 instrument (Applied Biosystems, Foster City, CA). The amplification reaction was performed according to the manufacturer's instructions.

### *2.4 HCV Genotyping*

HCV isolates of viral load positive samples were genotyped using universally accepted method of Ohno et al. (1997). Briefly, based on this method for HCV genotypes manifestation, two rounds of nested PCR was done by one step RT PCR kit (QIAGEN). Core specific primers, Sc2 and Ac2, were applied for the first- turn PCR and two mixtures of primers were used for the second- turn of Multiplex PCR. Primers of mixture A were specific for the detection of 1b, 2a, 2b, and 3b HCV genotypes (234 bp, 139 bp, 337 bp and 176 bp, respectively), while primers of mixture B were used for the detection of 1a, 3a, 4, 5a, and 6a HCV genotypes (208bp, 232bp, 99bp and 336bp, respectively). Meanwhile, the PCR programs of these processes described by Ohno et al. (1997) and primer sequences are shown in Table 1. The genotype specific band was visualized on a 2.5% agarose gel by ethidium bromide and UV light (Figure 1).

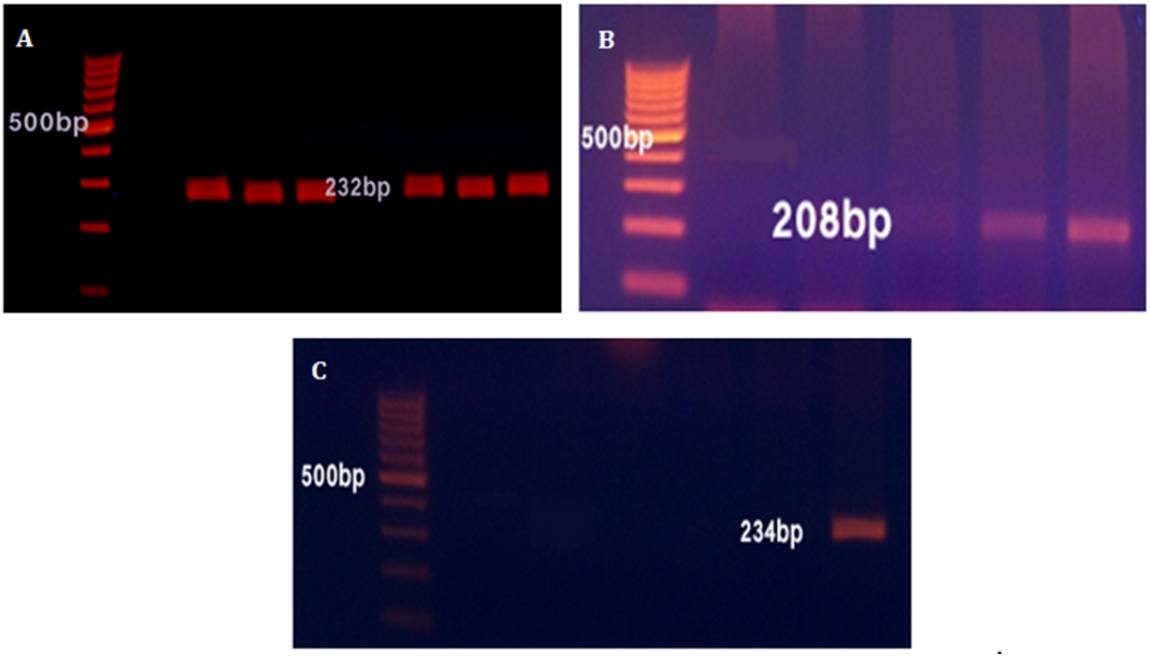


Figure 1. 2.5% agarose gel electrophoresis of the PCR products of the HCV DNA from different genotypes. DNA marker 500 bp (lane M); A: Genotype 3a; B: Genotype 1a; C: Genotype 1b

Table 1. PCR, sequencing, and genotyping Oligonucleotide primers (modified from Ohno et al. (1997)

| *Primer* | *Sequence (5'-3')* | *PCR round* |
|---|---|---|
| Sc2 | GGGAGGTCTCGTAGACCGTGCACCATG | 1st round PCR |
| Ac2 | GAG(AC)GG(GT)AT(AG)TACCCCATGAG(AG)TCGGC | 1st round PCR |
| S7 | AGACCGTGCACCATGAGCAC | 2nd -round PCR for sequencing |
| A5 | TACGCCGGGGGTCA(TG)T(GA)GGGCCCCA | 2nd -round PCR for sequencing |
| **Mix 1** | | |
| S7 | AGACCGTGCACCATGAGCAC | 2nd -round PCR for genotyping |
| S2a | AACACTAACCGTCGCCCACAA | 2nd -round PCR for genotyping |
| G1b | CCTGCCCTCGGGTTGGCTA(AG) | 2nd -round PCR for genotyping |
| G2a | CACGTGGCTGGGATCGCTCC | 2nd -round PCR for genotyping |
| G2b | GGCCCCAATTAGGACGAGAC | 2nd -round PCR for genotyping |
| G3b | CGCTCGGAAGTCTTACGTAC | 2nd -round PCR for genotyping |
| **Mix2** | | |
| S7 | AGACCGTGCACCATGAGCAC | 2nd -round PCR for genotyping |
| G1a | GGATAGGCTGACGTCTACCT | 2nd -round PCR for genotyping |
| G3a | GCCCAGGACCGGCCTTCGCT | 2nd -round PCR for genotyping |
| G4 | CCCGGGAACTTAACGTCCAT | 2nd -round PCR for genotyping |
| G5a | GAACCTCGGGGGGAGAGCAA | 2nd -round PCR for genotyping |
| G6a | GGTCATTGGGGCCCCAATGT | 2nd -round PCR for genotyping |

*2.5 Direct Sequencing*

To test the validity of PCR based genotyping, the nucleotide sequences of HCV core gene from 25 of the 180 specimens were sequenced by ABI 3130 genetic Analyzer instrument (Applied Biosystems, Foster City, Calif.).

*2.6 Statistical Analyses*

Data are presented as percentage (%) or number of patients. Chi-Square and Fisher's Exact tests were carried out by SPSS statistical package version 14.0 for windows. *P* values less than 0.05 were considered significant.

**3. Results and Discussion**

From 300 cases referred to Reference Clinical Laboratory 180 case shown viral load positive by Real time PCR

assay. Among these 180 HCV positive cases, 44 (24.4%) were female and 136 (75.6%) were male with an age range between 18 to 76 years old. There is a notable statistic; from 180 infected patients, 146 cases (81.1%) were connected with suspicious blood; 96 cases were addicted (IVDA), 43 cases had history of tattoo, 5 cases were health center staff, 2 cases had hemophilia and 34 cases (18.9%) have unknown risk factors (Figure 2) that maybe is related to high risk sexual behavior that didn't declare because of cultural reasons. One important thing in the mentioned data is that 7 cases means 3.9% of total patients, 5 cases of Health staffs and 2 hemophilia cases, are infected due to fault in Health systems because frequency of HCV infection in normal population is 0.5-1% (Merat et al., 2010). This is an unfortunate report that could be a catastrophe for a Health system. Interestingly, 82.36% of unknown routes group that may referred to high risk sexual behavior cases comprises of women. These cases are not IVDA but almost have addicted spouse.

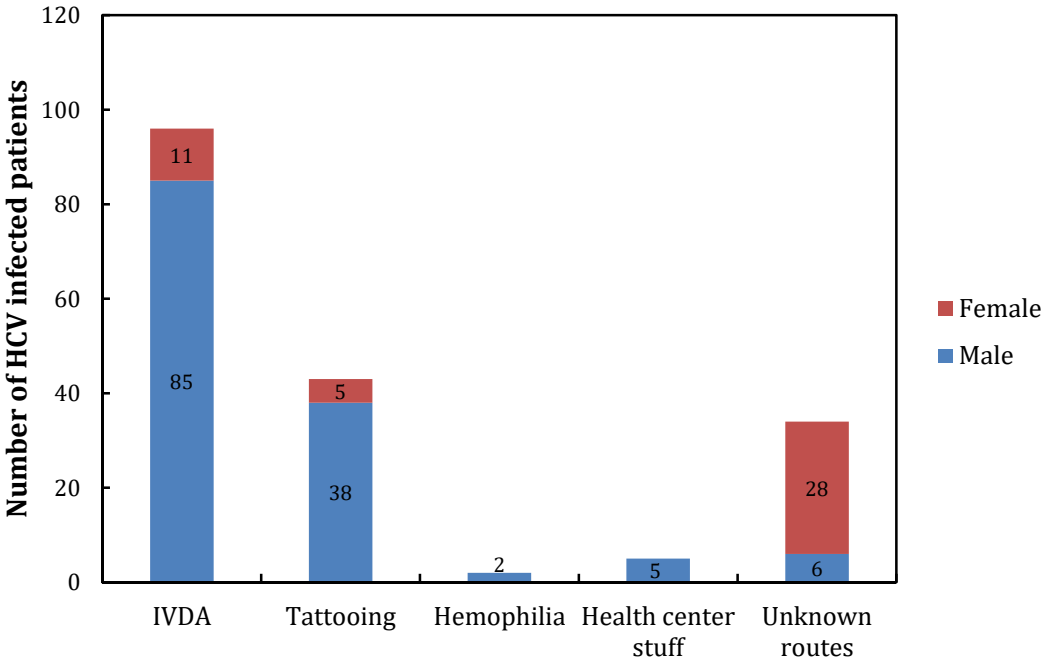


Figure 2. Routes of infection in HCV infected patients

As shown in Figure 3, RT-PCR genotyping results for 180 people infected by HCV showed that 138 people (76.6%) had 3a genotype, 35 people (19.4%) had 1a genotype, 3 (1.7%) had 1b genotype and finally 4 (2.2%) had 3a and 1b genotype. Genotypes 2a, 2b and 3b were not detected in any samples. Additionally, the results of direct sequencing for 25 cases, randomly selected, strongly confirmed RT-PCR results.

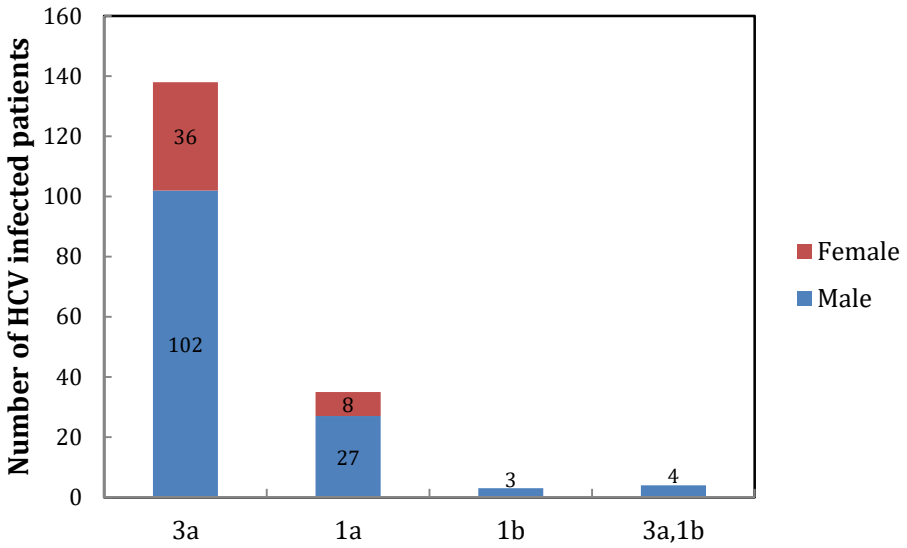


Figure 3. HCV genotype distribution in Kermanshah province

Classification of cases based on risk factors revealed that the predominant HCV genotype is 3a among drug abusers, tattooing and maybe high risk sexual behavior (unknown risk factors) groups (Table 2). These groups comprise of <45 years old cases which consist with previous study that demonstrate usually IVDA cases have 3a genotype (Pawlotsky et al., 1995).

Table 2. Hepatitis C virus genotype subtypes prevalence within transmission route groups

| Presentation of patients | HCV-genotype | | | | *Sig.* |
|---|---|---|---|---|---|
| | 3a (N = 138) | 1a (N = 35) | 1b (N = 3) | 3a,1b (N = 4) | |
| Male/Female (%male) | 102/36 (73.91) | 27/8 (77.14) | 3/0 (100) | 4/0 (100) | |
| Transmission routes | | | | | |
| IVDA | 77 | 16 | 0 | 3 | 0.05 |
| Tattooing | 29 | 10 | 3 | 1 | ns |
| Hemophilia | 0 | 2 | 0 | 0 | ns |
| Health center stuff | 4 | 1 | 0 | 0 | ns |
| Unknown routes | 28 | 6 | 3 | 0 | ns |

## 4. Conclusion

Different studies indicated that HCV is the most important etiological factor for transfusion-acquired and sporadic non-A, non-B hepatitis (Ohno et al., 1997; Alter et al., 1989). The variability in HCV genomes has been proven by comparative analysis of HCV isolates from different geographical regions. The variability in HCV genotypes leads to different serological reactivity and differences in treatment response (Zein, 2000; Simmonds et al., 2005; Casato et al., 1997; Martinot Peignoux et al., 1995; Manns et al., 2001; McHutchison et al., 2002). Based on sequence variation in both the coding and non-coding regions, several classification systems have been proposed. Ohno *et al.,* classified HCV to 1a, 1b, 1d, 2a, 2b, 3a, 3b, 4, 5a, and 6a isolates, based on the core region PCR with genotype-specific primers, that is widely acceptable (Ohno et al., 1997). Genotypes 1, 2, and 3 of HCV become manifest to have a worldwide distribution; however, their relative prevalence differs from one geographic region to another. 1b subtype, in Japan is responsible for about 73% of HCV infection (Takada et al., 1993). In the United States and Europe, 1a and 1b subtypes are the predominant genotypes (Zein et al., 1996; Dusheiko et al., 1994; Sallie, 1995; McOmish et al., 1994). HCV genotype 3a is more common among the abusers of intravenous drug in the United States and Europe (Pawlotsky et al., 1995). In Middle East genotype 4 of HCV is common (Abdulkarim et al., 1998; Chamberlain et al., 1997). HCV genotype distribution in Tehran, located in the center of Iran, indicated that 3a genotype was the most frequent type (46.6%), and then type 1 (43.2) is more common (Hajia et al., 2010). Another study in Northeastern of Iran shows 3a (40%) and 1a (39.2%) types are most prevalent (Vossughinia et al., 2012). A survey in Southern Iran shows 3a (26.2%) and 1

(11.1%) genotypes are common (Ziyaeyan et al., 2011). The present study in Western Iran, Kermanshah Province, revealed that 3a (76.6%) and 1a (19.4%) genotypes are prevalent.

In conclusion, these results indicate that type 3a of HCV is most prevalent in Kermanshah Province, Iran, which is different from other reports around the world.

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