Iraqi JMS

Published by Al-Nahrain College of Medicine
P-ISSN 1681-6579
E-ISSN 2224-4719
Email: iraqijms@colmed.nahrainuniv.edu.iq
http://www.colmed-alnahrain.edu.iq
http://www.iraqijms.net
Iraqi JMS 2022; Vol. 20(2)

Hepatitis B Virus Genotypes and Pre-core and Core Genes Mutations in a Sample of Iraqi Patients with Chronic Hepatitis B Infection

Hiba T. Hussain[1]*PhD*, **Arwa M. Al-Shuwaikh**[2]*PhD*, **Abbas M. Ahmed**[3]*PhD*

[1]Division of Biotechnology, Dept. of Applied Sciences, University of Technology, Baghdad, Iraq, [2]Dept. of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq, [3]Central Public Health Laboratory, Baghdad, Iraq

Abstract

Background Genomic studies of hepatitis B virus (HBV) diversity are becoming increasingly significant to understand how HBV mutations interact with a wide spectrum of clinical and pathological disorders.

Objective This study focused on identifying HBV genotypes and determining the status of pre-core (PC) and core promoter (CP) mutants.

Methods Nested polymerase chain reaction was used to identify the viral genotypes of 100 patients with chronic HBV infection. Only 30 samples out of 100 were selected to determine the prevalence of mutations in the PC and CP by Sanger sequencing.

Results Over 95% of the samples had only D genotype and mixed genotypes 5% (B+C+D) in chronic hepatitis B (CHB) patients. G1898A, G1901A, G1910A and G1915A mutations in PC gene were found in total 15 out of 30 (50%) samples, which were distributed in the following proportions: 12 out of 25 (48%) in patients with hepatitis B e antigen (HBeAg) -ve, 8 samples with mutants at G1898A, G1901A, G1910A and G1915A (four mutations), 4 samples with mutants at G1898A, G 1901A and G1910A (three mutations). In addition, 3 out of 5 (60%) in patients with HBeAg +ve, while no type of any mutation was detected in the core gene in patients with chronic hepatitis type B.

Conclusion The genotype D was predominantly prevalent among HBV in CHB patients with 95% and with 5% in mixed genotypes (B+C+D), while genotype B and C were relatively less prevalent among CHB patients than genotype D. The mutations were found in the PC gene at nucleotides G1898A, G1901A, G1910A and G1915A, while no type of mutation was detected in the core gene.

Keywords Genotypes, pre-core mutation, core mutation, HBV

Citation Hussain HT, Al-Shuwaikh AM, Ahmed AM. Hepatitis B virus genotypes and pre-core and core genes mutations in a sample of Iraqi patients with chronic hepatitis B Infection. Iraqi JMS. 2022; 20(2): 217-225. doi: 10.22578/IJMS.20.2.8

List of abbreviations: CHB= Chronic hepatitis B patients, CP= Core promoter, HBeAg= Hepatitis B envelope antigen, HBV= Hepatitis B virus, PC= Pre-core

Introduction

Hepatitis B virus (HBV) has infected two billion individuals worldwide. About 3.5% of the world's population, or 257 million people, have a chronic infection that increases their chance of developing severe liver disease, cirrhosis, and/or hepatocellular carcinoma [(1)]. HBV is a member of the family Hepadnaviridae, specifically the genus Orthohepadnavirus [(2)]. Blood or other bodily fluids from an infected individual can spread the virus, making it extremely infectious. For a

minimum of seven days, HBV can survive outside of the body [2,3].

HBV genomes have been classified into genotypes ranging from A to J based on their genetic variability (>8% for the entire genome) [4-6]. Each genotype was subdivided into multiple sub-genotypes [7]. Within specific populations and geographic locations, HBV genotypes are distributed differently [8,9]. There is a correlation between genotype/sub-genotype and severity of disease [9]. Numerous HBV genotypes and genome mutations have been identified as being associated with the progression of liver diseases. Their clinical significance, on the other hand, is still debatable [10].

Negative test for hepatitis B e antigen (HBeAg) in chronic hepatitis type B (CHB) patients is frequently associated with pre-core and core promoter mutants. The pre-core variation with the greatest prevalence is a G/A transition occurs at nucleotide (nt) 1898 (G1898A) and (G1899A), resulting in the formation of a premature stop codon that prevents the synthesis of the HBeAg [11]. Pre-core and core mutations down-regulate HBeAg synthesis, HBeAg can be detected in chronic hepatitis B patients as a marker of productive infection, the absence of HBeAg indicates a reduction in viral replication and infection due to the immune response of the host, however, the absence of HBeAg does not necessarily indicate a decline in HBeAg levels and viral replication, but in some cases, it is only a means of evading immune clearance caused by mutation at the pre-core and core, which results in the creation of a premature stop codon and HBeAg synthesis is no longer possible.

So, the aims of this study were to identify HBV genotypes, determine pre-core and core mutations in HBV and their impact on the subsequent course of liver disease and HBeAg expression.

Methods

The study enrolled 100 CHB patients (68 males and 32 females) ranging in age from 4 to 70 years and attending Al-Imamein Al-Kadhimein Medical City in Baghdad, Iraq between July and November 2020. This study was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University. Serum samples were collected from patients to determine the presence of hepatitis B surface antigen (HBsAg), HBeAg, anti-HBe, and anti-HBc IgG using enzyme-linked immunosorbent assay (ELISA) kits (Sure Bio-Tech, Hong Kong). Patients were classified into HBeAg +ve 13 (13%) and HBeAg -ve 87 (87%) CHB patients based on their HBeAg status. Then the serum was used to extract the viral DNA for genotyping using sets of primers to detect genotypes (A-F) by nested polymerase chain reaction (PCR). In addition, conventional PCR was used to amplify the pre-core and core region using specific primers, gel electrophoresis of the PCR products, and DNA sequencing to determine the presence of the mutations.

DNA extraction and HBV quantification

Viral DNA extraction from serum sample was performed by using the Relia Prep™ Blood gDNA Miniprep System (Promega, USA), which provides a fast and simple technique for the preparation of purified DNA. The stages of the method are lysis of cells, binding of nucleic acid to column membrane, washing the bound nucleic acid and elution of the nucleic acid.

HBV genotyping

To determine the genotypes of HBV, we used primers and applied PCR. Two rounds were made, the first round using a pair of outer primers, and the second round a pair of inner primers. The primers used in the study were designed based on the conserved nature of the nucleotide sequences in the pre-S1 to S gene regions, regardless of the genotype of the HBV [12]. The primers P1 (forward) and S1-2 (reveres) were used as outer primers (1,063 bases). To determine the genotypes, B2 was used as an inner primer (forward) in a combination known as mixture A, including A, B, C. We used (reveres) primers (BA1R) as (type A), BB1R as (type B), and BC1R as (type C); all these primers were in the mixture A. As for the determination of the genotypes (D, E, and F), B2R as (reveres)

primers were used in a combination known as mixture B, (forward) primers were used, BD1 (type D), BE1 (type E) and BF1 (type F) and all these primers were included in the B mixture. Variation in bands sizes specific to genotype types were determined on the basis of primers in the second-round combination using the polymerase chain reaction technique. Table 1 shows the strategy used to determine the types of genotypes of HBV.

The first round of the PCR was carried out in a tube containing the following components: a colorless ready to use master mixture 12.5 µl, 1 µl for each external primer, 1 µl of extracted DNA and 9.5 µl of nuclease-free water. The thermal cycler was set to run one cycle at 95°C for 10 minutes, followed by 40 cycles at 94°C for 20 seconds, 55°C for 20 seconds, and 72°C for 1 minute. The second round of PCR was carried out for each sample using B2 forward primer with mixture A that includes types A to C and reveres primer (B2R) with mixture B to determine the types D to F. As in the first reaction, 3 µl of the product of the PCR produced from the first round were added to two tubes, each tube containing the second group of pairs of internal primers with an amount of 0.5 µl for each B2, BA1R, BB1R and BC1R in addition to 7.5 µl of nuclease-free water and master mixture 12.5 µl to detect mix A genotypes, and in second tube the same composition but with another pairs of internal primers B2R, BD1, BE1 and BF1 to detect mix B genotypes, then it was amplified for 40 cycles as follows, preheating at 95°C for 10 minutes and 20 amplification cycles at 94°C for 20 seconds and 58°C for 20 seconds and 72°C for 30 seconds and additional cycles at 94°C for 20 seconds and 60°C for 20 second followed by 72°C for 30 seconds.

Table 1. The primers used for detection of HBV genotypes and the size of PCR products [12]

	Primers	The size of PCR products
1st round PCR	P1(F)/S1-2(R)	900 bp
2nd round PCR Mix A	B2+BA1R	68 bp
	B2+BB1R	281 bp
	B2+BC1R	124 bp
2nd round PCR Mix B	BD1+B2R	120 bp
	BE1+B2R	167 bp
	BF1+B2R	97 bp

HBV mutations in the pre-core and core promoter regions

The mutations in the pre-core and core genes of HBV were investigated using nested PCR with a set of primers (F and R) as given in Table 2. The first round of PCR was done using a ready to use 25 µl Taq colorless master mix, 1 µl of each external primers (P1 and P2), 5 µl of DNA extract and 18 µl of nuclease-free water. The reaction was carried out in a thermal cycler for 36 cycles of 1 minute at 94°C, 1 minute at 56°C, and 3 minutes at 72°C, followed by a 7-minute extension step at 72°C. For the second-round PCR, 2 µl of the first-round PCR product was added to 25 µl master mix, 1 µl of each internal primer (P3 and P4) and 21 µl of nuclease-free water. Five microliters of the second-round PCR products were electrophoresed in a 1% agarose gel stained with ethidium bromide (0.5 µg/ml). The gel electrophoresis apparatus was turned on and set to 72 volts for one hour before being photographed under ultraviolet light. All required precautions against cross-contamination were taken, and each test contained negative controls (the same composition of reaction mix omitted the DNA sample and replaced with nuclease-free water).

Table 2. Primers sequences used for amplify pre-core and core regions [13]

Nested PCR	Primer	Sequences	PCR product size (bp)
External primers	P1(F)	5´-TCGCATGGAGACCACCGTGA-3´ (Positions 1604-1623)	450 bp
	P2 (R)	5´-ATA GCTTGCCTG AGTGC-3´ (Positions 2076-2060)	
Internal primers	P3(F)	5´-CATAAG AGGACTCTTGGACT-3´ (Positions 1653-1672)	340 bp
	P4 (R)	5´-GGAAAGAAGTCAGAAGGC-3´ (Positions1974-1957).	

Sanger sequencing analysis

Purification of amplified PCR products using Qiaquick spin columns (Qiagen Inc.) was performed according to the manufacturer's instructions. The purified PCR products were then sent to Macrogen Corporation – Korea for Sanger sequencing using an ABI3730XL automated DNA sequences. The results were received by email and processed with the help of the genius prime software (version 2021).

Statistical analysis

The statistical package for social science (SPSS) software (version 25) was used to conduct the descriptive analysis. Frequency and mean± standard deviation (SD) of continuous data was calculated.

Results

Results presented in this study showed the HBV genotypes distribution of the collected samples revealed that genotype D was the most common genotype among patients (95%) followed by mixed (B+C+D) genotypes (5%). However, genotypes A, E and F were not identified in any patient (Table 3 and Figures 1 and 2).

On comparing between 87 HBeAg -ve CHB and 13 HBeAg +ve CHB patients regarding HBV genotypes, revealed that genotype D was the most predominant genotype in HBeAg -ve patients (82%) followed by mixed infection with (D,B,C) genotypes (5%), while in HBeAg +ve patients, it was found that the genotype D percentage was (13%) and no any infected patient with mixed infection (B+C+D) genotypes (data not shown).

Table 3. Hepatitis B virus genotypes of hundred chronic hepatitis B patients

HBV genotypes	CHB patients	
	No.	%
Genotype D alone	95	95.0
Mixed infection (B,C,D)	5	5.0
Mixed infection (A,E,F)	0	0.0
Total	100	100

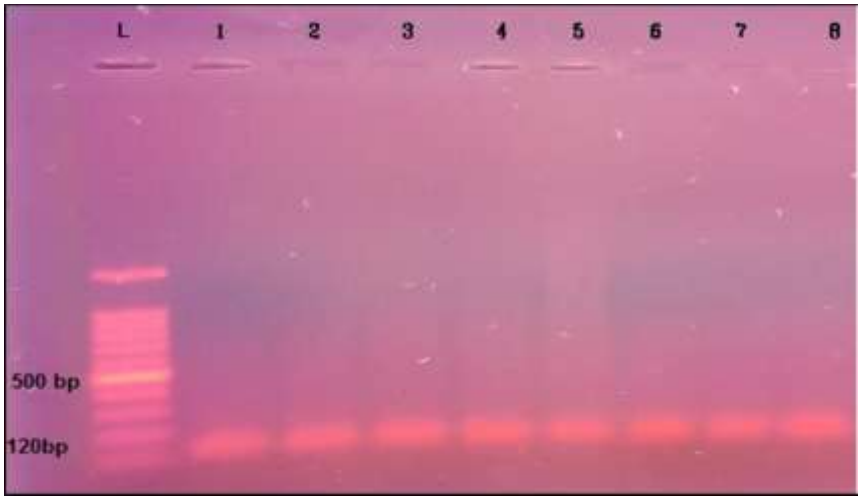

Figure 1. Eight chronic hepatitis B samples using gel electrophoresis was turned on and set to 72 volts for one hour, show amplification of mix B (120 bp genotype D) (L: 100-1000 pb ladder)

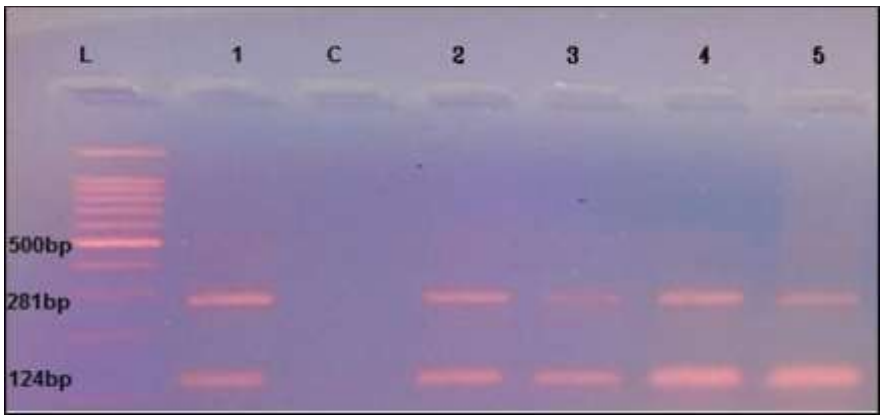

Figure 2. Gel electrophoresis on Mix A genotypes (2nd round) shows mix infection (genotype C, 124 bp) and (genotype B, 281 bp). C is negative control (no template DNA sample), L (100-1000 pb ladder)

Pre-core and core promoter mutants

G1898A, G1901A, G1910A and G1915A mutants were found in total 15 out of 30 (50%) samples. These mutations were detected in 12 of 25 (48%) of HBeAg -ve and 3 of 5 (60%) of HBeAg +ve samples. No mutations at core promoter positions were detected in this study, whether HBeAg -ve or HBeAg+ve. G1898A, G1901A, G1910A and G1915A mutants were distributed in the following proportions: 12 out of 25 patients (48%), 8 samples with mutants at G1898A, G1901A, G1910A and G1915A (four mutations), 4 samples with mutants at G1898A, G1901A and G1910A (three mutations) in HBeAg -ve patients, and 3 out of 5 (60%) in HBeAg +ve, as shown in table 4.

Patients with HBeAg -ve mutant viruses were older (30-65) years versus (20-48) years in HBeAg +ve. In comparison to HBeAg +ve patients, patients with HBeAg -ve had more mutations in the pre-core region at nucleotides G1898A, G1901A, G1910A and G1915A. While no mutations in the core region promoter at nucleotides A1762T and G1764A were detected in both groups, as shown in table 5.

HBV mutations and genotypes

In terms of genotypes, 25 of 30 patients (83.33%) were infected with HBV genotype D, whereas 5 of 30 patients (16.66%) were infected with HBV mixed genotype (B,C,D). In comparison to genotypes and mutations, this study found that genotype D-infected patients had more mutations at G1898A, G1901A, G1910A and G1915A than mixed genotype-infected patients. Additionally, demonstrated differences in age, sex, and the rate of HBeAg -ve and +ve between genotype D patients and mixed genotype (B+C+D) as shown in tables 4 and 5.

Table 4. The percentage of mutations in the pre-core and core promoter genes in 30 individuals with chronic hepatitis B virus with regard to HBeAg status and HBV genotypes

		Total	Mutation G1898A No. (%)	MutationG1901A No. (%)	MutationG1910A No. (%)	Mutation G1915A No. (%)	Mutation A1762T/ A1764G No. (%)
HBe Ag status	Positive	5	3 of 5 (60%)	3 of 5 (60%)	3 of 5 (60%)	3 of 5 (60%)	---
	Negative	25	12 of 25 (48%)	12 of 25 (48%)	12 of 25 (48%)	12 of 25 (48%)	---
Total		30	15 (50%)	15 (50%)	15 (50%)	15 (50%)	0
HBV genotypes	Genotype D alone	25	12 of 25 (48%)	12 of 25 (48%)	12 of 25 (48%)	12 of 25 (48%)	0
	Mixed (B,C,D)	5	3 of 5 (60%)	3 of 5 (60%)	3 of 5 (60%)	3 of 5 (60%)	0
Total		30	15 (50%)	15 (50%)	15 (50%)	15 (50%)	0

Table 5. The percentage of mutations in the pre-core and core promoter genes in HBeAg -ve and HBeAg +ve for 30 patients with chronic hepatitis B virus in relation to age and sex

		HBeAg (-ve) No. = 25 (83.33%)	HBeAg (+ve) No. = 5 (16.66%)
Age(yr)	mean±SD	33.92±1.44	24.6±1.22
Sex	(male/female) (n=30)	19M/6 F	3 M/2 F
Core(CP) mutation	A1762T	---	---
	A1764G	---	---
Pre core mutation	G1898A	12(48%)	3(60%)
	G1901A	12(48%)	3(60%)
	G1910A	12(48%)	3(60%)
	G1915A	12(48%)	3(60%)
HBV genotypes	Genotype D alone	20 (80%)	5 (100%)
	Mixed (B,C,D)	5 (20%)	0 (0%)

Discussion

The HBV genotypes distribution of the collected samples revealed that genotype D was the most common genotype among patients (95%) followed by mixed (B,C,D) genotypes (5%), while genotypes A, E and F were not identified in any patient, as shown in table 3. The foregoing findings are consistent with Khaled et al. [14] who reported that HBV infections were significantly linked to virus genotype D, which accounted for 87% of all patients, a high prevalence compared to mixed infections (D and F), which accounted for 13 percent of all samples. These results are not matched with those in Sulaimania, Iraq, where they reported that 100% of samples had mixed genotypes (25% with B,C,D genotypes and 75% with A,B,C,D genotypes)[15]. In Baghdad, one study reported that genotype D contributes to 80% of the infection, which is the most prevalent in CHB patients, and mixed genotypes F and D contribute to 20% of the infection [16]. These findings disagreed with Ali et al. [17] in Wasit (Iraq) who reported that there was no single HBV genotype infection and that 77.7% of their patients had mixed infection with five genotypes out of six. Also, another study in Baghdad that involved eighty CHB patients reported six HBV genotypes (A, B, C, D, E, F). The most frequently found genotypes were B and F (72.5% each), whereas the least frequently occurring genotype was E (12.5%). Therefore, they concluded that HBV genotypes B and F were the most prevalent in Iraqi CHB patients from Baghdad [18]. So, the results in this study appeared in agreement with some other results of studies conducted in Iraq and the same time are contradicted with others which conducted in Iraq as well. The reason is due to the difference in the number of samples used in each study, the method used in the diagnosis, where some studies relied on traditional methods using primers and some relied on the use of ready-made kits for diagnosis, and this is one of the reasons for the mismatch of results between studies within the same country.

In comparison to genotypes and mutations, this study found that patients with genotype D-infected patients had more frequency mutations at G1898A, G1901A, G1910A, and G1915A than patients with mixed genotypes. This was in disagreement with a study in Nigeria by Mbamalu et al. [19] who revealed that 11 (12.4%) of the 89 patients had genotype B, and 78 (87.6%) had genotype C, in addition, G1896A (23.6%), G1764A (9%), and A1762T (6.7%) were the most frequently occurring alterations. Pre-core mutations were found to be substantially more prevalent in genotype B patients than in genotype C patients (54.5% vs. 19.2%, p=0.01).

In this study in Iraqi CHB patients, the presence of mutations in the pre-core gene at the nucleotides (G1889A, G1901A, G1910A and G1915A) was 15 out of 30 (50%) divided into: 3 out of 5 (60%) representing HBeAg +ve and 12 out of 25 (48%) for patients with HBeAg –ve, as shown in table 4. As for the core gene, the study did not show any mutations in core gene. These results are in disagreement with another study in Baghdad by Kadham [20] who reported presence of mutations in core gene of HBV at nucleotide position A1762T and G1764A. The inconsistency of the results in this study with the study of Kadham [20]is due to the difference in the number of samples included in the study and the difference in the technique used to diagnose mutations in the pre-core and core gene. But at the same time present results agree with Kadham[20] who found mutations at nucleotide G1896A and G1898A, and these mutations indicate severe form of chronic liver disease. The present results are disagreed with results of a study in China regarding the absence of mutations in the core gene at nucleotides position A1762T and G1764A in the core gene promoter region[21]. The results differ due to the difference in the number of samples included in the study, the difference in the environment, the geographical location, and the possibility of a different pathological phase of the virus. The results of this study agreed with another study in Taiwan regarding the presence of mutations in the pre-core region, and disagreed regarding the absence of mutations in the core promoter region [11]; the difference may be due to dissimilarity in the virulence of the virus in its local isolates differs from the virulence of the virus in global isolates, as well as the difference in the environment and geographical location. The current study was consistent with another study conducted on patients with CHB in Korea

and found the frequency of mutations in the pre-core region at the nucleotides G1896A and G1898A, and also, present results did not agree with this Korean study regarding the absence of mutations in the core region at nucleotides A1764T, G1768A, and C1766T [22].

In conclusion, the genotype D of HBV was predominantly prevalent among CHB patients with 95% and with 5% in mixed genotypes (B,C,D), while genotype B and C were relatively less prevalent among CHB patients than genotype D. The mutations were found in the pre-core promoter gene at nucleotides G1898A, G1901A, G1910A and G1915A, while no type of mutation was detected in the core gene. The clinical significance of these mutations G1898A, G1901A, G1910A and G1915A, shows us the stages of disease development and whether the patient is in the stage of carrying the disease. And when we do the serological examination of the HBeAg gives negative results because of the mutations that occurred in the genome of the virus that led to the absence of the formation of HBeAg.

Acknowledgement

The authors would like to thank the employees of Dubai Specialist Laboratory in Baghdad, Al-Maghrib Street, Iraq.

Author contribution

As part of her PhD thesis, Dr. Hussain performed all laboratory work and wrote the draft of this paper. This work was designed and supervised by Dr. Al-Shuwaikh and Dr. Ahmed. The final version of this manuscript was read and approved by the authors.

Conflict of interest

The authors declare that there is no conflict of interest.

Funding

There is no financial support for this study from any institution.

References

1. Lago BV, do Espirito-Santo MP, Costa VD, et al. Genetic diversity of the hepatitis B virus subgenotypes in Brazil. Viruses. 2019; 11(9): 860. doi: 10.3390/v11090860.
2. International Committee on taxonomy of viruses (ICTV). Virus Taxonomy. URL: http://ictvonline.org/ (accessed February7, 2020).
3. Ribeiro CRA, Martinelli KG, de Mello VDM, et al. Cytokine, genotype, and viral load profile in the acute and chronic hepatitis B. Viral Immunol. 2020; 33(10): 620-7. doi: 10.1089/vim.2020.0176.
4. World Health Organization (WHO). Hepatitis B epidemiology. URL: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.2019.
5. Tian Q, Jia J. Hepatitis B virus genotypes: epidemiological and clinical relevance in Asia. Hepatol Int. 2016; 10(6): 854-60. doi: 10.1007/s12072-016-9745-2.
6. Yamani LN, Triani E, Amin M, et al. Prevalence and genotype distribution of hepatitis B virus among migrant workers in Lombok Island, Indonesia. Asian Pacific J Trop Med. 2020; 13(1): 8-16. doi: 10.4103/1995-7645.273568.
7. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol. 2006; 1: 23-61. doi: 10.1146/annurev.pathol.1.110304.100230.
8. Shi W, Zhang Z, Ling C, et al. Hepatitis B virus subgenotyping: history, effects of recombination, misclassifications, and corrections. Infect Genet Evol. 2013; 16: 355-61. doi: 10.1016/j.meegid.2013.03.021.
9. Zehender G, Ebranati E, Gabanelli E, et al. Enigmatic origin of hepatitis B virus: an ancient travelling companion or a recent encounter? World J Gastroenterol. 2014; 20(24): 7622-34. doi: 10.3748/wjg.v20.i24.7622.
10. Wang W, Shu Y, Bao H, et al. Genotypes and hot spot mutations of hepatitis B virus in Northwest Chinese population and its correlation with diseases progression. Biomed Res Int. 2019; 2019: 3890962. doi: 10.1155/2019/3890962.
11. Chen CH, Lee CM, Lu SN, et al. Clinical significance of hepatitis B virus (HBV) genotypes and precore and core promoter mutations affecting HBV e antigen expression in Taiwan. J Clin Microbiol. 2005; 43(12): 6000-6. doi: 10.1128/JCM.43.12.6000-6006.2005.
12. Naito H, Hayashi S, Abe K. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J Clin Microbiol. 2001; 39(1): 362-4. doi: 10.1128/JCM.39.1.362-364.2001.
13. Chan HL, Hussain M, Lok AS. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. Hepatology. 1999; 29(3): 976-84. doi: 10.1002/hep.510290352.
14. Khaled IA, Mahmoud OM, Saleh AF, et al. Prevalence of HBV genotypes among Egyptian hepatitis patients. Mol Biol Rep. 2011; 38(7): 4353-7. doi: 10.1007/s11033-010-0562-8.
15. Rashid PMA, Salih GF. Identification and genotyping of hepatitis B virus by PCR assay using genotype specific primers. Europ Sci J. 2014; 10(9): 424-33.

16. Mohsen RT, Al-azzawi RH, Ad'hiah AH. Hepatitis B virus genotypes among chronic hepatitis B patients from Baghdad, Iraq and their impact on liver function. Gene Reports. 2019; 17: 100548. doi: https://doi.org/10.1016/j.genrep.2019.100548.
17. Al-Suraifi ASK, Al-Rubaie ADJ, Al-Mayahie SMG, et al. Unusual HBV mixed genotype infections among hepatitis type B Iraqi patients in Wasit Province/Iraq. Int J Biomed Engin Clin\ Sci. 2016; 2(1): 1-7. doi: 10.11648/j.ijbecs.20160201.11.
18. Ahmed AM. Determination of hepatitis B virus genotypes among Iraqi chronic hepatitis B patients and inactive HBV carriers. PhD Thesis, Genetic Engineering and Biotechnology Institute, Baghdad University, Iraq. 2013.
19. Mbamalu C, Ekejindu I, Enweani I, et al. Hepatitis B virus precore/core region mutations and genotypes among hepatitis B virus chronic carriers in South-Eastern, Nigeria. Int J Health Sci (Qassim). 2021; 15(2): 26-38.
20. Kadham M. A study of Precore and basal Core Promoter mutations of Hepatitis B virus in relation to drug resistance chronic infections in some Iraqi patients. PhD Thesis, College of Science, University of Mustansiriya, Iraq. 2018.
21. Shi M, Zhang Y, Zhang J, et al. Hepatitis B virus genotypes, precore mutations, and basal core promoter mutations in HBV-infected Chinese patients with persistently normal alanine aminotransferase and low serum HBV-DNA levels. Braz J Infect Dis. 2012; 16(1): 52-6. doi: 10.1016/s1413-8670(12)70274-x.
22. Yoo BC, Park JW, Kim HJ, et al. Precore and core promoter mutations of hepatitis B virus and hepatitis B e antigen-negative chronic hepatitis B in Korea. J Hepatol. 2003; 38(1): 98-103. doi: 10.1016/s0168-8278(02)00349-5.

Correspondence to Dr. Hiba T. Hussain
E-mail: hiba.thamir80@gmail.com
Received Jan. 16th 2022
Accepted Apr. 18th 2022