

Immunoassay and Genotyping of Hepatitis C virus in Ramadi City

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ABSTRACT

The study aimed to detect the prevalence of hepatitis C virus in two groups of population, hemodialysis patients and blood donors by different diagnostic methods and also to identify the dominant genotype of this virus in our area. All the samples that collected from blood donors and hemodialysis patients were tested by Rapid test and ELISA technique to detect the positive cases of hepatitis C virus. Only the positive samples were tested by real time PCR for genotyping. 75 hemodialysis patients 27 were infected with HCV and only 13 were blood donors from total number 4560. The total number of positive HCV were 40 samples, all the 40 cases were positive by Rapid and ELISA tests while only 38 were positive by real time PCR. The genotypes of this virus within our cases include genotype 4 (26) and genotype 1a (12). This study concludes that rapid test, ELISA and real time PCR are highly sensitive in detection of this virus. The dominant genotype in our area is genotype 4.

Keywords: HCV, HCV genotyping, blood donors, hemodialysis patients

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INTRODUCTION

The virus of hepatitis C is one of the Flaviviridae family genus Hepacivirus and it is a hepatotropic virus with ribonucleic acid (RNA). This virus was cloned in 1989 and firstly described as infectious agent of Hepatitis other than A and B hepatitis. It causes serious and long-lasting type of hepatitis in the human and monkey with great predisposition for chronicity (1). Hepatitis C virus is the only participant of the type Hepacivirus within the family Flaviviridae. Due to its extraordinary inherited changeability, HCV is classified into seven genotypes and other than one hundred subtypes (2).

The genome of HCV includes 9.8 kb of single strand opened RNA molecule of positive polarization, which encompasses a single extended exposed sense structure that is flanked by 5' and 3' untranslated areas (UTRs). Both UTRs are very designed and are occupied in viral RNA copying, while an inner ribosome access location confined in the 5' UTR facilitates conversion of the positive-part RNA viral genome (3). Hepatitis C virus in chronic type that may be remain without treatment can lead to cirrhosis and carcinoma of liver in some population (4). The occurrence of HCV infection differs during the world, with the maximum number of infections informed in Egypt. The reasons greatest related with infection are inoculation-medication usage and delivery of a blood transfusion previously 1990, on the other hand in particular cases no risk factors can be known (5, 6).

Hepatitis C virus have seven genotypes from one to seven and many subtypes of this virus. Infection forms and variation of subtypes are differing according to countries. The dissimilarity among percentage of infections in different endemic area is around 20% (7). The most prevalent genotypes of this virus are 1 and 3, involving 46% and 30% of all cases, correspondingly. The genotypes 2, 4, 5, and 6 consists 9%, 8%, 1%, and 6% of cases, correspondingly. While the genotype 7 has been establish in merely a few persons from Central Africa (8).

Hepatitis C virus genotypes are recognized a discrete geographical dissemination and medical signs. For

instance, the genotype 1 is dominant in Americas, Japan and Europe. The variation within the genotypes of HCV was depend on the transmission methods and the oldness of infected patients. Such as, the HCV gt 3a and 1a are exceedingly symbolized amongst intravenous drug consumers and the HCV gt 1b is repeated between patients who received blood transfusions (9).

Dissimilarities in genotypes and subtypes of HCV made the treatment of this infection more complicated. Therefore, the death caused by HCV infection stays to rise. Detection of HCV genotypes and subtypes that are responsible for certain infection in certain area is very necessary for appropriate antiviral treatment and identification of the HCV genotype and sub-genotype is crucial for an appropriate antiviral treatment and preserve of HCV-infected individuals (10).

The changeability in the genome of the strains of this virus is about 65.8%–68.7% nucleotide sequence characteristics of floor-length arrangements for types, while for the subtypes is 76.9%–80.1% nucleotide sequence characteristics of full-length sequences, and it is about 90.8%–99% nucleotide order uniqueness of full-length sequences for isolates and quasi species) 11).

Identification of the present of infections with HCV involves screening tests for the occurrence of antibodies to HCV (anti-HCV) monitored by verification of recent infection both by using nucleic acid testing (NAT) for HCV RNA or an immunoassay (IA) for HCV core antigen that are accessible.

Rapid diagnostic tests (RDTs) exclude the requirement for greatly skilled healthcare staffs, sample carriage, and offer fast TAT (12). Many procedures support in HCV identification containing advanced type of ELISA tests that recognize the antibodies against HCV. This test consists of numerous antigens of this virus with successive decrease of the important period of HCV infection, window period with enhancement in revealing of HCV exposed patients (13).

Although that ELISA test is sensitive in detection of the virus antibodies, but it cannot measure the virus loads in the samples as serum and blood. Molecular methods that

used in detection of HCV as real time PCR is highly sensitive and specific assay that measure the HCV loads in the samples with high accuracy. Quantitative assay of virus RNA by real time PCR is more defined and reproducible (14).

PATIENTS AND METHODS

A total of 40 patients infected with hepatitis C virus were enrolled in this study. The blood samples were collected from dialysis patients and blood donors in Ramadi city. Out of 75 dialysis patients, twenty-seven were infected with hepatitis C virus while only thirteen one was blood donors from total of 4560. The blood samples were collected in gel tubes, centrifuge and tested by Rapid (CTK Biotech) and ELISA tests (Advanced company) to detect the positive samples from negative one. The remaining serum samples were kept in -20C for real time genotyping. The genotyping of HCV was carried by using Bosphore kit (Anatolia, Turkey) by Stratagene Mx 3005P instrument.

The kit that used for genotyping of HCV is (Bosphore kit V1) that identify the genotypes in the serum of patients, detect four main and most dominant genotypes of this virus that include (1,1a ,1b, 2, 3, 4). The analytic sensitivity is 100 IU/ml. A region within the 5'UTR is amplified, and fluorescence detection is accomplished

using the FAM filter. Bosphore® HCV Genotyping Kit v1 is composed of Real-Time RT PCR reagents and positive and negative controls.

Four detection mix were used, each one contain forward, reverses primers and dual-labeled probe specific for HCV genotyping that include 1(1c, d,e,f,g,h,i,j,k), 1b,1a, 2, 3, 4 in addition to internal control.

The methods of this work were accomplished according to manufacturer's instruction. The thermal protocol required to run with the Detection Mix 1, 2 and 3 is in the following: for 50 cycle.

Reverse Transcription 50°C 30:00 min.

Initial denaturation 95°C 14:30 min.

Denaturation 97°C 00:30 min.

Annealing 54°C 0 1:20 min.

(Data Collection) Synthesis 72°C 0:15 min.

Hold 22°C 05:00

RESULTS

The sample of this study include 40 patients infected with HCV, their mean age was (43.1), most of them were males 23 (57.5%), while female constitute 17 (42.5%) from the total number as in figure 1. The percent of dialysis patients infected with hepatitis C virus was (36%) while the percent of blood donors who infected with this virus was (0.29%) from the total number.

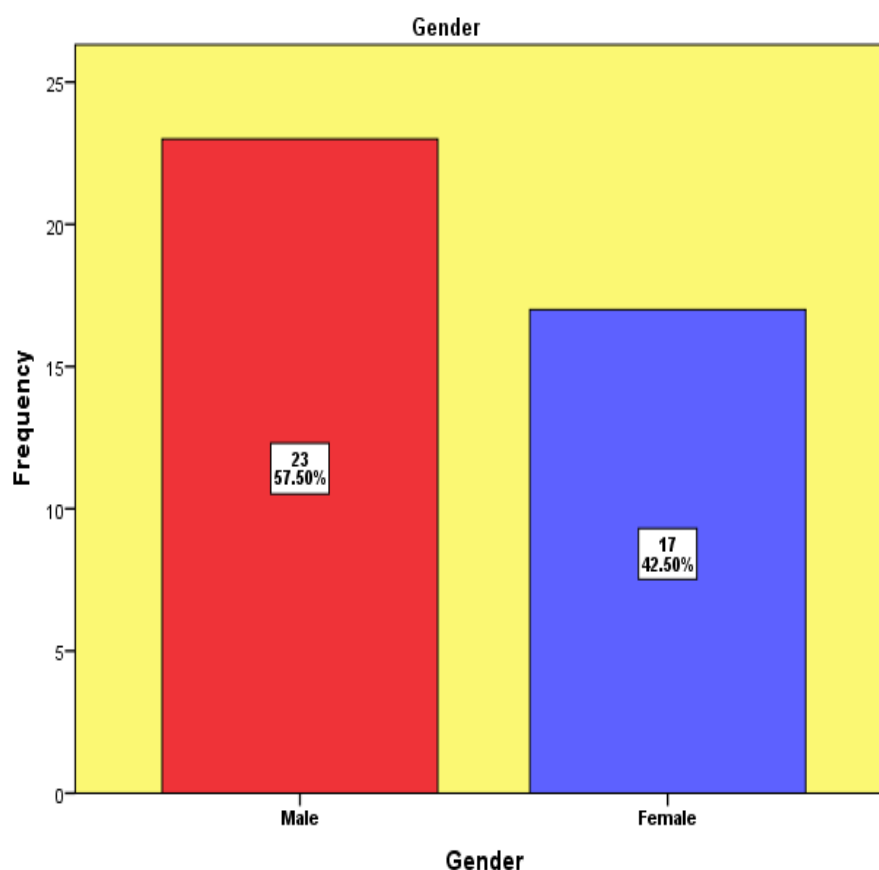


Figure 1. show the percent and frequency of gender in study group.

Most of the patients of hepatitis C were dialysis patients 27 (67.5 %) while the remaining one were blood donors 13 (32.5 %) as in figure 2.

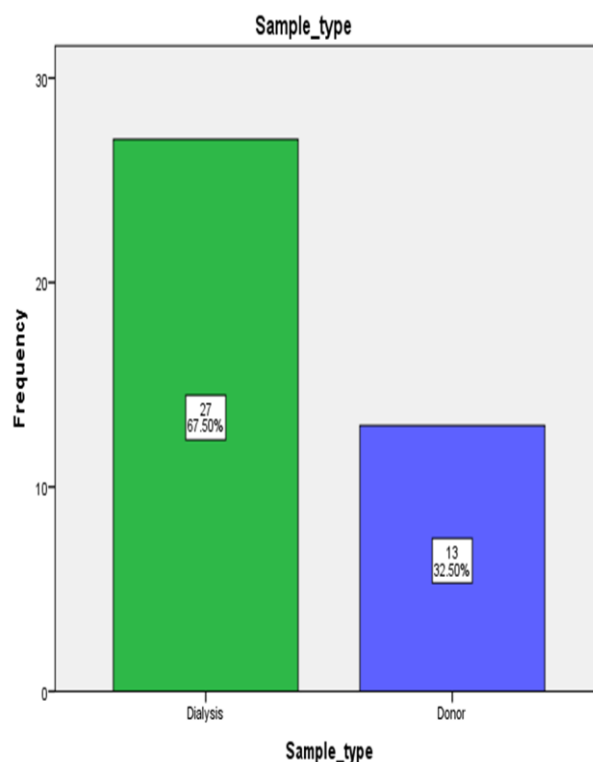


Figure 2. show the percent and frequency of HCV infected patients according to the type of patients.

Hepatitis C virus diagnosis was accomplished by detection of antibodies against the HCV by Rapid test and ELISA as primary test to screen the infections and real time PCR technique as a golden typical test, the results show that 100% of study sample give positive outcomes of HCV by ELISA and Rapid test techniques. Real time test was positive in 38 (95 %), only two samples (5%) were not detected by this test as in figure 3.

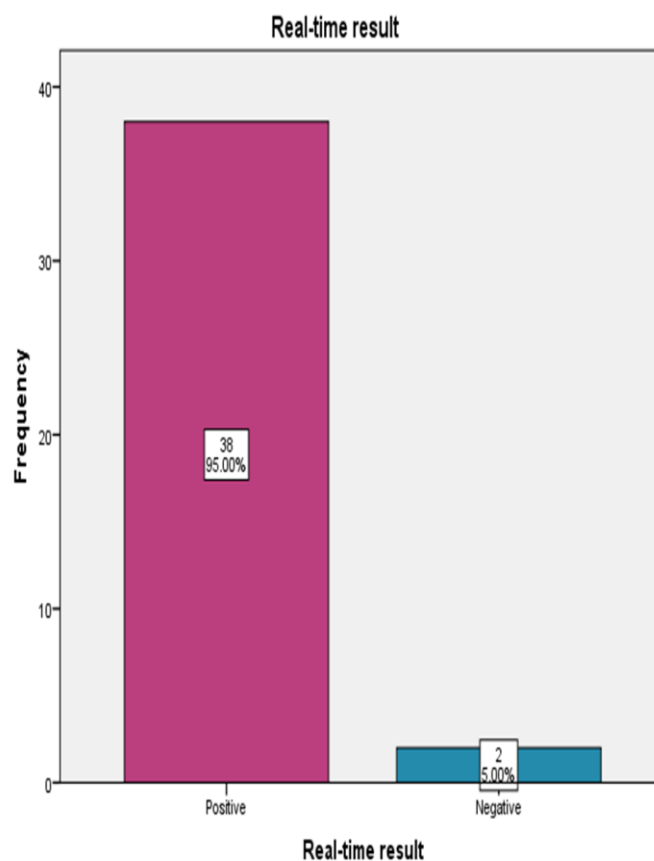


Figure 3. Show the results of real time PCR among the patients.

Frequency of genotypes in this sample of the study was 26 (65%) of genotype **4**, 12 (30 %) of **1a** genotype while two of patients 2(5%) already were negative as in figure 4.

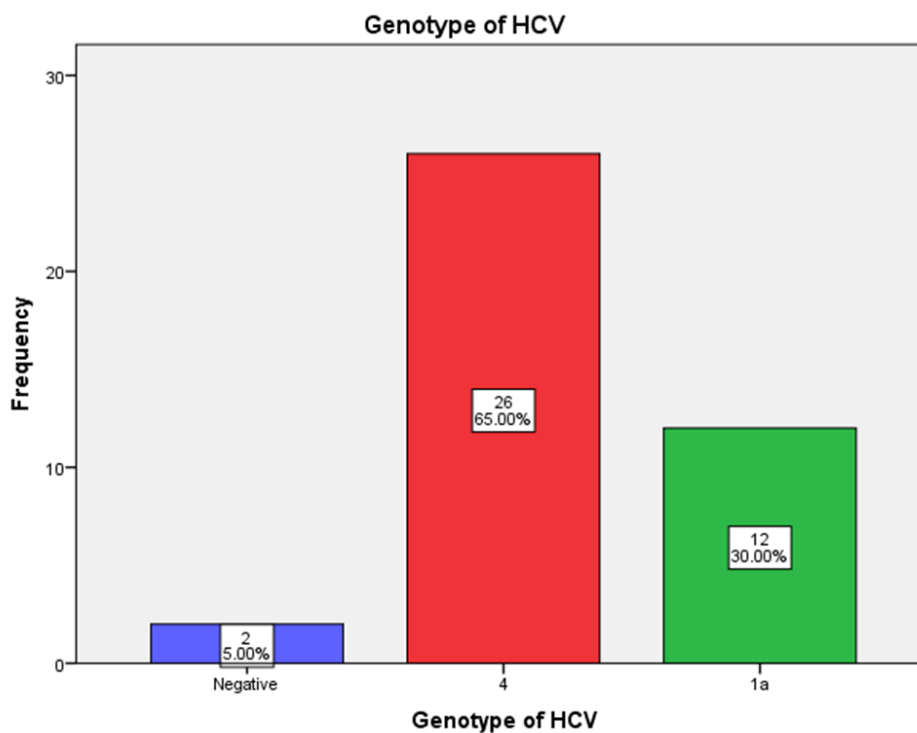


Figure 4. The distribution of Hepatitis C virus genotypes among patients

There are no significance differences between gender and distribution of genotypes P value > 0.005 as in figure 5.

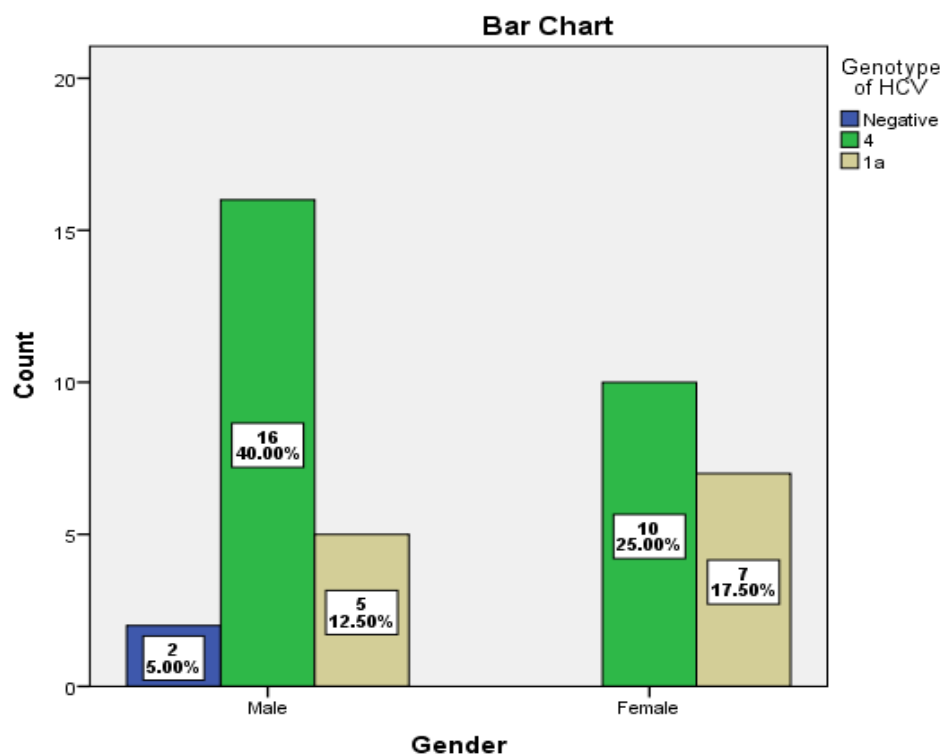


Figure 5. The relation of gender with genotypes distribution

There are no significance differences between genotypes distribution and sample types as in the table 1.

Table 1. show the relation of genotypes distribution with the sample types.

			Genotype of HCV			Total
			Negative	4	1a	
Sample_type	Dialysis	Count	0	19	8	27
		% within Sample_type	0.0%	70.4%	29.6%	100.0%
	Donor	Count	2	7	4	13
		% within Sample_type	15.4%	53.8%	30.8%	100.0%
Total		Count	2	26	12	40
		% within Sample_type	5.0%	65.0%	30.0%	100.0%

DISCUSSION

One of the important blood infectious agents is HCV. There are 120-130 million of the world population are infected with hepatitis C virus. This infection considers a main global health threat problem because HCV is the causative agent of chronic hepatitis that may be led to a cirrhosis and carcinoma of liver (15). Hepatitis C virus transmission routs are differing according to countries. In developed countries the virus transmission occurs by intravenous medicine abuse, while in less developed one the virus transmission occurs mainly by injection therapies with contaminated tools (16). This study shows that percent of male infected with hepatitis C virus more than female. This may be due to that most blood donors were male thus the percent of infected male with this virus higher than female. This result agreed with other study in Iran by Fatemeh Farshadpour *et al* (17).

One of the important risk factors for HCV is a long hemodialysis, this indicated by our result in this study that show the percent of dialysis patients infected with HCV was (36%) from total number of all dialysis patients in our city. This percent was increased from previous years and this contracted with other study as in Daniel *et al* (18). The increase in percent of infection of HCV may be due to contaminated surfaces, unsuitable handling of instruments by medical staff in the hemodialysis center in addition to incorrect giving of parenteral drugs to patients. The infection with HCV among hemodialysis patients varies in prevalence in various countries around the world, as well as between centers in the same region and range from 5% to 60% in developing countries. The percent of HCV infection in Egypt was 50.7% of hemodialysis patients and this result not consistent with this study (19).

This study show that percent of blood donors was (0.29%) from total number of 4560 donors, this result was differ of Seva Öner *et al* (20), Fatemeh Farshadpour *et al* (17) while consistent with Atallah *et al* (21). The differences in distribution of HCV infection among blood donors in different countries may be due to variation in health level of country, education of population, economic status, percent of infection in general people and the correct of donor selection program (22). The genetic heterogeneity in the HCV has high level. This has permitted the strain of this virus to be categorized into many of different genetic groups, that include genotypes, subtypes and isolates (23). There are many genetic material tests as restriction fragment length polymorphism, real time PCR, line-probe assay and heteroduplex mobility analysis, that identify the HCV genotypes in different samples. (24).

This study shows two genotypes of HCV that include 1a and 4 genotypes, this finding accomplished by using Real time PCR technique. The predominant genotype in our sample of study is 4 genotype 26 (65 %) while the remaining patients have 1a genotype 12 (30%). This

finding not consistent with Matthias Schröter *et al* (25) that found the genotype 4 was the lowest one. The result of genotyping in this study is consistent with Nawfal *et al* (26), Sallam *et al* (27), Al-Shamahy and Abdu (28), the distribution of genotypes is differing according to region and this variation due to the mutation in the virus genome, transmission methods and residents' study (29). The genotype 1 is the dominant one around the world and constitute of 83.4 million cases from all cases of this virus, only about 1/3 of percent are found in East Asia. Genotype 3 is more major after genotype 1 and include 53.3 million cases of all HCV cases. The genotypes 2, 4, 6 are constitute about 22.8% from all cases while the genotype 5 include the residual proportion (>1%). The genotypes 1 and 3 are most prevalent when compared with other genotypes, while the 4 and 5 genotypes are present in less advanced nations (29). Detection of genotypes of HCV is necessary in our city because the treatment and control of this virus in all world depend on the genotypes that predominant and the treatment will differ according to genotype distribution for instance, three treatments are suggested for the management of patients infected with HCV genotype 1. These schedules are simeprevir plus sofosbuvir with or without ribavirin, ledipasvir/sofosbuvir and ombitasvir/paritaprevir/ritonavir plus dasabuvir with or without ribavirin (30).

This study conclude that hemodialysis patients were risky for infection with HCV, three methods that used in this study were highly sensitive in detection of HCV. The genotypes that detected in our samples were genotype 4 followed by genotype 1a.

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