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Detection of Hepatitis C Virus Genotype 4 (HCV-4) Using Real Time PCR in Iraqi Patients Infected with HCV

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Abstract: Hepatitis C virus (HCV) infection is world problem that cause great liver damage approximately effecting 70 million people worldwide which has 6 genotypes and HCV genotype 4 (HCV-4) is the most virulence among the other genotype where it is responsible for a major cause of liver cirrhosis, which leads to liver failure and it is the root cause of hepatocellular carcinoma. The goal of this study is to assess the percentage rate of HCV-4 which has a great effect on the type and duration of medication that used to cure HCV infection, and its an attempt to select which drugs should be available in the hospital. A total of 100 patients with HCV infection and 90 control subjects were involved in this cross-section study during the period from February 2019 to October 2019 in Al-Yarmouk Teaching Hospital and Special Nursing Hospital . The detection of HCV was carried by ELISA and the qualitative real time PCR Technology. The HCV was found with 99% in serum that gave noticeable increase when compared with the control group and the results that showed a high percentage rate (66%) of HCV genotype 4 (HCV-4) while HCV-1a and HCV-1b gave moderate to low percentage rate (32%) and (1%)respectively. Patients with HCV infection show a significant high prevalence of predominance percentage rate of HCV genotype 4 among other genotypes in our patients.

Keywords: Real time PCR, HCV, Genotype, RNA.

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Introduction

Hepatitis C virus (HCV) infection is a world problem that causes great liver damage approximately affecting 70 million people worldwide (1). Its RNA enveloped viruses characterized by a high spontaneous mutation rate, that results in a heterogeneous group of viruses based on different nucleotide sequences and according to that, it classified to six major genotypes and a series of subtypes like Genotypes 1,2,3,4,5 and 6. The subtype labeled with small letters for example genotype 1a and 1b (2) that is the genotype has a great effect on the type and duration of medication used in the treatment of HCV (3).

HCV genotype 4 (HCV-4) is the most virulence among the other genotype, it is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections that considered a major cause of liver cirrhosis, which leads to liver failure and it is the root cause of hepatocellular carcinoma (4,5).

HCV can be transmitted through intravenous drug use (IVDU), blood to blood contact, use of unsterilized injection needles or unsterilized instruments for nose, ear piercing, tattoos, sexual exposure and blood products (6,7). Virus cannot be transmitted from one person to other by sharing drink or food with an infected individual (8, 9). Egyptian people have the highest prevalence of (15%)worldwide of HCV where the HCV-4 are responsible for (90%) of infections, while Saudi Arabia has 1-3% with a predominance of genotype HCV- 4 (62%) of infections(10,11).The HCV-4 has become increasingly prevalent in some southern European countries on the Mediterranean Sea. Italy, France, Greece, and Spain, where higher prevalence rates of 10–24% have been reported (12).

This study conducted to estimate the rate percentage of HCV genotype 4 among another genotype for Iraqi patients which have a great effect on the type and duration of medication that used to treat HCV infection in an attempt to provide these medications in the hospital.

Materials and methods

This study was conducted in Al-Yarmouk Teaching Hospital and Special Nursing Hospital during the period from February 2019 to October 2019. That includes 100 patients with HCV infection, whom give a positive result to surface antigen HCsAg in Enzyme Linked Immune Sorbent Assay test (ELISA), age range 18-65 years and 90 healthy subjects whom give a negative result to surface antigen HCsAg in ELISA test were selected from the virology lab that done before any surgical intervention. It's done by the collection of 190 venous blood sample from100 patients who have the symptoms of HCV infection which also have positive results to surface antigen HCsAg in ELISA test and 90 samples of a healthy subject as a control. About 5 ml of venous blood was collected by using disposable needles and syringes. The serum obtained by centrifugation at 4500 rpm for 10 minutes and kept frozen at (-20) until the time of its use.

Detection of HCV surface antigen HCsAg in the serum by using ELISA technique, according to the HCsAg ELISA Test Kit manufactured by American CTK Biotech, specialized for detection of HCsAg in the blood serum, while the detection of the genotype of the virus is done by polymerase chain reaction (PCR) technology that’s based on primer-specific extension analysis (PSEA) of HCV PCR amplicons, called PSEA-HCV. Primers specific for genotypes 1, 2, 3, and 4 and subtypes 1a, 1b, 2a/c, and 2b were designed to bind to variable regions R1 to R7 within the amplified 5_ UTR of the HCV genome. Each PSEA-HCV primer was 5_ fluorescently labeled to facilitate detection (Sigma-Genosys, Woodlands, TX *a* 5_ fluorescent labels: HEX, 6-carboxy-2_, 4, 4_, 5_, 7, 7_-hexachlorofluorescein; FAM, 6-carboxyfluorescein).

The primer sequences and the dye labels as following:

Identifier	Sequence
PSEA-HCV-1	5_ HEX- AAG GAC CCG GTC GTC CTA 3'
PSEA-HCV-2	5_ FAM- TAT CCA AGA AAG GAC CCA 3'
PSEA-HCV-3	5_ FAM- CAA CAC TAC TCG GCT AGT 3'
PSEA-HCV-4	5_ HEX- CAT GGC GTT AGT ATG AGT GTT 3'
PSEA-HCV-1a	5_ HEX- ACT CGG CTA GCA GTC TT 3'
PSEA-HCV-1b	5_ FAM- ACT CGG CTA GCA GTC TCT 3'
PSEA-HCV-2a/c	5_ HEX- GAG TAC ACC IGA ATT GCC GGG 3'
PSEA-HCV-2b	5_ FAM- TGA GTA CAC CGG AAT TMC CG 3'

The PCR reaction system through the following thermal regimen:

The initial denaturation at 94°C for 20 s, followed by 30 cycles of 94°C for 1mint, 54°C for 45 s, and 72°C for 2 mints.

Test Sensitivity

10-100,000,000 IU/ml.

The Xpert HCV Viral Load quantifies HCV genotype from (1-6),1a, 1b, 1a/1b, 2, 2a, 2b, 2a/c, 3, 4. The Xpert HCV Viral Load is ultrasensitive that the limit of detection (LOD) was 4.0 lU/ml in EDTA plasma (95% cl 2.8-5.2) and LOD of 6.1 lU/ml in serum (95% cl 4.2-7.9). RNA purity more than (2.2).

Statistical Analysis

Data were collected and analyzed using SPSS version 10.0 for windows (SPSS, Chicago, Illinois, USA). The statistical test used according to the need P- value < 0.05 was considered as statistically significant.

Results

The HCV was found with 99% in serum that gave noticeable increase when compared with the control group person whom gave a negative results in the same test.

The result of real time PCR for detection the genotype of HCV showed a high percentage rate (66%) of HCV genotype 4 (HCV-4) among another genotypes while HCV-1a showed moderate percentage rate (32%) and HCV-1b showed low percentage rate (1%) as in table (1) and figure (1).

Table (1): The results of Real time PCR of HCV infected patients and control subjects

HCV genotype and subgenotype	Patient withHCV N0.(100)	Control subjects N0.(90)
HCsAg(no.) Mean ± SD %	100	0
Real time PCR(no.) Mean ± SD %	99	0
HCV-4(no.) Mean ± SD %	66	0
HCV-1a(no.) Mean ± SD %	32	0
HCV-1b(no.) Mean ± SD %	1	0

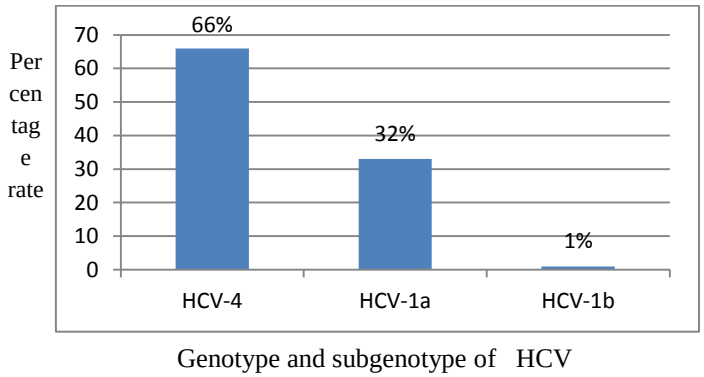

Figure (1): Distribution of different genotypes of HCV among infected patients.

Discussion

With the time the frequency of HCV infections is increasing in different parts of the world which is a major global health issue affecting several individuals around the globe that is a major cause, which may end in liver cirrhosis and hepatocellular carcinoma (13). The prevalence of HCV genotypes varies all over the world due to virus mutation, route of infection and population analysis (14). Sensitive and accurate detection of genotype of HCV infections has become an increasingly important requirement of genotyping assays. First, the severity of HCV and patient response to current antiviral therapies seem, at least in part, to be determined by the genotype of the infecting HCV strain(15). Second, one of genotype may be more common than other genotype reported given the typical routes of transmission of HCV infection (16). The method is simple and rapid and can be easily performed by any laboratory equipped to perform PCR analysis, also PSEA-HCV assay does not require the user to re-extract the sample or repeat the amplification that offers several advantages, including cost and time savings(17). PSEA-HCV is designed to multiplex the most common genotypes in one reaction, it can effectively provide HCV genotyping =(i.e., 1, 2, 3, or "other") within hours of an HCV-positive result. In additional individual reactions that require to subtype HCV genotype 1 or 2 can be set up as necessary by using the internal control. It is debatable whether HCV subtyping information is clinically relevant or even reliable for HCV genotypes 1a and 1b when using the 5_ UTR (18)and could be omitted from routine service work. The results of study were compared with other study like KSA and Egypt and others show proximity same result with predominance of genotype HCV-4 . Egypt has the highest prevalence of HCV worldwide (15%) and the highest frequency of HCV-4 responsible for almost 90% of infections(10,19). The prevalence of HCV in Saudi Arabia is 1–3%, with a predominance of genotype 4 (62%) (11). Similarly, studies from other parts of the Middle East also suggest a high prevalence of HCV-4. For example, 36–46% of HCV-infected Lebanese patients have HCV- 4 (20), 59% of Syrian patients (21) and 27% of HCV-infected Jordanian patients on dialysis have HCV-4 (22).

Conclusion

The result of present study demonstrated that the HCV-4 infection

in Iraqi patients have a higher prevalence among other genotypes with a predominance percentage rate about 66%.

Reference

1. Daniele, L. and Anna, R. (2019). Hepatitis C Virus Genetic Variability and Genome Polymorphisms. *Cells* ; 305:12-22.
2. Kuiken, C. and Simmonds, P. (2009). Nomenclature and numbering of the hepatitis C virus. *Methods Mol. Biol*; 510: 33–53.
3. Ciuffreda, D.; Lewis, L.; Kasprowicz, V.; Nolan, B.; Streeck, H. and Aneja, J. (2012). Primed during acute hepatitis C infection, but rapidly disappear from human blood with viral persistence. *J. Exp. Med*; 209: 61–75.
4. World Health Organization 2010. Hepatitis C. Geneva, Switzerland: WHO fact sheet 164. Available at http://www.who.int/ media Centre/factsheets/fs164/en/print. html.
5. Mohamed, M.; Abdel-Hamid, M.; Mikhail, N.; Abdel-Aziz, F.; Medhat, A.; Magder, L., *et al*. (2015). Intrafamilial transmission of hepatitis C in Egypt. *Hepatology*; 42:683–687.
6. Ali, S.; Ali, I.; Azam, S. and Ahmad, B. (2011). Frequency distribution of HCV genotypes among chronic hepatitis C patients of Khyber Pakhtunkhwa. *Virol J*.; 8:63-67.
7. Stoszek, S.K.; Abdel-Hamid, M.; Narooz, S.; El Daly, M.; Saleh, D.; Mikhail, N., *et al*. (2006). Prevalence of and risk factors for hepatitis C in rural pregnant Egyptian women. Trans R Soc Trop. *Med. Hyg*.; 100:102–107.
8. Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. *Clin. Microbiol Infect*; 17: 10–15.
9. Maheshwari, A. and Thuluvath, P. (2010). Management of acute hepatitis C. *Clin. Liver Dis*; 14:169–76. 5.
10. Elkady, A.; Tanaka, Y.; Kurbanov, F.; Sugauchi, F.; Sugiyama, M.; Khan, A., *et al*. (2018). Genetic variability of hepatitis C virus in South Egypt and its possible clinical implication. *J. Med. Virol*.; 81 (6): 1015–1023.
11. Nazir, I. and Mohammed, E. (2018). Changing pattern of hepatitis viral infection in Saudi Arabia in the last two decades. *Journal of Hepatology*; 54: 1250–1262 .
12. Esteban, J.; Sauleda, S. and Quer, J. (2008). The changing epidemiology of hepatitis C virus infection in Europe. *J. Hepatol*; 48:148–162.
13. Sanaa, M.; Naseer, I. and Hepatitis, C. (2008): what we know and what we don't yet know. *Hepatology*; 47:1371–83.
14. Mederacke, I.; Wedemeyer, H. and Manns, M. (2009). Boceprevir, an NS3 serine protease inhibitor of hepatitis C virus, for the treatment of HCV infection. *Curr. Opin. Investig Drugs*; 10: 181–189.
15. Zein, N. (2016). Clinical significance of hepatitis C virus genotypes. *Clin. Microbiol. Rev*. 13:223–235.
16. Quarleri, J. F.; Bussy, M. V.; Mathet, V. L.; Ruiz, V.; Iácono, R.; Lu, L., *et al.* (2018). In vitro detection of dissimilar amounts of hepatitis C virus (HCV) subtype-specific RNA genomes in mixes prepared from sera of persons infected with a single HCV genotype. *J. Clin. Microbiol*. 41:2727–2733.
17. Liew, M.; Erali, S.; Page, D.; Hillyard, C. and Wittwer, A. (2017). Hepatitis C genotyping by denaturing high-performance liquid chromatography. *J. Clin. Microbiol*. 42:158–163.
18. Weck, E. and Chen, Z. (2017). Hepatitis C virus genotyping: interrogation of the 5_ untranslated region cannot accurately distinguish genotypes 1a and 1b. *J. Clin. Microbiol*. 40:3127–3134.
19. Faisal, N. and Shumaila, N. (2018). An overview on hepatitis C virus genotypes and its control. *Egyptian Journal of Medical Human Genetics*. 80: 1016–1030.
20. Al-Faleh, F. (2011). Changing pattern of hepatitis viral infection in Saudi Arabia in the last two decades. *Journal of Hepatology*; 54 : 1250–1262 .
21. Antaki, N.; Haddad, M.; Kebbewar, K.; Abdelwahab, J.; Hamed, O.; Aaraj, R., *et al*. (2018). The unexpected discovery of a focus of hepatitis C virus genotype 4 in a Syrian province. *Epidemiol Infect*;17:1–6.
22. Abdel-Hamid, M.; Abdel-Aziz, F. and Bdour, S. (2018). Hepatitis C virus infection in Jordanian hemodialysis units: serological diagnosis and genotyping. *J. Med Microbiol*; 51:700–704.