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Hepatitis B virus genotypes among chronic hepatitis B patients from Baghdad, Iraq and their impact on liver function

Rana T. Mohsen[a], Raghad H. Al-azzawi[a], Ali H. Ad'hiah[b,*]

[a] Department of Biotechnology, College of Science, University of Anbar, Ramadi, Iraq
[a] Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq
[b] Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

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ABSTRACT

Hepatitis B virus (HBV) infection is a significant global health problem. Populations of different ethnicities show great heterogeneity in HBV genotype frequency distributions. A cross-sectional study was conducted during June–October 2018 to determine frequency of HBV genotypes among chronic HBV patients from Baghdad, Iraq. The method of detection was nested polymerase chain reaction system. Further, the study assessed the impact of HBV genotypes on serum level of liver-function tests: total serum bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. Eighty chronic HBV patients were enrolled in the study. Six HBV genotypes were identified (A, B, C, D, E and F). The most frequently encountered genotypes were B and F (72.5% each), while E genotype was the least observed with frequency of 12.5%. In addition, the majority of patients (77.5%) showed mixed HBV genotypes (two, three, four or five) with different genotypic combinations. Single HBV genotype patients represented only 17.5%. When patients positive for mixed HBV genotypes were inspected for serum level of liver-function parameters, only alkaline phosphatase showed a significant difference. It exhibited a gradual increase median that positively paralleled the number of positive genotypes; patients positive for one genotype showed the lowest median (87.0 U/L), while patients positive for five genotypes demonstrated the highest median (130 U/L). In conclusion, the HBV genotypes B and F were the most frequent among the investigated sample of Iraqi chronic HBV patients from Baghdad.

1. Introduction

Hepatitis B virus (HBV) infection is a global health challenge. It has been estimated that 257 millions of humans are infected with the virus, resulting in an estimated annual mortality of 887,000 due to liver cirrhosis and/or hepatocellular carcinoma (Schweitzer et al., 2015; WHO, 2017a). A community-based study in Iraq conducted on 9610 household reported a prevalence of 1.6% for hepatitis B surface antigen (HBsAg). In addition, the anti-hepatitis B core antigen (HBc) and anti-HBs antibodies were reported at prevalence of 9.7 and 17%, respectively (Tarky et al., 2013). Accordingly, the endemicity of HBV in Iraq was considered to be low/intermediate level.

Molecular evolutionary analysis of HBV-DNA sequences revealed a classification of ten genotypes designated A–J, with > 8% of genetic variability (Choga et al., 2019). These genotypes show heterogeneous global frequency distributions. Specifically, A genotype is very common in sub-Saharan and Western Africa and Northern Europe, whereas B and C genotypes occur more frequently in Asia. D genotype is predominantly detected in Africa, Mediterranean countries, India and Europe, while E genotype is well-presented in central and Western Africa. F genotype is predominantly observed in Latin America and Alaska. G genotype is recorded in Germany, France and the United States of America. H genotype is most frequently observed in Central and South America. Finally, the genotypes I and J have been identified in Vietnam and Japan, respectively (Sunbul, 2014; Tian and Jia, 2016; Velkov et al., 2018). Among Iraqi HBV patients, D genotype was the most frequently encountered (reviewed by Al-mhanah, 2018).

Assessing the role of HBV genotypes in the outcome of infection revealed inconsistent observations. However, an increasing number of studies demonstrated that progression and severity of liver disease, seroconversion rate and outcome of antiviral medication are impacted by the virus genotype (Kmet Lunaček et al., 2018; Mojsiejczuk et al., 2019). In addition, HBV genotypes may influence liver functions (Kumar et al., 2011). Accordingly, there is an increased necessity to

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBc, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; *p*, probability; TSB, total serum bilirubin

* Corresponding author at: Tropical-Biological Research Unit, College of Science, University of Baghdad, Al-Jadriya, Baghdad, Iraq.
E-mail address: dr.ahadhiah@sc.uobaghdad.edu.iq (A.H. Ad'hiah).

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determine HBV genotype frequencies in populations of different geographical regions and ethnicities. Therefore, the present study aimed to determine HBV genotype frequencies in chronic HBV patients living in Baghdad. A correlation of these genotypes with gender and age of patients was also conducted. Further, the impact of HBV genotypes on liver-function parameters was also explored.

2. Patients and methods

2.1. Patients

A cross-sectional study was conducted during June–October 2018 to assess HBV genotypes in Iraqi patients with chronic HBV infection. The Ethics Committee at the Department of Biology (University of Baghdad) and the Iraqi Ministry of Health approved the study protocol (Reference: BEC/1019/002). A written informed consent was taken from all participants. The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). The WHO and European Association for the Study of the Liver (EASL) guidelines on HBV testing were followed (Lampertico et al., 2017; WHO, 2017b). Eighty chronic HBV cases (50 males and 30 females) were recruited from the Specialized Center for Gastroenterology and Hepatology in Baghdad. The mean age of patients was 40.7 years (standard deviation = 13.8).

2.2. Laboratory tests

Sera of patients were qualitatively screened for anti-HBc IgM, -HBc IgG and -HbsAg antibodies using ELISA kits (MyBioSource, USA). The sera were also quantitatively assessed for total serum bilirubin (TSB), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using commercial kits (Linear Chemicals, Spain). Instructions recommended by the manufacturers were followed.

2.3. HBV genotyping

DNA was isolated from whole blood using a rapid blood genomic DNA extraction kit (Bio Basic, Canada) employing standard procedure recommended by the manufacturer. The HBV genotyping was subsequently performed using specific nested polymerase chain reaction (PCR) system previously described by Naito et al. (2001). This PCR detection system identifies six major genotypes of HBV (A, B, C, D, E and F). The isolated DNA was subjected to two rounds of nested PCR cycling. In the first round, universal primers (P1: sense primer and S1–2: antisense primer) were used to amplify pre-S1 through S genes of HBV. The PCR reaction mixture (20 μL) consisted of 2 μL for each of 10 pmol forward and reverse primers, 11 μL of free-nuclease water, and 5 μL of DNA. This reaction mixture was then combined with the lyophilized PCR master mix (AccuPower PCR Premix, Bioneer, Korea). PCR cycling was performed using initial denaturation step at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 20 s and elongation at 72 °C for 1.5 min. This was followed by a final elongation step at 72 °C for 5 min. The products of first round were subjected to a second round of PCR amplification. Two nested PCRs were set-up for each sample; Mix A for genotypes A, B and C, and Mix B for genotypes D, E and F. Each reaction consisted of a mixture of 20 μL containing 1 μL of the first-round PCR product, 1 μL of each of 10 pmol primers and 15 μL of nuclease-free water. This mixture was then combined with the lyophilized PCR master mix (AccuPower PCR Premix, Bioneer, Korea). The thermocycling conditions were 40 cycles consisting an initial denaturing at 94 °C for 5 min, 20 cycles of amplification at 94 °C for30 s, 58 °C for 30 s, and 72 °C for 40 s. This was followed by further 20 cycles of 94 °C for 20 s, 60 °C for 30 s, and 72 °C for 40 s and a final extension step at 72 °C for 5 min. The PCR products were electrophoresed on 2% agarose-gel at 5 V/cm^2 for 60 min and stained with diamond dye. The migrating PCR products were then visualized using gel-documentation system. The HBV genotypes were determined on the basis of the PCR product-size migration alongside a pattern of DNA ladders.

2.4. Statistical analysis

HBV genotypes were presented as percentage frequencies. Statistically significant differences were assessed using either two-tailed Fisher's exact probability (p) or Pearson Chi-square test. For liver-function parameters, the data were given as median and range. The differences were assessed using Kruskal–Wallis test. False discovery rate (FDR) was applied to correct the p-value due to multiple comparisons. A corrected p-value ≤ 0.05 was considered significant. All statistical analyses were conducted using SPSS statistical package (version 19.0).

3. Results and discussion

ELISA serum assessments for anti-HBc IgM, -HBc IgG and -HbsAg antibodies revealed that all patients were seropositive for anti-HBc IgG and -HbsAg antibodies and were seronegative for anti-HBc IgM antibody. Such profile is consistent with the diagnosis of chronic HBV infection (Loh and Kew, 2007; Lampertico et al., 2017).

Of the six detected genotypes, B and F were the most frequently encountered and represented 72.5% for each. This was followed by A and D genotypes (40 and 30%, respectively). The genotypes C and E were the less frequently observed (20.0 and 12.5%, respectively). The majority of patients (77.5%) exhibited mixed genotypes (two, three, four or five) with different genotypic combinations, whereas single-genotype patients represented only 17.5% of the sample (Table 1). Consistent with these findings, previous studies from Iraq reported that most of HBV patients had mixed genotypes. However, the genotype D was the most frequently encountered in these studies (reviewed by Al-mhanah, 2018); an observation that is not supported by the present results. In neighboring countries, the D genotype was also predominantly observed in HBV patients from Iran, Saudi Arabia, Syria and Jordan (Asaad et al., 2015; Nodeh et al., 2018). The high prevalence of B genotype is consistent with what has been commonly reported in other Asians populations such as Indonesia (Wahyuni et al., 2019). However, the high frequency of the F genotype was unexpected as it was predominantly reported in HBV patients of Latin America and Alaska (Velkov et al., 2018). None of the previous published Iraqi studies detected F genotype in HBV patients (reviewed by Al-mhanah, 2018). An exception was a study from Samara (a city located 135 km north the capital Baghdad), in which one case of chronic HBV infection (out of 87 cases) was reported to have the mixed genotypes C and F (Abdulrazaq and Al-azaawie, 2017). The unanticipated increased frequency of the F genotype especially in the population of the capital Baghdad can be attributed in part to the open human movement policy adopted by the Iraqi government during the last 15 years. In this context, the repatriations of thousands of Iraqi peoples living abroad in addition to the immigration of people of different ethnicities (Asians, Europeans and Americans) seeking better work opportunities in Iraq might have increased the probability of transferring the HBV-F genotype to Iraq. It was also observed that 5% of chronic HBV patients showed no detectable genotypes; although a band of 1063 bp (region of HBV *S* gene) was amplified in the first-round PCR. This is probably due the used PCR system, which is not designed to identify other genotypes (*i.e.* G, H, I and J) that require other sets of primers.

When patients positive for mixed HBV genotypes were inspected for serum level of liver-function parameters, only ALP showed a significant difference (p-value = 0.048). It exhibited a gradual increase median that positively paralleled the number of positive genotypes; patients positive for one genotype showed the lowest median (87.0 U/L), while patients positive for five genotypes demonstrated the highest median (130 U/L) (Table 2).

Table 1
Hepatitis B virus genotype numbers and percentage frequencies in patients distributed according to gender and age groups.

	Cases (N = 80)	Gender			Age group (year)			
		Male (N = 50)	Female (N = 30)	p_1-Value	< 40 (N = 32)	40–50 (N = 30)	> 50 (N = 18)	p_2-Value
HBV genotype; N (%)								
A	20 (40.0)	14 (28.0)	6 (20.0)	NS	8 (25.0)	7 (23.3)	5 (27.8)	NS
B	58 (72.5)	36 (72.0)	22 (73.3)	NS	24 (75.0)	25 (83.3)	9 (50.0)	NS
C	16 (20.0)	10 (20.0)	6 (20.0)	NS	9 (28.1)	3 (10.0)	4 (22.2)	NS
D	24 (30.0)	10 (20.0)	14 (46.7)	NS	8 (25.0)	8 (26.7)	8 (44.4)	NS
E	10 (12.5)	6 (12.0)	4 (13.3)	NS	3 (9.4)	3 (10.0)	4 (22.2)	NS
F	58 (72.5)	36 (72.0)	22 (73.3)	NS	25 (78.1)	23 (76.7)	10 (55.6)	NS
Not detected	4 (5.0)	4 (8.0)	0	NS	1 (3.1)	2 (6.7)	1 (5.6)	NS
Mixed genotypes[a]								
Two	30 (37.5)	16 (32.0)	14 (46.7)	NS	13 (40.6)	8 (26.7)	9 (50.0)	NS
Three	22 (27.5)	10 (20.0)	12 (40.0)	NS	7 (21.9)	8 (26.7)	7 (38.9)	NS
Four	6 (7.5) 5	4 (8.0)	2 (6.7)	NS	4 (12.5)	1 (3.3)	1 (5.6)	NS
Five	4 (5.0)	4 (8.0)	0	NS	2 (6.3)	2 (6.7)	0	NS

HBV: hepatitis B virus, N: number, NS: not significant (corrected p-value > 0.05), p: probability, p_1: males *versus* females, p_2: age groups comparison.
[a] Patients positive for more than one genotype.

Table 2
Liver-function parameter medians in hepatitis B virus patients distributed according to number of positive genotypes (mixed genotypes).

Genotype number	N	Median (range)			
		TSB (μmol/L)	ALP (U/L)	ALT (U/L)	AST (U/L)
Not detected	4	6.0 (5.3–6.7)	87.5 (85.0–90.0)	16.5 (11.0–22.0)	39.0 (36.0–42.0)
One (A, B, D and F)	14	6.0 (1.8–11.0)	87.0 (57.0–130.0)	17.5 (12.0–30.0)	44.0 (27.0–94.0)
Two (BC, BD, BF and EF)	30	7.2 (2.3–11.6)	90.5 (82.0–150.0)	19.0 (10.0–30.0)	42.0 (23.0–52.0)
Three (ABD, ABF, ACD, BCF, BDE and BEF)	22	6.6 (3.5–9.9)	98.0 (57.0–120.0)	21.5 (12.0–31.0)	45.0 (19.0–52.0)
Four (ABCF and BCDF)	6	7.5 (5.1–9.9)	96.5 (67.0–100.0)	19.0 (13.0–22.0)	43.0 (40.0–51.0)
Five (ABDEF and BCDEF)	4	6.2 (5.6–6.9)	130.0 (107.0–153.0)	14.5 (13.0–16.0)	29.0 (13.0–46.0)
p-value		NS	0.048	NS	NS

ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, N: number, NS: not significant (corrected p-value > 0.05) p: Kruskal–Wallis test probability, TSB: total serum bilirubin.

4. Conclusions

This study concluded that the HBV genotypes B and F were the most frequent among the investigated sample of Iraqi chronic HBV patients from Baghdad. These results contradict findings of previous studies in which the D genotype was predominantly observed. However, consistent with other studies, our findings showed great heterogeneity of the HBV genotypes per individual patient. This genetic heterogeneity might be driven by the recent influx of carriers of HBV people to Iraq. Such possibility would be better verified if the HBV genotypes are determined through DNA sequencing, and thus the phylogenetic tree can be constructed. However, it is important to emphasize that the findings of present study were based on a relatively small sample size, which limits its generalization to the broader community in Iraq. Further studies on larger sample sizes are required to confirm or refute the presented results.

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Author contributions

The three authors were equally contributed, and they were involved in designing the project, carrying out laboratory and data analysis, and writing manuscript.

Declaration of competing interest

None.

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