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Frequency distribution of hepatitis B virus (HBV) genotypes in Iraqi patients

Laith A.I.K. Al-Kaif
Department of Microbiology, College of Medicine, University of Babylon, Babylon, Hilla, Iraq and Dept. of Medical Laboratory Techniques, Al Mustaqbal University College, Babylon province, Iraq.
Email: laith1992.alkaif@gmail.com

Alaa H. Al-Charrakh
Dept. of Microbiology/ College of Medicine / University of Babylon, Hilla, Iraq
ORCID: http://orcid.org/0000-0002-6533-6395
Corresponding author email: ahani67@gmail.com

Mohammad A. K. Al-Saadi
Dept. of Microbiology/ College of Medicine / University of Babylon, Hilla, Iraq
Email: mbmc.kadhum70@gmail.com

***Abstract*---**Infection with the hepatitis B virus can lead to asymptomatic carrier status, developing into severe chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Ten genotypes (A-J) and 46 sub-genotypes have been discovered. As a result, the current study was conducted to determine HBV genotypes in Iraqi patients. RT-PCR was used to determine the highest viral load in infected subjects and then used for screening HBV genotyping. The results showed that HBV genotype distribution among HBV infected patients was as follows: genotypes C, A, B, and D (8.5%, 5%, 4.3%, and 2.1%, respectively). In addition, the value of the viral load was higher with genotype C compared to A, B, and D HBV genotyping. This study concluded that mono-genotypes prevalent in patients infected with HBV and genotype C were more prevalent. In addition to the existence of a relationship between viral load and genotyping.

***Keywords*---**Liver infection, HBV, Viral load, HBV genotyping, RT-PCR.

Introduction

Acute hepatitis B virus (HBV) and other manifestations of chronic liver disease may be caused by molecular characteristics of the virus, which account for a

significant percentage of liver diseases. A decrease in functioning liver cells is linked to chronic liver disease (CLD). As a result, medicines that undergo hepatic metabolism will be less effective in CLD patients [1, 8]. The cytochrome P450 (CYP) enzyme is the primary metabolizer of most medicines [2-6].

Several earlier investigations have shown that liver illness has a varied influence on CYP activity [4, 7]. HBV is a double-stranded circular DNA genome with a size of about 3200 base pairs (bps) that belongs to the Hepadnaviridae family. Unlike that of other DNA viruses, HBV replication includes a crucial reverse transcription step. [8, 9]. This step involves RNA-dependent DNA polymerase, lacks proofreading, and error-prone viral replication. The formation of genotypes and sub-genotypes is influenced by a crucial molecular component of error-prone replication. Genotype I is a unique tri-recombinant of genotypes A, C, and G that serves as proof. Genotype J resembles gibbon genotypes and human genotype C [10, 11]. As a result, understanding the impact of HBV genotype evolution on the pathophysiology and outcome of HBV infection requires a comprehensive molecular investigation [11].

The phylogenetic analysis of the entire viral genome is used to classify HBV. The degree of nucleotide divergence over the entire genome is a molecular criterion for genotype and sub-genotype classification. As a result, HBV genotype prevalence differs by region. Ten genotypes (A-J) and 46 sub-genotypes have been identified to date. [A (1-6), B(1-5), C(1-16), D(1-9), E, F(1-4), G, H, I(1-2), and J], [12, 13]. Whole-genome sequencing followed by phylogenetic analysis is a gold standard for HBV genotyping, identifying predominant, novel, and recombinant genotypes [14]. Identifying HBV genotype is essential for many reasons, including epidemiological studies and its primary distribution in different regions. Variations in genotype distribution can be seen between countries and within countries. Furthermore, there are links between clinical outcomes and patient treatment efficacy. [15].

In Iraq, many studies were conducted to determine the epidemiological status of the disease, considering that Eastern Mediterranean Region (EMR) countries have intermediate endemicity of viral B hepatitis with carrier rates of 2% to 5% in their general population [16]. In Basrah, a study on blood donors in 2013 showed that 2.3% of them had serological evidence of hepatitis B virus infection. In 2016 the percentage was 2.1 [17]. Another study on blood donors in Babylon governorate showed a seroprevalence of 0.7%. In a survey of the general Iraqi population, the occurrence was 1.6% [18]. In a study conducted in Najaf governorate about screening of blood donors for the detection of hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV), and HIV antibody. The results showed that the prevalence of HBV, HCV and HIV infection among 1305 blood donors (during 2017-2018) were 3%, 0.5%, and 0.06% respectively. Most of the seropositive donors were HBV positive (84.3%) and only (13.9%) were HCV positive [19].

The aim of this study was to identify hepatitis B virus genotypes in Iraqi patients as well as to determine if there is any relationship between viral load and virus genotyping.

Materials and Methods

Design study and participants

A hundred and forty-one patients were enrolled in this study who admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad Medical City, Center of artificial Kidney, and Center of Hepatology and Gastroenterology Hospital in Marjan Medical City, Babylon province during the period extending from March 2021 to June 2021. They included 80 males and 61 females. The patients in this study were aged 13-76 years old, divided into young, middle-aged adults, and the elderly.

Ethical Approval

The research was carried out by the ethical principles outlined in the Declaration of Helsinki. Before the sample was taken, the patient gave their verbal consent. Furthermore, the Babylon and Baghdad Health Directorate and the committee on publishing ethics at the College of Medicine, University of Babylon, Iraq, examined and approved the study protocol and patient permission forms under reference number BMS 0298 016.

Samples collection

After blood collection in the EDTA tube, these specimens were spun at 2500 r.p.m. for 15 minutes. Then, separated plasma specimens were collected, distributed in 1-1.5ml quantities in sterile containers (Eppendorf, size 1.5 ml), labeled, and stored at - 20 °C until used.

DNA extraction and Detection of HBV genotyping

This method was introduced using the viral DNA purification kit provided by the manufacturer (Bio-comma limited, 518118P.R. China). After determining the viral load value for subjects with HBV, HBV genotyping was performed according to the HBV Real-Time PCR kit to detect and differentiate hepatitis B virus genotypes A, B, C, and D in HBV-positive clinical material supplemented by the manufacturing company (Sacace 22100 – Como – Italy).

Results and Discussion

The database of characteristics of the study population are illustrated in (Table 1). The HBV genotypes were investigated among 60 out of 141 patients using RT-RCR (Table 2). Eighty-one patients were not tested by RT-PCR genotyping for HBV selectively as it had low viral load values in the plasma of the tested patients. HBV genotypes distributions found a prevalence among HBV infection patients with genotypes C, A, B, D (8.5%, 5%,4.3%, 2.1%, respectively).

(Table 3) shows HBV viral load. The current results showed a significantly different HBV viral load between HBV genotyping. From the results above and according to previous studies in neighboring countries, HBV genotype D appears to be the only or dominant type in Jordan, Iran, Syria, Saudi Arabia, and Turkey

[20]. In addition, HBV genotypes have a distinct geographic distribution. For example, Genotype D is found worldwide, but it is most common in the Mediterranean, Middle East, and southern Asia [21-23]. Furthermore, host genetic factors, including Hepatitis B virus genotype, are widely viewed as the everyday basis of the different outcomes of HBV infection [24]. This is inconsistent with our study in which a difference was observed between the A, B, C, and D genotypes of the diagnosed individuals. Also, in Baghdad, disagreement with Ahmed [25] found that genotype D was the predominant genotype among chronic hepatitis B infection (80%).

Regarding gender, age groups, and their relationship to the distribution of HBV genotyping, according to our study, the distribution was recorded with respect to gender which is genotype A (2 male, 5 female), genotype B (4 male, 2 female), genotype C (8 male, 4 female), genotype D (3 male), while the distribution according to age was genotype A above 50 years, genotype B under 30 years and genotype C ranged from 35-50 years, while genotype D was the focus in the elderly.

The recent findings suggest that the affected person's gender and age group are important factors in their susceptibility to viral infection. Other studies have linked HBV infection with a considerable rise in HBV seropositivity among males and the elderly, in addition to relative genotype distribution. This has been attributed to changes in the infecting genotype as well as mutations in the B-cell and T-cell epitopes in the S-gene of the HBV genome that may contribute to breakthrough infections even among the vaccinated [26-30]. Differences in circulating genotypes, behavioral factors, and host genetic factors, as well as differences in the application of HBV management methods, could explain the differences between our findings and those of the previous study.

Gender is a well-known factor associated with acute and chronic HBV infection; it identified a sex difference in HBV viral load in families that had HBsAg-positive siblings, and the finding was that viral load generally higher in males than females siblings, suggesting that sex plays an important role in HBV viral load [31].

The high viral load level in a patient over 50 years old can be explained by the elderly patient's immune system being weaker, allowing the virus to replicate efficiently. In 2019, a study of multiple viruses demonstrated a persistent infection by evolving evasion mechanisms of the host immune system, certain viruses can establish latency at low levels of viral replication and also be reactivated to cause devastating symptoms in the absence of appropriate immunity. So aging, a complex biological process, results in profound alterations in the immune system, and these changes can accumulate to produce a progressive deterioration in the ability to respond to pathogens and develop proper and durable immunity after vaccination [32].

Interestingly, in the cases that can be associated with SARS-CoV-2, the mechanisms of HBV reactivation are mostly due to a disrupted equilibrium between the host's immune system and viral replication. Therefore, the intensity of glucocorticoids or immunosuppressive medications and the host's baseline

virological markers is a primary risk factors for HBV reactivation [33-35]. Although HBV reactivation is possible with SARS-CoV-2 infection, the overall risk is modest to low. Therefore, the HBV genotype is not affected by infection or a slight change as a result of SARS-COV-2 infection or when some treatments are used.

Differences in pathogenicity between HBV genotypes are now partially recognized. HBV DNA levels inside cells and HBV DNA and HBeAg levels outside cells were found to be greater in genotypes B and C than in genotypes A and D. HBV DNA and viral antigens accumulated intracellularly may have a role in the development of cellular damage in hepatocytes [36, 37]. Furthermore, genotype C was a high replication capability that could cause increased genotype-related severe liver damage. Through *in vitro* studies, the following was seen [37]: First, Intracellular HBV core protein expression was raised when a pre-core (PC) or basal core promoter (BCP) region mutation altered HBeAg expression in genotype C. Second, Intracellular HBV surface protein expression was lower in PC wild-type HBV genotype C patients than in HBV genotype B patients. Third, in PC-mutant patients, extracellular HBV DNA was lower. Fourth, HBsAg production was minor in HBV genotype C than in genotype B. Fifth, in HBV genotype B, HBeAg secretion was lower than in genotype C.

The fundamental conclusions shown by previous studies are about the relationship between HBV genotypes, disease severity, and HCC development. Genotype A is more severe than genotype D and highly prevalent in the asymptomatic group and D in liver cirrhosis. Genotype B is an independent factor for HBeAg seroconversion, associated with higher HBeAg loss. Genotype C is associated with HBe Ag positivity, and the prevalence of genotype C increases from asymptomatic (AS), chronic hepatitis (CH), liver cirrhosis (LC) to hepatocellular carcinoma (HCC), in contrast to genotype B. in addition to it is associated with HCC. While Genotype D is more related to acute infection than to A. Genotype D was associated with higher HBV recurrence and mortality after transplantation than genotypes A and C. Death resulting from liver complications was more common in genotype F patients than in A and D [38-40].

Our results were incompatible with Ni *et al.* [41], who found no difference in the baseline viral load between HBV genotypes in 460 HBV carrier children but agreed with both Lindh *et al.* [42] and Prasad *et al.* [43], who found a significant correlation between a very high level of viral load and patients with HBV genotype.

Conclusion

This study concluded that the prevalence of mono-genotypes in patients infected with HBV and genotype C was more prevalent. In addition to the existence of a relationship between viral load and genotyping. Therefore, we recommended conducting a broad study of genotypes in Iraq for affected people to know the prevalence of the most common genotype, as well as management of the clinical case and the use of appropriate treatments according to each genotype, especially for patients with progressive stages or coinfection or infected with autoimmune diseases.

Conflict of interest
The authors declare that there is no conflict of interest.

Authors' Declaration
As a result of this, the authors declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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Table 1: Database characteristics of the HBV infected patients involved in the study

Variable	Frequency (%)
Patient No.	141 (100%)
Gender	
Male	80 (56.74%)
Female	61 (43.26%)
Age groups	
Young (< 30)	46 (32.6 %)
Adult (30 - 60)	84 (59.6 %)
Elderly (> 60)	11 (7.8 %)
HBV contact	
House	44 (31.2%)
Work	3 (2.1%)
Other	94 (66.7%)
Disease status	
Acute	27 (19.2%)
Chronic	100 (70.9%)
Chronic active	8 (5.7%)
Autoimmune	6 (4.2%)
Liver cirrhosis	5 (3.6%)
HBV Vaccinated	12 (8.5%)
Blood transfusion	6 (4.2%)
Other diseases	
Hemodialysis	1 (0.7%)
Diabetes mellitus	10 (7.1%)
Hypertension (%)	12 (8.5%)
SARS-CoV-2	115 (81.6%)
Leukemia	1 (0.7%)
HCV	3 (2.1%)
Thalassemia	1(0.7%)
Kidney disease	1(0.7%)
Lymphadenopathy	1(0.7%)
Surgery	1(0.7%)

Table 2: The patient's HBV Infection Distribution according to HBV genotyping

HBV genotypes	Frequency	%	P. value
Not detected	81	57.4	
Genotype A	7	5.0	
Genotype B	6	4.3	
Genotype C	12	8.5	< 0.001
Genotype D	3	2.1	
Undetermined	32	22.7	
Total	141	100.0	

Table 3: Comparison of genotyping with viral load in HBV infected patients

HBV Genotyping	Frequencies	Viral load	
		HBV IU/ml	
		Mean ± SD	P-value
Not detected	81	224.37 ± 99.59	
Genotype A	7	777.14 ± 100.16	
Genotype B	6	832.83 ± 115.23	
Genotype C	12	1520.58 ± 798.92	< 0.001
Genotype D	3	737.67 ± 56.96	
Undetermined	32	214.25 ± 65.20	
Total	141	396.64 ± 456.78	