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Article

Prevalence of Hepatitis C Virus Genotypes with Correlation of Viral Load and Liver Functions in Diwaniya City, Iraq

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Abstract: Aim of the study: Hepatitis C virus (HCV) exhibits significant genetic diversity, influencing disease progression and treatment outcomes. This study aimed to determine the prevalence of HCV genotypes and their association with viral load and liver function in patients from Diwaniya City, Iraq. Methods: A total of 96 HCV-positive patients were recruited from private clinics in Diwaniya City. Blood samples were collected for viral load and genotyping quantification using real-time PCR. Liver function tests, including alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and bilirubin levels were assessed using an automated system. Results: Genotype 1a was the most prevalent (64.6%), followed by 1b (25%) and 4a (10.4%). A higher viral load was observed in males, although this difference was not statistically significant. A significant variation in viral load was found among different genotypes. Correlation analysis revealed a non-significant positive association between viral load and Liver function tests. Conclusion: Genotype 1a is the predominant HCV genotype in Diwaniya City. While viral load may influence liver function, further investigation is warranted to confirm this observation. These findings contribute to a better understanding of HCV epidemiology and clinical management in the region

Keywords: hepatitis C virus, genotyping, viral load, liver functions

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1. Introduction

Globally, it is estimated that 1.3 million people died due to viral hepatitis in 2022, compared to 1.1 million in 2019 making it the second leading infectious cause of death, on par with tuberculosis. Furthermore, WHO reported that there are 50 million people live with chronic Hepatitis C (HCV) globally. It contributes to roughly 17% of deaths attributed to viral hepatitis, translating to around 221,000 deaths in 2022 (1). HCV is a positive-sense RNA virus of the family Flaviviridae belonging to genus *Hepacivirus*. HCV infection can cause acute hepatitis C and more than half of the cases is progressed to chronic cases which may developed to a chronic inflammatory disease, that could cause liver fibrosis, liver cirrhosis and finally hepatocellular carcinoma (2, 3). HCV spreads mainly through contact with infected blood. or medical procedures (3, 4). Molecular analysis confirmed that there are seven major HCV genotypes displaying antigenic variability and sub-classified into seventy-six recognized subtypes (1a, 1b, etc.) (5). HCV Response to treatment is different among genotypes, it seems that determination of hepatitis C genotype, as a key tool, is crucial in to control therapeutic regimen, for example Direct Acting Antiviral (DAA) therapies showed that HCV genotypes 1, 3 and 4 have high SVR rates (6). On the other hand, Evaluation of HCV viral load is not a main marker of disease severity; it could be major predictive marker of response to HCV antiviral therapy, low viral load may indicate

to High SVR rate (7, 8). Relationship between liver function with viral load may be act as a predictive marker to evaluate disease progression in hepatitis C patients. (9). Different studies investigate the prevalence of HCV genotypes in different regions in Iraq, however, the present study aimed to investigate the prevalence of HCV genotypes and association of viral load to liver functions in chronic Hepatitis patients in Iraq/ Diwaniyah city.

2. Methodology

Patients and sample collection: Sample collected from individuals who were clinically diagnosed as Hepatitis patients and that were serologically positive to HCV in private clinics of Diwaniya city during 2023. Information of Each patient was taken regarding with gender and age. Approximately, 5mL venous blood was taken, about 2 ml placed in EDTA tube for plasma extraction, the other placed in gel tube for serum extraction. Plasma and serum were extracted within 2 hours of blood collection through centrifugation.

HCV viral load: Gene Expert system (Cepheid/ U.S.A) used for measurement of HCV viral load using Xpert® HCV Viral Load kit (Cepheid/ U.S.A) begins with the collection of a blood sample, from which plasma were separated according to manufactures' instructions. Briefly, A total of 1000 μL of plasma is added to a sample cartridge containing reagents that lyse the virus releasing its genetic material. This cartridge is inserted into the Xpert® MTB/RIF system, which employs real-time polymerase chain reaction (PCR) to amplify the HCV RNA. Fluorescent probes specific to HCV RNA are utilized for the detection and quantification of the amplified viral nucleic acid. The system generates a quantitative result expressed in copies of HCV RNA per milliliter (copies/mL). Results are interpreted according to pre-established clinical thresholds, with undetected results indicated for viral loads below 4 IU/mL (0.6 log10 IU/mL), detected for loads below 10 IU/mL (1.0 log10 IU/mL), and quantifiable results for loads between 1.0 log10 IU/mL and 8.0 log10 IU/mL. The upper limit of quantification is set at above 108 IU/mL (8.0 log10 IU/mL).

HCV Genotyping: Sacace HCV Real-TM Qual Dx PCR Kit/ Italy was used to detect and differentiate HCV genotypes qualitatively according to manufactures' instructions. In brief, Four PCR tubes were prepared for each sample and labeled accordingly. Each tube received 15 μL of the master mix, after which 10 μL of the respective samples were added: cDNA, negative control, positive control, and cDNA with the HCV genotype. The tubes were then sealed and loaded into a simple PCR machine. Timeline according to steps Specified temperature profile.

Liver Functions: Liver functions measured using Beckman Coulter AU480 Chemistry Analyzer/ Germany according to manufactures' instructions. Briefly, the appropriate reagents for the specific liver function are loaded into the designated positions on the analyzer. The analyzer is calibrated using standardized solutions to ensure accurate results. The prepared serum samples are loaded onto the analyzer's sample tray. 10 μL of serum and 200μL reagents are mixed in reaction cuvettes. The mixture is incubated to allow the chemical reactions to occur. The results are typically viewed and printed.

Statistical Analysis: - Data were analyzed and presented by using statistical package for social science (SPSS version 26) and Microsoft office Excel 2010. Numeric data were presented after performance of Kolmogorov's normality test to evaluate if the data is normally or not normally distributed, Mann-Whitney-U test used to compare between two means that not normally distributed. Kruskal Wallis Test used to compare between mor than means for data that not normally distributed. Correlation coefficient used to determine correlation between two numeric variables. Chi-square test was used to evaluate association between two variables that had categorical property. level of the significance is considered at 0.05 or less and highly significant level at 0.01 or less(10).

3. Results and Discussion

Demographic Characterization: A total of 96 HCV patients were enrolled in the present study. The study population comprised 41 (42.7%) males and 55 (57.3%) females, resulting in a male-to-female ratio of approximately 1:1.3. The age of participants ranged from (18 – 80) years, with a mean age of 49.35 years. Notably, the majority of patients (53.1%) were aged 50 years and older, indicating a higher prevalence of HCV infection among the elderly population, table (1). The present study included a relatively balanced gender distribution, with a slight female predominance. however, this result is compatible with other study in Iraq which stated that the male patients were 46.68% while female was 53.32%. (11) Conversely, other study in Iraq reported a slight predominance of male among chronic hepatitis patients, About 57 % (2). Notably, results indicated that higher prevalence of HCV infection among elderly population which may be due to delayed diagnosis, immune compression or Pre-existing conditions.

Genotyping of HCV: Genotyping of HCV revealed a diverse distribution, with genotypes 1a, 1b, and 4a being identified. Genotype 1a was the most prevalent, accounting for 64.6% (62/96) of cases, followed by genotype 1b (25%) and genotype 4a (10.4%). Statistical analysis indicated a significant association between HCV genotype distribution and gender ($p=0.001$). Additionally, a significant difference in genotype distribution was observed across age groups ($p<0.05$), table (1), figure (1). Response to treatment is different according to HCV genotypes, determination of HCV genotype is essential for successful treatment, as each genotype responds differently to antivirals. determination the specific genotype allows to precise treatment plans for optimal effectiveness, improving patient outcomes. In addition, understanding the prevalence of HCV genotypes within a population is crucial for public health efforts, enabling targeted interventions and the development of effective vaccines to combat this global health challenge (3). Our findings align with previous studies conducted in Iraq, which have consistently identified genotype 1a as the predominant circulating strain (12, 13, 14). However, it is noteworthy that other investigations within Iraq have reported genotype 4 as the most prevalent (11,15). This discrepancy underscores the potential for regional variations in HCV genotype distribution within the country. Furthermore, our observation of genotype 1a as the predominant strain is consistent with global trends. Genotype 1, particularly 1a, is recognized as the most widespread HCV genotype in regions of Meddle East like Iran and Palestine (16). It accounts for a substantial proportion of global HCV infections and exhibits a broad geographic distribution, encompassing regions such as North America, Europe, and South America (17). The observed significant differences in genotype distribution across genders and age groups within our study may be attributed, in part, to the relatively small sample size. However, it is crucial to acknowledge that these findings warrant further investigation with larger cohorts to establish robust conclusions regarding the influence of these demographic factors on HCV genotype distribution within the Iraqi population.

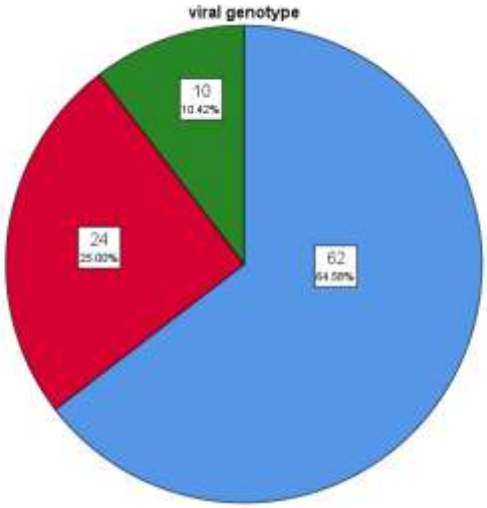

Figure 1. HCV genotypes in Diwaniya city

Tabel 1. Gender and Age Distribution of HCV Genotype in Diwaniya City

variable			viral genotype			Total	*P* value
			1a	1b	4a		
Gender	Female	Count	27	20	8	55	0.001* S**
		% of Total	28.1%	20.8%	8.3%	57.3%	
	Male	Count	35	4	2	41	
		% of Total	36.5%	4.2%	2.1%	42.7%	
Total		Count	62	24	10	96	
		% of Total	64.6%	25.0%	10.4%	100.0%	
Age group	<20	Count	4	0	1	5	0.000* S**
		% of Total	4.2%	0.0%	1.0%	5.2%	
	20-29	Count	2	0	7	9	
		% of Total	2.1%	0.0%	7.3%	9.4%	
	30-39	Count	12	3	0	15	
		% of Total	12.5%	3.1%	0.0%	15.6%	
	40-49	Count	13	2	1	16	
		% of Total	13.5%	2.1%	1.0%	16.7%	
	>50	Count	31	19	1	51	
		% of Total	32.3%	19.8%	1.0%	53.1%	
Total		Count	62	24	10	96	
		% of Total	64.6%	25.0%	10.4%	100.0%	

*= Chi square test with 0.05 significance level, **significant difference

Evaluation of HCV Viral Load: The mean viral load among HCV patients was found to be 6,137,333.33 IU/ml. Table (2) shows a comparison of viral load between male and female patients and revealed a mean of 11,377,000 IU/ml and 2,644,222.2 IU/ml, respectively. However, statistical analysis indicated no significant difference in viral load between the two groups (p=0.195). The viral load was higher more among males although statistically non-significant association, this finding was also recorded in northern area of Iraq by Bakir et al (13). Another study in China showed that the viral load of HCV in male group was higher than in female (18). This may be explained by finding that estrogens may have an antiviral effect, and their decline post-menopause could contribute to worsening HCV-related liver disease in older women. (3)

Tabel 2. Distribution of Viral load according to gender

gender	N	Mean	Std. Deviation	*P** value
male	41	11377000	19375908.7	0.224 NS**
female	55	2644222.2	4797179.322	
Total	96	6137333.33	9838266.8	

* Mann-Whitney U TEST with 0.05 significance level, **non-significant difference

The mean HCV viral load among patients infected with genotype 1a, 1b, and 4a was found to be 5,681,806.5 IU/ml, 199,500 IU/ml, and 5,914,500 IU/ml, respectively. Statistical analysis indicated a significant difference in viral load among these genotype groups (p=0.001). These findings suggest that different HCV genotypes may exhibit distinct viral load profiles, potentially influencing disease progression and treatment outcomes, table (3). This is agreed with studies in China and France that showed HCV genotype was significantly correlated with viral load (19-20)

Tabel 3. Distribution of Viral load according to genotypes

Genotype	**Mean**	**Std. Deviation**	**N**	***P** value**
1a	5681806.5	11621558	62	0.001 S**
1b	199500	100566.22	24	
4a	5914500	6736265.6	10	
Total	6137333.33	9838266.8	96	

* Kruskal Wallis Test with 0.05 significance level, **significant difference

Evaluation of liver function of HCV patients: The mean levels of total bilirubin, direct bilirubin, indirect bilirubin, AST, ALT, and ALP were found to be 1.7402 mg/dL, 0.4299 mg/dL, 1.3103 mg/dL, 59.947 U/L, 70.418 U/L, and 51.7239 U/L, respectively. table (4).

Tabel 4. Evaluation of Liver Function in HCV Patents

variable	**N**	**Range**	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Total Bilirubin	96	3.68	0.52	4.20	1.7402	1.07475
Direct bilirubin	96	1.15	0.15	1.30	0.4299	0.32433
In-Direct bilirubin	96	2.55	0.35	2.90	1.3103	0.77094
AST	96	119.8	17.7	137.5	59.947	26.8027
ALT	96	95.3	19.0	114.3	70.418	27.5064
ALP	96	221.0	43.2	264.2	109.084	51.7239

Statistical analysis showed that there are no significant differences in distribution of all liver testes of HCV patients in male and female groups. p values were 0.570, 0.511, 0.997, 0.686, 0.634 and 0.935 for AST, ALT, ALP, Total Bilirubin, In-Direct bilirubin and Direct bilirubin respectively, table (5). These findings suggest that gender may not be a significant determinant of liver function test abnormalities in HCV-infected individuals. The generalizability of these findings may be limited by the sample size of the study. Larger studies with more diverse populations are needed to confirm these observations. In addition, the study did not account for the stage or severity of liver disease among the patients. Liver function tests can vary significantly depending on the extent of liver damage, which may be influenced by factors other than gender.

Tabel 5. Distribution of Liver Function Tests in Male and Female Groups

Variable	Gender	N	Mean	Mean Rank	*P* Value*
AST	Male	41	59.0	46.63	0.570 NS**
	Female	55	60.7	49.89	
	Total	96	59.947		
ALT	Male	41	67.9	46.34	0.511 NS**
	Female	55	72.3	50.11	
	Total	96	70.418		
ALP	Male	41	109.0	48.49	0.997 NS**
	Female	55	109.2	48.51	
	Total	96	51.7239		
Total Bilirubin	Male	41	1.82	49.83	0.686 NS**
	Female	55	1.68	47.51	
	Total	96	1.7402		
In-Direct Bilirubin	Male	41	1.37	50.06	0.634 NS**
	Female	55	1.26	47.34	
	Total	96	1.3103		
Direct Bilirubin	Male	41	.45	48.77	0.935 NS**
	Female	55	.42	48.30	
	Total	96	.4299		

* Mann-Whitney U Test with 0.05 significance level, **non-significant difference

Association of viral load and liver tests: To assess the association between viral load and various biochemical parameters, correlation analysis was performed. The results indicated a non-significant positive correlation between HCV viral load and AST, ALT, ALP, total bilirubin, indirect bilirubin, and direct bilirubin, table (6).

Tabel 6. Association of viral load and liver parameters in HCV patents

value	AST	ALT	ALP	Total Bilirubin	Direct bilirubin	In-Direct bilirubin
R*	0.147	0.24	0.086	0.165	0.164	0.161
*P***	0.601	0.389	0.759	0.556	0.56	0.566

*Correlation coefficient, **P value at 0.05 significance level

A positive correlation suggests that as HCV viral load increases, the levels of these biochemical markers tend to increase as well, albeit not significantly. A study showed that liver dysfunction is associated with the HCV-RNA levels. another study indicated that HCV nucleic acid titers were higher in chronic HCV patients and cirrhosis significantly, compared to patients with mild histological changes. (21, 22). This may be due to liver inflammation or damage viral replication. However, Researches indicated that HCV viral load is not significantly associated with the elevated some liver biochemical parameters (23, 24).

4. Conclusion

This study found that genotype 1a is the most common HCV strain in Diwaniya City, Iraq. Our findings suggest a possible link between viral load and liver function, although

further research is needed to confirm this. These results have implications for treatment strategies and highlight the importance of continued HCV surveillance in the region.

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