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# Genotyping of Hepatitis C Virus (HCV) in Patients of Basra Province/Iraq

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**Abstract:** Hepatitis C virus (HCV) infection has reached epidemic proportions. Worldwide, more than one million new cases of infection are reported annually, and HCV is believed to be more prevalent than hepatitis B virus infection. The study was carried out to genotyping of Hepatitis C virus (HCV) in patients of Basra province /Iraq. This study included 102 HCV infected patients 49 males and 53 females the age groups between 5-75 years. The selection of the patients for genotyping were based on the presence of HCV RNA in plasma by using Real Time-PCR, then detected HCV genotyping by using reverse transcription polymerase chain reaction (RT-PCR).The results showed prevalence of genotypes 1a, 1b, 2 and 3a in 37.25%, 17.65%, 4.90% and 0.98% respectively. Also these study determinate mixing genotypes (14.7%). Furthermore, distribution of HCV genotypes on studied groups showed high prevalence of 1a genotype in digestion system patients (14.71%), hemodialysis unit patients (11.76%), oncology children/child hospital (5.88%) and Center of hereditary blood disease patients (4.90%), then 1b genotype (9.80%) in digestion system patients, (3.92%) in hemodialysis unit patients, (0.98%) in oncology children/child hospital and (2.94%) in Center of hereditary blood disease patients. Also a 2 genotype was found only in digestion system patients (3.92%), hemodialysis unit patients (0.98%). while 3a genotype has 0.98% in digestion system patients only. Based on these results, it could be concluded genotype (1a and 1b subtypes) was a predominant of HCV genotypes in patients.

**Keywords**: **HCV, genotypes, viral LOAD**

## I. INTRODUCTION

Hepatitis C virus (HCV) is a positive sense, single stranded RNA virus, grouped in the *Hepacivirus* genus of the Flaviviridae family which also includes many arthropod-borne human pathogens of the *Flavivirus* genus such as yellow fever virus, West Nile virus and dengue virus (**1**). However, there is no vaccine yet available. Discovered in 1989 (**2**). Hepatitis C virus constitutes a significant health burden worldwide. Indeed, this virus has a high propensity for establishing a chronic infection and it is estimated that 130-170 million people suffer from chronic hepatitis C. In the long-term, this can lead to advanced liver fibrosis, cirrhosis and hepatocellular carcinoma. As a consequence, HCV is the most common indication for liver transplantation in developed countries (**3**). As a result, ALT levels and a positive HCV serology result are not adequate for the diagnosis of chronic HCV; instead, detection of HCV RNA is required to establish the diagnosis. Results from longitudinal viremia studies have indicated that spontaneous resolution of chronic HCV infections occurs at a rate of 0.50% to 0.74% per person-year annually (**4**). Unfortunately, up to 20% of individuals with chronic hepatitis C eventually develop liver cirrhosis, which may be complicated by hepatocellular carcinoma, hepatic decompensation, or death (**5**). At least six major viral genotypes (1 to 6) have been identified (**6**). Genotypes display differences in nucleotide sequences below 30–35%, and within a genotype genomes are allocated into different subtypes if their sequences differ by over 20–25%. Furthermore, viral isolate(s) present in an infected individual can mutate into quasi-species (**6**, **7**). The determination of the viral genotype is important for transmission studies, and has major therapeutic implications. Patients infected with genotype 1 have a significantly lower rate of

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response to antiviral therapy compared to other genotypes (**8**). Epidemiologically, the prevalence rates of different virus genotypes vary from region to region and there are different risk groups exposed to different genotypes of the virus (**9**).

## II. MATERIALS AND METHODS

Present study was conducted on the following study groups during the period between 21/9/2014 into 23/9/2015. For the first time in Basra Province /Iraq, in this study was determined the genotypes of HCV of 102 Basra patients, consisting of 49 males, 53 females with age ranged 5-75 years, attending to Molecular lab/ general Al-Faiha hospital. HCV RNA was extracted by using QIA amp ® Viral RNA Mini Handbook, Germany according to the manufacturers' instructions, then tested by using Real-Time PCR (7500 Applied Biosystems, Singapore) according to a sensitive commercially available Real-Time PCR kits (Real Time Kit for the Quantitative detection of HCV (Sacace Biotechnologies, Italy) for detecting HCV RNA viral loading. Consecutively, patients with high viral load were detected of HCV genotyping by using PCR technique (Thermo cycler, Cleaver Scientific, UK) by using a sensitive commercially RT-PCR kits (Sacace , Italy) according to the manufacturers' instructions, then PCR products were electrophorased in 2.5 % agarose gel and stained with ethidium bromide, The amplicon was observed and photographed by using gel documentation system. The DNA ladder was coelectrophorased to verify the size of the amplicon. The four genotypes or subtypes were distinguished from one another by the size of PCR products: 338 bp for subtypes 1a; 395 bp for subtypes 1b; 286 bp for genotype 2 and 227 bp for subtypes 3a. SPSS was used to analyze data. Data of this study was analyzed by the ANOVA and chi square tests. Quantitative variables were expressed as min.-max. (Mean±SD).A p value $\leq 0.05$ was considered statistically significant.

## III. RESULTS AND DISCUSSION

Hepatitis C virus infection has reached epidemic proportions. Worldwide, more than one million new cases of infection are reported annually, and HCV is believed to be more prevalent than hepatitis B virus infection (**10**). Of 102 patients, the present results showed that 49 (48.04%) and 53 (51.96%) were males and females respectively. The mean age of patients was 28.77±13.58 years. A few factors associated with outcome of HCV infection. These include age at infection, gender, genotype/subtype, viral load, and mode of infection (**11**). Some authors consider that female gender is associated with higher rates of viral clearance (40% in women compared to 19% in men) (**12**). Most sera containing anti-HCV antibodies are also HCV PCR-positive, indicating that these antibodies are markers of ongoing infection and do not correlate with resolution or clearance of infection. The ability of serum antibodies to recognize all HCV quasispecies is restricted, and neutralizing anti-HCV antibodies have not yet been identified (Walker CM 1999). The results of present study (Table, 1; Figure 1, 2 and 3) showed high prevalence of 1a genotype (37.25%), then 1b genotype (17.65%), also first recorded in Iraq, 2 genotype (4.90%), while 3a genotype has 0.98%. Furthermore, these study determinate a mixing genotypes (14.7%).

Reverse transcription polymerase chain reaction (RT-PCR or PCR): PCR is a laboratory method used to detect circulating HCV RNA in blood. PCR can be quantitative or qualitative, and under optimal conditions qualitative PCR can detect 100 international units (IU)/mL or less of circulating virus (**13**).Because the absence of viremia in anti-HCV-antibody positive patients is associated with little or no risk for HCV infectivity (**14**). The HCV viral load also provides important information in relation to treatment success. In particular if the HCV viral load is <400,000 IU/ ml in a person with HCV genotype 1, treatment success approaches that for HCV genotype 2 and 3. The HCV viral load does not correlate with liver disease progression risk. While different genotypes and subtypes share basic biological and pathogenic features, they differ in terms of response to treatment and epidemiology (**15**). A relationship has been suggested between HCV type, subtype, and serum HCV RNA levels (**16**). Genotype 1 is the most prevalent genotype worldwide, with a higher proportion of subtype 1b in Europe and 1a in the USA. Genotype 3a is highly prevalent in the European population of people who inject drugs (PWID). This group is currently experiencing an increasing incidence and prevalence of infections with HCV genotype 4. Genotype 2 is found in clusters in the Mediterranean region, while 5 and 6 are rare in Europe (**17**). The novel genotype 7 was identified in patients from Canada and Belgium, possibly infected in Central Africa (**18**). Genotype la, initially described as an American variant, is distributed worldwide. So is genotype lb (Japanese variant), the most widespread worldwide, and so are types 2 and 3. Genotype 4 is mainly found in Africa and the Middle-East. Type 5 has mainly been isolated in South Africa and Central Africa. Type 6 strains are essentially distributed in the Far East (**19**). Several studies have shown that infection with genotype 1, with a prevalence of 70–80% in most western countries, is associated with a poor response to IFN-α therapy (**20**). According

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to this explanation, HCV genotype 1b is a marker for more severe HCV-associated liver disease, because it reflects a longer time of infection rather than a more aggressive form of hepatitis C virus (**21**).The HCV genotypes do not seem to be directly implicated in the progression of liver disease, although specific genotypes are associated to distinct histopathological manifestations (**22**). According to distribution of HCV genotypes on studied groups (Table ,2) the results showed high prevalence of 1a genotype in digestion system patients (14.71%), hemodialysis unit patients (11.76%), oncology children/child hospital (5.88%) and Center of hereditary blood disease patients (4.90%), then 1b genotype (9.80%) in digestion system patients , (3.92%) in hemodialysis unit patients, (0.98%) in oncology children/child hospital and (2.94%) in Center of hereditary blood disease patients. Also a 2 genotype was found only in digestion system patients (3.92%), hemodialysis unit patients (0.98%). while 3a genotype has 0.98% in digestion system patients only. Furthermore, these study determinate mixing genotypes (14.7%). Patients with HCV genotype 1a tend to have higher relapse rates than patients with HCV genotype 1b with certain regimens. Genotype 1 HCV infection that cannot be subtyped should be treated as genotype 1a infection. For HCV genotype 1a–infected, treatment-naïve patients, there are three regimens of comparable efficacy: ledipasvir/sofosbuvir (**23**). Several studies generally conclude that the transmission of the virus to hemodialysis (HD) patients is generally nosocomial with possible risk factors being failure to disinfect devices between patients, sharing of single-use vials for infusions, poor sterile technique, poor cleaning of dialysis machines, and poor distance between chairs (**24**). Epidemiological studies about HCV infection among HD patients in Iraq have reported a prevalence of 7.1%-62% in different cities (**25**). While the reported prevalence in the general population in Iraq is ranges from (0.2%) to (0.5%) (**26**), and is (8.18%) in blood bank (**27**). They coexist in various geographic locations with different prevalence and it has been suggested that this might lead to differences in pathogenesis, outcome of disease, responses to antiviral therapy and might induce different serological reactivates (**28**).

## IV. CONCLUSION

The predominant of HCV genotypes in Basra patients was genotype 1a followed by genotype 1b. HCV genotype determines the length, and soon the type, of treatment and likely response to current medications. Further studies are needed to better determine the possible role of genotype in the outcome of HCV-related liver disease.

**Table (1).The percent of HCV genotypes with distribution gender, age and Viral Load among hepatitis C patients**

| Genotypes | No. (%) | Gender (M/F) | Age(Years) | | Viral Load IU/ml | |
|---|---|---|---|---|---|---|
| | | | Range | Mean ±SD. | Min. _Max. | Mean ±SD. |
| **Single genotypes** | | | | | | |
| 1a | 38(37.25) | 16/22 | 5-72 | 26.29±19.05 | Less than 250_greater than 5E+7 | 83448178.37±200742545.5 |
| 1b | 18(17.65) | 9/9 | 6-75 | 35.5±18.62 | Less than 250_greater than 5E+7 | 47721197.11±482647571 |
| 2 | 5(4.90) | 2/3 | 10-47 | 22.8±12.61 | Less than 250_greater than 5E+7 | 21674050±23178465.36 |
| 3a | 1(0.98) | 0/1 | 24 | 24±0 | 298000 | 298000±0 |
| **Mixed genotypes** | | | | | | |
| 1b,3a | 6(5.88) | 3/3 | 8-65 | 35.16±21.91 | Less than 250_36400000 | 6086666.67±13556604.14 |
| 1a,3a | 6(5.88) | 3/3 | 12-50 | 30.5±15.1 | Less than 250_greater than 5E+7 | 12705125±20038962.85 |
| 1b,2 | 1(0.98) | 1/0 | 11 | 11±0 | Greater than5E+7 | 51000000 ±0 |
| 1b,2,3a | 2(1.96) | 0/2 | 26-60 | 43±17 | Greater than5E+7 | 79000000±41012193.31 |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Total mixed genotypes | 15(14.7) | 7/8 | 11-65 | 30.75±12.70 | Less than 250_greater than 5E+7 | 37197947.92±1865194008 |
| Unknown | 25(24.51) | 15/10 | 5-75 | 30.68±20.44 | Less than 250_greater than 5E+7 | 41428896.12±40268094.41 |
| P Value | P≤0.01 | | | | | P>0.05 |
| Final total | 102(100 ) | 49/53 | 5-75 | 28.77±13.58 | Less than 250_greater than 5E+7 | 89151345.92±91271604.06 |

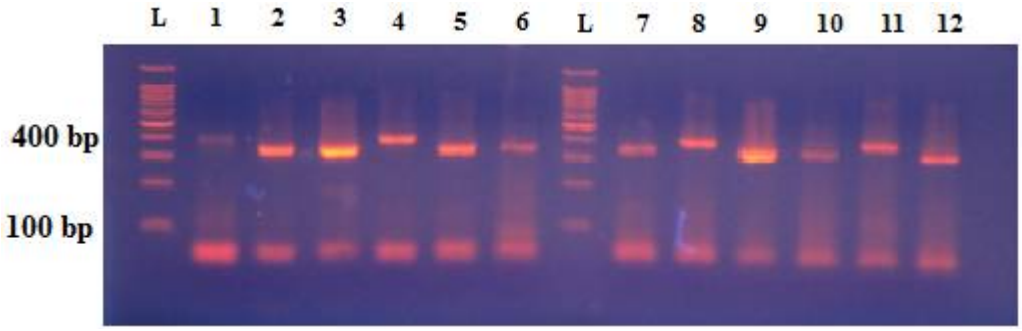


**Fig. 1 Electrophoresis of RT-PCR products**
**L=DNA Ladder; Lane 1=(Positive Control) cDNA 1b;Lane 4, 8, 11= 1b genotype ; Lane7=(Positive Control) cDNA 1a; Lane 2, 3, 5, 6, 9, 10, 12=1a genotype**

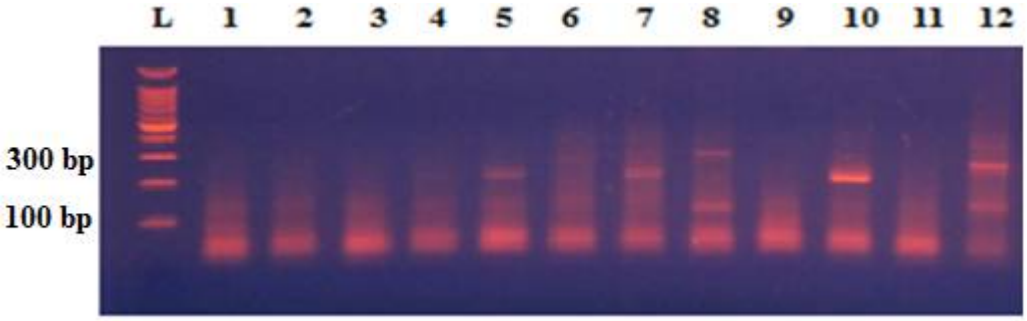


**Fig. 2 Electrophoresis of RT-PCR products**
**L=DNA Ladder; Lane 1, 2, 3, 4, 9, 11= unknown genotypes; Lane 5, 7, 10=3a genotype; Lane 6, 8, 12= 2 genotype**

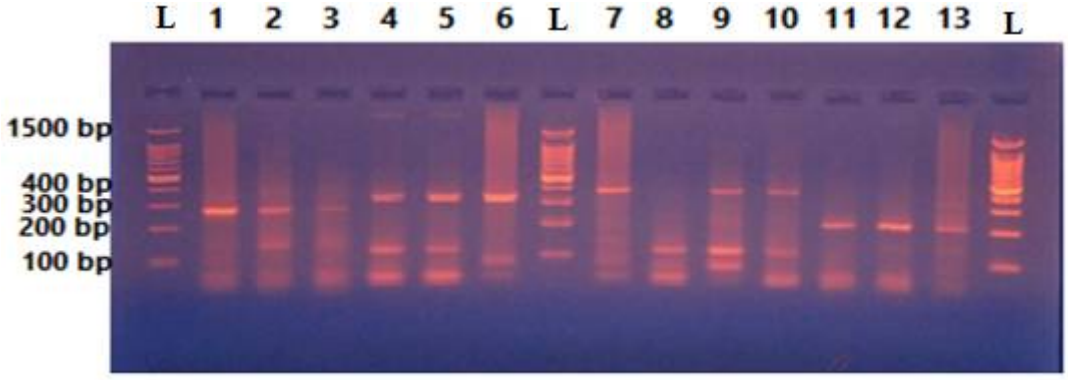


**Fig. 3 Electrophoresis of RT-PCR products**

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**L=DNA Ladder; Lane 1= (Positive Control) cDNA 2; Lane 2, 3 =2 genotype; Lane 4, 5 =1a genotype; Lane 6= (Positive Control) cDNA 1a; Lane 7=(Positive Control) cDNA 1b; Lane 8= unknown genotype; Lane 9, 10 =1b genotype; Lane 11, 12 =3a genotype; Lane 13=(Positive Control) cDNA 3a**

**Table (1).Distribution of HCV genotypes among studied groups**

| Studied groups | 1a No. (%) | 1b No. (%) | 2 No. (%) | 3a No. (%) | Mixing genotypes | | | | Unknown (%) | P Value | Total No. (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1b,3a | 1a,3a | 1b,2 | 1b,2,3a | | | |
| Gastrointestinal Patients (GIT) | 15 (14.71) | 10 (9.80) | 4 (3.92) | 1 (0.98) | 3 (2.94) | 3 (2.94) | 1 (0.98) | 1 (0.98) | 8 (7.84) | ≤ 0.01 | 46 (45.1) |
| HD Unit Patients | 12 (11.76) | 4 (3.92) | 1 (0.98) | – | 1 (0.98) | – | – | 1 (0.98) | 12 (11.76) | ≤ 0.01 | 31 (30.39) |
| Child Hospital Patients | 6 (5.88) | 1 (0.98) | – | – | – | 2 (1.96) | – | – | 3 (2.94) | > 0.05 | 12 (11.76) |
| Thalassemia Patients | 5 (4.90) | 3 (2.94) | – | – | 2 (1.96) | 1 (0.98) | – | – | 2 (1.96) | > 0.05 | 13 (12.75) |
| *P* Value | > 0.05 | ≤ 0.05 | >0.05 | | > 0.05 | >0.05 | | > 0.05 | ≤ 0.05 | | ≤ 0.01 |
| Total | 38 (37.25) | 18 (17.65) | 5 (4.90) | 1 (0.98) | 6 (5.88) | 6 (5.88) | 1 (0.98) | 2 (1.96) | 25 (24.51) | | 102 (100) |

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