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Investigation and Prevalence of Hepatitis C Virus Genotypes in Pregnant Women

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ABSTRACT Hepatitis C virus (HCV) causes severe liver infection and is spread through blood transmission from infected person to healthy individuals. HCV is more common in less developed countries due to poor hygienic conditions. This condition can be worse in pregnant women, where HCV can infect the fetus and may lead to chronic infections and may cause cirrhosis and carcinoma. Therefore, the purpose of this research was to study the distribution and prevalence of HCV in pregnant women in the Pakistani population, where such data are unavailable. Blood from 72 HCV-positive pregnant women was collected, RNA was extracted and nested PCR was performed for genotyping using genotype-specific primers. The most frequent genotype was found to be 3a (79%), followed by 3b (4%), 1a (4%), 1b (2%) and mixed genotypes (2%). The severity of HCV, reaction to therapy, and prognosis depend on several factors and one of the most important factors is genotype. Hence, this study will pave the way for the adoption of efficient therapeutic models to control HCV in high-risk populations.

INTRODUCTION

Hepatitis denotes any inflammation of the liver and six hepatotropic viruses have been discovered to date in liver inflammation (Gillcrist 1999). HCV is the most common blood-borne virus, and it is a major health problem in developing countries such as Pakistan (Hanafiah et al. 2013). HCV is widespread in Pakistan and the problem is likely to rise due to the extensive use of unsafe medical procedures (Umer and Iqbal 2016). Worldwide, approximately 130-150 million peoples are chronically infected with the hepatitis C virus whereas hepatitis B infected about 2.5 billion people (Irvem et al. 2017; Raza et al. 2007; Khan et al. 2008). Globally, HCV infection affects approximately 8 percent of pregnant women. There are at least seven genotypes of the HCV, and all genotypes differ from each other by 31-33 percent of the whole genome (Hughes et al. 2017; Ghaderi-Zefrehi et al. 2016; Okamoto et al. 1992). Hepatitis C virus is mainly transmitted through blood transfusion and unsafe medical practices (Millbourn et al. 2020; Ragusa et al. 2020).

HCV is a member of the family Flaviviridae and RNA virus. The whole genome comprised 9,500 nucleotides and 3000 amino acids structural proteins containing core, E1 and E2 while (NS) proteins which are non-structural (Afridi et al. 2009; Rajaguru and Nettleman 2011). In pregnant women, hepatitis C can affect the mother and fetus, which may lead to chronic infection in infants with cirrhosis and carcinoma (Jahan et al. 2020). Acute infection is diagnosed infrequently and these individuals are 70-80 percent asymptomatic (Rajaguru and Nettleman 2011; McCaughan et al. 1992). In chronic infection, the perseverance of hepatitis C virus for at least 6 months in the blood is affected by age, sex, and ethnicity. It is previously stated that most abundant genotype is 3a ranging 70-80 percent then 3b and 1a (Idrees et al. 2008; Idrees and Riazuddin

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2008; Chen and Morgan 2006). In Punjab, Pakistan, genotype 3a is most abundant which is 86.46 percent followed by untypable genotype (7.17%) (Haqqi et al. 2019). Genotype is important for therapeutic and duration and response therapy, and there is need to determine HCV genotype distribution in different region to design better strategy according to viral genotype (Haqqi et al. 2019).

Objectives

The main objective of the current study was to investigate the prevalence and distribution of hepatitis C genotypes in pregnant women and the age wise distribution of hepatitis C genotypes in pregnant women.

MATERIALS AND METHODS

The research work was conducted at the Pathology laboratory, INMOL, PAEC and The University of Lahore. Blood sampling was carried out at Jinnah Hospital, Lahore, Lady Atchison Hospital, Lahore and Shiekh Zayad Medical Complex, Lahore.

Samples Collection

Blood samples (2-3ml) from 72 pregnant women were collected from the patients of hepatitis C with the help of expert technician and qualified medical staff. These samples were taken with the signed consent of the patients and a detailed questionnaire was filled with patient's general and clinical information. The study was approved from the local ethical committee of the University of Lahore Pakistan.

Serum Separation

Serum of the samples were separated by centrifugation at 3000 rpm for 10 minutes and then stored in a refrigerator at -20°C prior to processing.

Screening of Patients Samples

Serum from HCV patients was collected and was screened through ELISA. Qiagen Viral RNA mini Spin Protocol was followed to isolate RNA from 100ul serum sample and cDNA was synthesized using reverse transcriptase. Nested PCR was done using Taq polymerase in a total reaction of 25 ul for the detection of viral RNA and amplified products analyzed on 2% agarose gel.

Genotyping

HCV samples were genotypes using genotype-specific primers as defined by Ohno et al. (1997) using two rounds of amplification (Ohno et al. 1995). First of all, about 4 ul of RNA was used for the synthesis of cDNA using MMLV-RT at the temperature of 37^{O}C for 50 minutes. The first round of PCR was used for confirmation of the active virus. One microliter synthesized cDNA was used for the 1st round of PCR which was subjected to two 2nd round nested PCR. Mix-1 primers set and Mix 2 primers set in a reaction volume of 25ul had specific genotype primers set as mentioned respectively (Bashir et al. 2013) (Table 1). Then, the re-

Table 1: Oligonucleotides used for HCV genotype

S. No.	*Name*	*Primer sequence (5′ - 3′)*	*Position*
1	SC2	5 'GGGAGGTCTCGTAGACCGTGCACCATG 3'	3-24
2	AC2	5' GAGNGGKATRTACCCCATGAGRTCGGC 3'	391-417
		MIX 1	
3	S71	5' AGACCGTGCACCATGAGCAC 3'	8-12
4	S2A	5' AACACTAACCGTCGCCCACAA 3'	40-60
5	G1b	5' CCTGCCCTCGGGTTGGCTAR 3'	203-222
6	G2a	5' CACGTGGCTGGGATCGCTCC 3'	159-178
7	G2b	5' GGCCCCAATTAGGACGAGAC3'	306-325
8	G3b	5' CGCTCGGAAGTCTTACGTAC 3'	145-164
		MIX 2	
9	S72	5' AGACCGTGCACCATGAGCAC 3'	8-12
10	G1a	5' GGATAGGCTGACGTCTACCT 3'	177-196
11	G3a	5' GCCCAGGACCGGCCTTCGCT 3'	211-220
12	G4	5' CCCGGGAACTTAACGTCCAT 3'	58-87
13	G5	5' GAACCTCGGGGGGAGAGCAA 3'	289-308
14	G6a	5' GGTCATTGGGGCCCCAATGT 3'	315-334

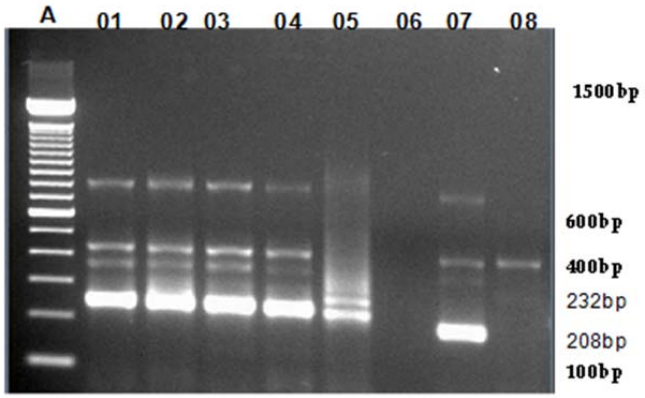

Fig. 1. Gel electrophoresis showing different genotypes of HCV in Mix 2; Lane 01, 02, 03, 04 and 05 (3a); Lane 06 (negative control; Lane 07 (1a); Lane A (DNA Ladder)

Table 2: Prevalence of various genotypes of HCV in pregnant women

Name of genotype	*1a*	*1b*	*3a*	*3b*	*Mixed*	*Untypable*
Number of patients infected with specific genotype	2	1	46	2	1	6
% age	4	2	79	3	2	10

Table 3: Age wise distribution of genotypes in pregnant women

Age in years	*Genotypes*						
	1a	*1b*	*3a*	*3b*	*Mixed*	*Untypable*	*Total*
15-20	0	0	**1**	0	0	0	1 (1.72%)
21-25	**1**	0	**14**	**1**	0	**3**	19 (32.75)
26-30	**1**	0	**14**	0	**1**	**3**	19 (32.75%)
31-35	0	0	**12**	0	0	0	12 (20.68%)
35-40	0	**1**	**5**	**1**	0	0	7 (12.06%)
Total	2	**1**	**46**	**2**	1	6	**58**

sults were analyzed through agarose gel electrophoresis and DNA ladder were run in a separate well.

RESULTS

Analysis of 72 ELISA positive samples was performed through polymerase chain reaction which was from different areas of Punjab. Out of 72 samples, 58 HCV RNA-positive samples were detected through PCR. The net percentage for PCR negative was 14 (19%) and positive samples were 58(81%) which further led to genotyping by using type-specific primers. Figure 1 represents different genotypes which were obtained after amplification through PCR.

Table 2 displays the circulation of different genotypes in pregnant women. The most predominant genotype was 3a (79%), as 46 samples were positive, then genotype 1a (4%), 3b (3%), 1b (2%) , and untypable (10%). Mixed infection by two different genotypes was observed in the patient as 3a/3b (2%).

According to age groups in the Table 3, in age group 1 of 15-20 years old, only one sample show genotype 3a. In age group 2 of 21-25 shows 14 samples of genotype 3a, followed by only 1 sample of genotype 1a and 3b and furthermore, followed by 3 samples are untypable. Then in

age group 3 of 26-30 years old shows 14 samples of genotype 3a, followed by the 1 sample of genotype of 1a and 1 was the mixed type and furthermore, 3 samples show untypable. In age group 4 of 31-35 years old only 12 samples of genotype 3a were found. Furthermore, in age group 5 of 35- 40 years old shows 5 samples of 3a, followed by 1 sample of 1b and 3b. The genotype 3a was most prevalent in the age group 21-35 years. Results show that a high HCV infection rate of 32.75 percent was found in two age groups, that is, 21-25 and 26-30 years which represent that 65.50 percent of HCV pregnant women were found in the age range between 21-30 years while only 1 percent of patients were observed in the age group 10-20 years.

DISCUSSION

Various studies have been conducted in several parts of the world as well as in Pakistan to see the occurrence of different genotypes of hepatitis C virus in general population but pattern of genotype distribution in pregnant women is not previously reported in Pakistan. This verified that the harshness of disease, reaction to therapy and prognosis rely on several factors and genotype is an important factor (Jahan et al. 2011). According to WHO, HCV infection will be eliminated worldwide in the end of 2030 (WHO 2016).

As the present study in which anti-HCV positive samples in pregnant women were taken, to perform these samples by using PCR and to confirm them as they were HCV-RNA positive. In the history of HCV pregnant patients, most physicians recommend some test for their future information as anti-HCV, HbsAg, hemoglobin and complete urine test. Because if they were HCV or HBV positive, then these patients would be treated appropriately during delivery and their medical instruments would be separate and physicians would handle them more carefully and properly.

Messina et al. (2014) presumed that genotype 1 is the most well-known genotype in 85 of the 117 nations, and is exceptionally predominant around the world. They observed that of the 53 percent of genotype 1 cases subtypes were (1a and 1b (31% and 68%, respectively). They also observed that genotype 2 ruled in West Africa, genotype 3 in south Asia and parts of Scandinavia, genotype 4 in Central and North Africa, Genotype 5 in South Africa, and genotype 6 in SE Asia (Messina et al. 2015).

Another study conducted by Ramia and Eid-Fares (2006) revealed that genotypes 1, 2, and 3 are the most commonly reported genotypes globally. In North America and Northern Europe, 1a is the most reported subtype followed by 2b and 3a. In Southern and Eastern Europe the most prevalent genotype is 1b, followed by 2 and 3 respectively. However subtype 3a, is most common genotype in South East Asia (Ramia and Eid-Fares 2006).

Chappel et al. (2018) conducted a research on 87924 pregnant women and observed the HCV prevalence of 1.2 percent. Genotyping method used to be classified into different genotypes based on PCR for core gene is most popular in Japan as defined by (Okamoto et al. 1992). So, this further verified in areas in which HCV types 3 to 6 are common to further authenticate genotype protocol of (Ohno et al. 1995). Ruiz-Extremera et al. (2020) conducted a research on 21870 pregnant women and found that genotypes 1b and 3 are most prevalent (25 % each), and then 4c in 12.5 percent patients and unknown genotype in 37.5 percent (Ruiz-Extremera et al. 2020).

The present study of HCV positive samples in pregnant women in different hospitals of Punjab revealed that out of 72 samples of anti- HCV ELISA positive, 81 percent were positive by PCR, which then proceeded further for genotyping. The distribution pattern of HCV genotyping in pregnant women in Punjab was 3a 79 percent. The study reveals that HCV is a major concerned for infection in pregnant women and proper preventive measures are needed to prevent them from HCV infection. The research work also revealed the frequency of major HCV genotypes in Punjab province. Fortunately, major Hepatitis C virus genotype prevalent was found 3a, which has very good response towards interferon therapy which are contradictory to previous results that HCV genotypes 1 is the most predominant among non-Arab Countries (Haqqi et al. 2019).

The results show that a high HCV infection rate of 32.75 percent was found in two age groups as 21-25 and 26-30 years which represent that 65.50 percent of HCV pregnant women which was in agreement with the study carried out that high seroprevalence of anti HCV was between

age 26-30 years (Kausar Jilani et al. 2017). Genotype is important for therapeutic and duration, response therapy and there is need to determine HCV genotype distribution in different region to design better strategy according to viral genotype (Haqqi et al. 2019). Henceforth the most common genotype is 3a in Punjab, Pakistan which infects pregnant women and may be due to unhygienic medical procedures during previous surgical cesarean delivery (C-section) and blood transfusion**.**

CONCLUSION

HCV confirmatory test is very important in pregnant women to save the lives of the mother and infant. HCV may be transmitted due to previous delivery procedures (C-section) and/or during blood transfusion. In this study, we identified various HCV genotypes in pregnant women in Punjab area of Pakistan, where the most common genotype was type 3a. The severity of disease and reaction to therapy can be controlled through proper information of genotypes and proper sanitization of medical instruments during labor process.

RECOMMENDATIONS

In future more studies are needed with larger groups to design better strategy to control HCV in pregnant women. The effect of medicine therapy should also be studied in patients with long-term monitoring of the patients and in subsequent pregnancies.

LIST OF ABBREVIATIONS

HCV	Hepatitis C virus
cDNA	complementary DNA
WHO	World Health Organization

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