

Research Article

Phylogenetic Tree and Genotyping of Hepatitis B Virus Isolated From Blood Donors at Thi-Qar Province

Ahmed Hieran Ramadhan Al-Jabery¹, Ali N Alsharefi² and Ali Obeid Ibrahim Alkafaji³¹*Virology, Ministry of Health, Thi-Qar Health Department - Main blood bank, Thi-Qar, Iraq.*²*Microbiology, National University for Science and Technology. Iraq.*³*Hematopathology, Ministry of Health, Thi-Qar Health Department -Main blood bank, Thi-Qar, Iraq.***Corresponding author: Ahmahmeedd19@gmail.com,***Article Info****Keywords:** *HBV, genotype, Nested PCR, Phylogenetic tree, Thi-Qar province .***Received:** 21 July 2024**Accepted:** 25 September 2024**Published:** 6 November 2024**Abstract**

Hepatitis B virus infection is a major health problem around the world due to its serious effects on the liver. The current study, which lasted from January to October 2024, aimed to determine the genotype of hepatitis B virus, found that the genotype of hepatitis B in Thi-Qar province, southern Iraq, is D. By extracting the DNA of twenty-four samples that were positive by ELISA, the genotype was determined by sequencing and using a HBV database.



© 2024 by the author's. The terms and conditions of the Creative Commons Attribution (CC BY) license apply to this open access article.

1. Introduction

Hepatitis B is a liver infection caused by the hepatitis B virus. The infection can be acute (severe and short-term) or chronic (long-term) [1]. The infection may be transmitted through contact with the body fluids of an infected person, such as blood, saliva, vaginal fluids, and semen. It can also be transmitted from a mother to her child [2]. Hepatitis B can cause chronic infection and increases people's risk of death from cirrhosis and liver cancer. Infection spread of hepatitis B virus significantly all over the world, there are two billion people have exposed to HBV globally [3]. HBV can be classified according to distribution, geographical area and mutation into ten genotypes (A-J) [4]. Various subtypes have been discovered with specific geographical distributions, for example genotype A is diffuse in Africa, Europe and America, while genotype B and C is popular in Asia. On the contrary genotype D is worldwide distribution but is common in Mediterranean region, Africa and India [5]. Genotype E identified in west Africa and genotype F restricted in south America [6]. Genotype G is common in Europe especially in France and Germany, while genotype H popular in Mexico. However genotype I was isolated in Vietnam. The modern genotype J was found in Japan [5]. The most studies showing the genotype A is common with chronic hepatitis B virus, while genotype D is found with patients with acute liver failure [7]. Also genotype B is prevalent in asymptomatic carrier and liver failure patients, while genotype C is isolated in patients have liver cirrhosis and hepatocellular carcinoma [8]. This study was aimed to determine the genotype of HBV distribution in Thi-Qar province southern of Iraq.

2. Material and Methods

Samples collection

Two ml of blood was isolated in EDTA tube for the samples to appear positive in ELISA test, and they were placed in the centrifuge at 1500 rpm for 20 minutes. Then the plasma was isolated from it, and it was placed in a Eppendorf, size 2 ml and stored at - 20 °C until the DNA was extracted.

DNA extraction

The DNA of twenty-four samples was extracted according to the instructions of the Geneaid/Taiwan No. QAIC/TW/50077. It was positive in ELISA test, and the Nested PCR test was performed using the primers HBVS1 (sense, 5 CTG CTG GTG GCT CCA GTT C 357-75), and HBVS2 (antisense, 5 AGA AAA TTG GTA ACA GMG GYA 3, 814-794) for the first round, and HBVS3 (sense, 5 GCG GKG TKT TTC TTG TTG ACA A 3,205-226) and HBVS4 (antisense, 5 GGG ACT CAA GAT GYT GYA CAG 3,789-769) for second round to amplify S gene, according to the protocol pre-heating at 94 °C for 15 min, 35 cycles including denaturation at 94 °C for 20 s, annealing at 60 °C for 20 s, and extension at 72 °C for 30 s then at 72 °C for 5 min.

HBV Genotyping Detection

Genotyping of HBV were identified by sequencing and using the HBV database available at the link <https://hbvdb.lyon.inserm.fr/HBVdb/HBVdbIndex>.

3. Results

Four positive samples only were found using Nested PCR after DNA extraction from twenty-four samples was positive by ELISA such as Figure 1.



Figure 1: Agarose gel electrophoresis of HBV seropositive sample for S gene at (564 bp) PCR product, with DNA ladder (M) 100-3000 bp.

Phylogenetic Tree Analysis of HBV Isolates

The sequence analysis was shown by using the MEGA7 program to analyze the Phylogenetic tree of the HBV in the Neighbor-Joining method, where there was a clear affinity for the nucleotides of the isolates of current study which is registered in the gene bank under the accession numbers (LC831905, LC831906, LC831907 and LC831908) with the isolates in the database, which bear accession numbers (MW999641, GU938314, FJ349228, DQ412337, HM348638, JN040778, MK213873, FJ349210, JN040770 and JF754594) respectively, as in the figure 2.

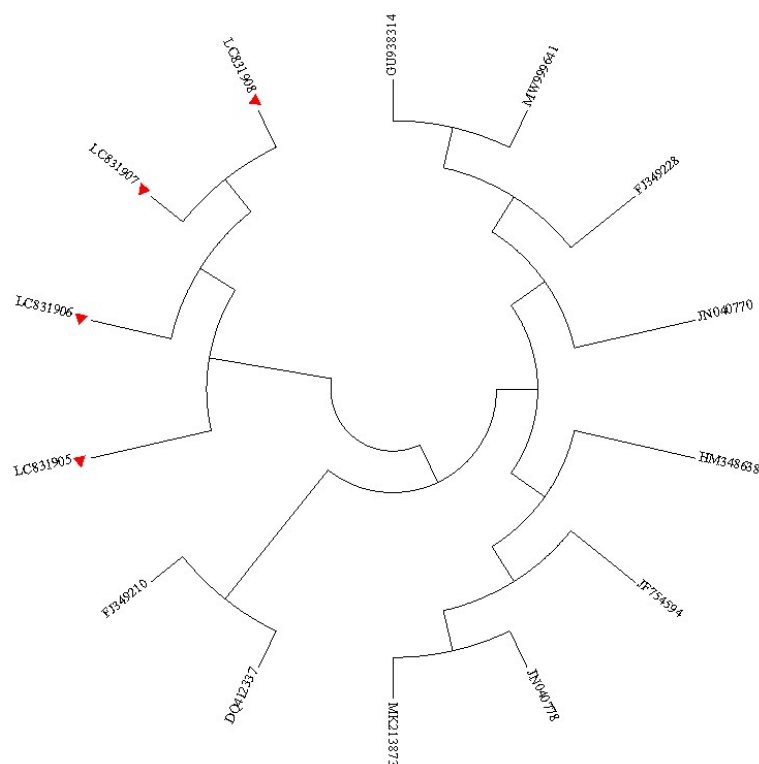


Figure 2: Phylogenetic tree of HBV by MEGA7 software depended on S gene sequence.

4. Discussion

It is important to determine the genotype of HBV because it can affect the management and monitoring of chronic hepatitis B, predict disease outcomes and response to antiviral drugs, as well as its importance in epidemiological studies [9]. It is also important in clarifying the pathogenesis, course of infection and virulence [10, 11]. Therefore, studying the genotype is important to shed light on the relationship with treatment protocols. It has been found in the context of the administration of antivirals such as (Lamivudine, Emtricitabine and tenofovir) that it may be possible to inhibit one genotype without the other [12]. Perhaps distinguishing between genotypes will help in developing more effective vaccines against specific genotype of the virus. Therefore, knowing the dominant genotype in a particular area helps in understanding the dynamics of geographical spread, tracking virus strains and planning for its control. The course of an infection can be influenced by a person's genotype. Since different genotypes do not circulate within the same population, it is frequently difficult to compare them. Even after a patient has been cleared of chronic HBV infection, it will be crucial to keep an on them for signs of HCC progression as new treatments become available. The effectiveness of antiviral treatments may be affected by HBV genotype. More in-depth and large-scale studies of genotypes are urgently needed to achieve the WHO goal of eliminating HBV worldwide as a public health burden by 2030 [13].

HBV genotypes are good markers for clinical epidemiological assessment [14]. HBV differs in virulence, immunological reactivity, pathogenicity, and resistance to treatment [15]. For example, genotype C is associated with a faster progression to cirrhosis, a higher rate of hepatocellular carcinoma development, and causes more liver malignancies than genotype B, while genotype D is more severe than genotype A, but genotype F is associated with higher mortality rates. The response of genotypes to interferon varies greatly, with genotype A responding better to treatment than D [16].

Infected with genotype A are asymptomatic, while genotype D causes liver damage associated with acute infection more than genotype A. Reinfection after liver transplantation and death are associated with genotype D more than genotypes A and C [17].

Genotype D represents an important and widely prevalent variant with some distinct clinical characteristics that clinicians should be aware of when managing hepatitis B patients, knowing that genotypes have different geographical distributions and are strongly associated with ethnic groups [18].

Data on genotypes in Iraq are scarce because in some cases differences in the distribution of genotypes can be found within one country, so the genotype must be determined in all regions of the country. There is no previous study in Thi-Qar on the genotype of hepatitis B, so we focused on studying the genotypes of hepatitis B in Thi-Qar province in southern Iraq.

The results of the current study conducted in Thi-Qar province in southern Iraq showed that the genotype of hepatitis B virus is D, which is consistent with the study of [9] and [19], those who mentioned that genotype D is the most prevalent in Baghdad province, also in Basra province they mentioned that genotype D is the most prevalent [20].

And what was mentioned by [21] in Mosul city, as well as what was mentioned in Diyala province [22], and [23] in Dohuk province found that genotype D constitutes 99.2% by examining 134 samples that were positive for the HBsAg test.

The results of the current study differed with [24] in Samarra, they found C+F to be the most prevalent, and in Wasit B was the most prevalent, followed by C and then G in one sample [25].

They also found [26] that C is the most prevalent in Baghdad, but [27] stated that B and F are the most prevalent in Baghdad, while E is the least prevalent.

At the level of Arab countries, the predominant genotype in Egypt was genotype D [28]. The predominant genotypes in Morocco are D (91.04%), A (7.55%), and E (1.41%) [29]. Genotype D was found in all 80 hepatitis B patients in Syria [30]. Genotype D is common in

Palestine [31]. Genotype analysis revealed that 90% have genotype D among Saudis [32, 33]. The predominant genotype among patients in the Kingdom of Bahrain was genotype D (61%), followed by genotype A (10%) [34].

The most common genotype in Asia is C, with genotypes B and D following at 17.8% and 15.4%, respectively. Whereas genotype B was most common in Vietnam, genotype C was most common in Lebanon, and genotype D was most common in Jordan [14]. Genotype B and D were the most prevalent genotypes among Yemeni hepatitis B patients [35, 36]. All patients who could be sequenced in Turkey were identified as genotype D [37]. Also, the predominant hepatitis B genotype among breast cancer patients was genotype D in Iran [38].

Genotype D was most prevalent among Pakistanis [39]. Genotype D was most prevalent among blood donors in Canada. Genotype F was the most prevalent, followed by genotype E in Ecuador. While genotype F is most prevalent in Latin America and the Caribbean [40].

Tests of HBV treatment efficacy have focused primarily on the most common genotypes in Europe and the United States. Thus, information on the drug susceptibility of genotype F remains limited. Patients with HBV genotypes Fanfiction have been shown to respond well to interferon therapy [41].

Genotype C1 was the most prevalent subgenotype among pregnant women in northern Thailand, accounting for 77.4% of cases, followed by putative subgenotypes B9, C2, B2, and B4 [42]. Genotype F and H are endemic in the Americas [43]. Although the US and Japan have reported cases of genotype H, its main distribution centers are in Nicaragua and Mexico [44].

Worldwide treatment standards as they stand do not account for genotypes. This highlights the critical need for additional thorough studies of HBV genotypes. So that doctors can treat their patients properly and researchers can learn more about how their treatments work.

5. Conclusion

The current study, which lasted from January to October 2024, aimed to determine the genotype of hepatitis B virus, found that the genotype of hepatitis B in Thi-Qar province, southern Iraq, is D.

References

- [1] World Health Organization. Archived from the original on 9 april 2024. January 2024.
- [2] W. J. Jeng and A. S. Lok. How to achieve a functional cure for chronic hepatitis b infection. *Clinical Liver Disease*, 23(1):e0134, 2024.
- [3] Y. Min, X. Wei, X. Xia, Z. Wei, R. Li, J. Jin, others, and X. Peng. Hepatitis b virus infection: An insight into the clinical connection and molecular interaction between hepatitis b virus and host extrahepatic cancer risk. *Frontiers in Immunology*, 14, 2023. Article 1141956.
- [4] B. O. Wandera, D. M. Onyango, and S. K. Musyoki. *Hepatitis B virus genetic multiplicity and the associated HBV lamivudine resistance mutations in HBV/HIV co-infection in Western Kenya: A review article. Infection, Genetics and Evolution*, 98, 105197. , 2022.
- [5] C. L. Lin, T. C. Tseng, and J. H. Kao. What can we learn from hepatitis b virus clinical cohorts? *Liver International*, 35, . :91–99, 2015.
- [6] Z. Liu, Y. Zhang, M. Xu, X. Li, and Z. Zhang. Distribution of hepatitis b virus genotypes and subgenotypes: A meta-analysis. *Medicine*, 100(50):e27941, 2021.
- [7] O. E. Anastasiou, M. Wiedera, S. Westhaus, et al. Clinical outcome and viral genome variability of hepatitis b virus-induced acute liver failure. *Hepatology*, 69:993–1003, 2019.
- [8] X. Wu, S. F. Wei, N. Xu, Q. Su, X. Li, and Z. H. Zhang. Meta-analysis on distribution of hepatitis b virus genotypes and related clinical outcomes in China [in chinese]. *J Clin Hepatol*, pages 271–5, 2017.
- [9] A. R. Alheany and S. F. Abdullah. Hepatitis b virus genotypes in iraqi patients with chronic hepatitis b infection. *Biochemical Cellular Archives*, 22(1), 2022.
- [10] A. Valsamakis. Molecular testing in the diagnosis and management of chronic hepatitis b. *Clinical microbiology reviews*, 20(3): 426–439, 2007.
- [11] Y. C. Chuang, K. N. Tsai, and J. H. J. Ou. Pathogenicity and virulence of hepatitis b virus. *Virulence*, 13(1):258–296, 2022.
- [12] K. P. Singh, M. Crane, J. Audsley, A. Avihingsanon, J. Sasadeusz, and S. R. Lewin. Hiv-hepatitis b virus coinfection: Epidemiology, pathogenesis, and treatment. *Aids*, 2017. doi: 10.1097/QAD.0000000000001574.
- [13] R. M. Toyé, C. L. Loureiro, R. C. Jaspe, F. Zoulim, F. H. Pujol, and I. Chemin. The hepatitis b virus genotypes e to j: The overlooked genotypes. *Microorganisms*, 11(8):1908, 2023.
- [14] K. E. Bello, T. N. A. Mat Jusoh, A. A. Irekeola, N. Abu, N. A. Z. Mohd Amin, N. Mustaffa, and R. H. Shueb. A recent prevalence of hepatitis b virus (hbv) genotypes and subtypes in asia: A systematic review and meta-analysis. *Healthcare*, 11(7):1011, April 2023.
- [15] H. T. Hussain, A. M. A. Al-Shuwaikh, and A. Ahmed. Hepatitis type b virus genotypes in chronic hepatitis b patients (chbp). In *AIP Conference Proceedings (Vol, No. 1)*. AIP Publishing. , December 2023. 2834.
- [16] S. Raimondi, P. Maisonneuve, S. Bruno, and M. Mondelli. U.(2010). is response to antiviral treatment influenced by hepatitis b virus genotype? *Journal of hepatology*, 52(3):441–449.
- [17] M. Khan, S. Khan, M. F. Gondal, S. Bibi, B. T. Khan, A. Majid, , et al. Genetic diversity in enhancer ii region of hbv genotype d and its association with advanced liver diseases. *PLoS ONE*, 17(1):e0261721, 2022.

- [18] Y. Xiao, Z. Cui, L. Sun, X. Zhou, L. Li, L. Wu, others, and X. Cui. Lack of geographical and ethnic distribution of hepatitis b virus genotypes in hainan island, China. *Infection, Genetics and Evolution*, 107:105401, 2023.
- [19] A. M. Ahmed. *Determination of Hepatitis B Virus Genotypes among Iraqi Chronic Hepatitis B Patients and Inactive HBV Carriers*. PhD thesis, The genetic engineering and biotechnology institute, 2013. 195pp.
- [20] S. A. K. Al-Aboudi and H. A. Al-Hmudi. Some of immunogenetic status of hbsag negative, hbcab positive blood donors in basra province-iraq. *ann biol res*. 2015; 6(4):47-54.
- [21] A. Dawood, G. Hasan, and A. Hayawi. Determination genotype d of hepatitis b virus amongst patients in mosul-iraq. *Int J Sci Tech Res*, 8(9):1218–1220, 2019.
- [22] M. H. Mustafa and Z. J. Jameel. Detection of hepatitis b virus (hbv) genotypes by nested polymerase chain reaction (pcr) technique in diyala province. *Academic Science Journal*, 1(4):45–57, 2023.
- [23] I. M. Abdulla and A. A. Goreal. Detection of hepatitis -b virus genotypes among chronic carriers in duhok – iraq. *J Fac Med Baghdad*, 58(2):170–175, 2016.
- [24] G. Abdulrazaq and A. F. Al-azaawie. Molecular and immunological study of hepatitis b virus infection in samara city, iraq. *Cihan Univ. Sci. J.*, 2017:97–116, 2017. doi: 10.24086/cuesj.si.2017.n2a9.
- [25] S. H. Hanash, K. H. Ghali, and A. D. Jabbar. Hbv genotyping distribution among iraqi patients in wasit province. *Eur J Mol Clin Med*, 7(2):4263–78, 2020.
- [26] L. A. Al-Kaif, A. H. Al-Charraikh, and M. A. Al-Saadi. Frequency distribution of hepatitis b virus (hbv) genotypes in iraqi patients. *International journal of health sciences*, 6(S9):2656–2665, 2022.
- [27] R. T. Mohsen, R. H. Al-azzawi, and A. H. Ad'hiah. Hepatitis b virus genotypes among chronic hepatitis b patients from baghdad, iraq and their impact on liver function. *Gene Reports*, 17:100548, 2019.
- [28] M. R. Masoud, G. A. Aboufarrag, A. A. Hmed, A. R. Sofy, and K. A. El-DougDoug. Genotyping study on hepatitis b virus among egyptian patients. *Al-Azhar Medical Journal*, 51(4):1867–1884, 2022.
- [29] S. Madihi, H. Charoute, S. Boukaira, H. Bouafi, W. Baha, A. Zyad, and A. Benani. Virological characterization of hepatitis b virus infection in morocco: a ten-years study (2014–2023). *Diagnostic Microbiology and Infectious Disease*, 116502. , 2024.
- [30] M. S. Mhmood. The distribution of hepatitis b virus genotypes and subgenotypes in acute and chronic infections and the identification of amino acids substitutions in addition to allele frequency. *Journal of Medical Genetics and Clinical Biology*. , 2024.
- [31] elkader. *ElOttol, A., Abdulla, M., Sharif, F., Ashour, M., Alashi, S., Altaher, A.* Abuzeid, N. Genotype/Subgenotype Distribution of Hepatitis B Virus in Hemodialysis Centers from Gaza Strip, Palestine. , 2023.
- [32] A. Alshabi, N. Fatima, A. Marwan, K. G. Oraibi, E. A. Qubaisi, H. O. Arif, others, and I. A. Khan. Epidemiology screening and genotyping analysis for hepatitis b virus in southwestern region of saudi arabia. *Journal of infection and public health*, 14(2):187–192, 2021.
- [33] M. Di Stefano, G. Faleo, T. Leitner, W. Zheng, Y. Zhang, A. Hassan, others, and T. A. Santantonio. Molecular and genetic characterization of hepatitis b virus (hbv) among saudi chronically hbv-infected individuals. *Viruses*, 15(2):458, 2023.
- [34] E. M. Janahi, Z. Ilyas, S. Al-Othman, A. Darwish, S. J. Sanad, B. Almusaifer, others, and S. Perna. Hepatitis b virus genotypes in the kingdom of bahrain: prevalence, gender distribution and impact on hepatic biomarkers. *Medicina*, 55(10):622, 2019.
- [35] A. M. Othman, H. A. Al-Shamahy, and A. A. M. Al-Ankoshy. *PREVALENCE OF DIFFERENT HEPATITIS B VIRUS GENOTYPES AND RISK FACTORS ASSOCIATED AMONG SELECTED YEMENI PATIENTS WITH CHRONIC HEPATITIS B INFECTION*. ,
- [36] G. Gençdal and C. Yurdaydin. Epidemiology of hepatitis b virus in the middle east. *Liver Cancer in the Middle East*, pages 91–99, 2021.
- [37] Şayan M. Çetin S. Telli E. Ugur M. Aydin E. ... YETKİN M. ŞAHİN, A. Acute hepatitis b outbreak in a nursing home for the disabled in türkiye. *Eastern Mediterranean Health Journal*, 30(4), 2024.
- [38] V. F. Sueini, M. P. Nahad, A. Talaeizadeh, M. Moradi, A. Alborzi, R. Pirmoradi, others, and M. Makvandi. Prevalence of hepatitis b virus markers among the women with breast cancer. *Asian Pacific Journal of Cancer Prevention: APJCP*, 25(2):547, 2024.
- [39] K. Gilani, U. Kareem, R. Badar, A. Rafique, S. Khurshid, S. Manawar, others, and M. Ishaq. Investigation and prevalence of hepatitis b virus genotypes in southern and northern districts of punjab, pakistan. *Migration Letters*, 21(S7):374–389, 2024.
- [40] C. Osiowy, E. Giles, C. F. Lowe, N. Matic, D. G. Murphy, S. Uzicanin, others, and S. F. O'Brien. Hepatitis b virus genotype surveillance in canadian blood donors and a referred patient population, 2016–2021. *Vox Sanguinis*, 119(3):232–241, 2024.
- [41] M. Venegas, J. Poniachik, F. Fuster, C. Hurtado, R. A. Villanueva, and J. Brahm. *Genotype F of hepatitis B: response to interferon*. , 2015.

- [42] S. Sirilert, P. Khamrin, K. Kumthip, R. Malasao, T. Tongsong, and N. Maneeakarn. Hepatitis b virus genotypes associated with pregnant women in northern thailand. *Journal of Infection and Public Health*, 17(3):406–411, 2024.
- [43] F. Pujol, R. C. Jaspe, C. L. Loureiro, and I. Chemin. Hepatitis b virus American genotypes: Pathogenic variants? *Clinics and research in hepatology and gastroenterology*, 44(6):825–835, 2020.
- [44] J. M. Wolf, V. R. Z. B. Pereira, D. Simon, and V. R. Lunge. Evolutionary history of hepatitis b virus genotype h. *Journal of medical virology*, 93(6):4004–4009, 2021.