Available online on 15.07.2021 at <http://ujpr.org>
Universal Journal of Pharmaceutical Research
 An International Peer Reviewed Journal

Open access to Pharmaceutical research
 This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial Share Alike 4.0 License which permits unrestricted non commercial use, provided the original work is properly cited

Volume 6, Issue 3, 2021

Open Access

RESEARCH ARTICLE

PREVALENCE OF DIFFERENT HEPATITIS B VIRUS GENOTYPES AND RISK FACTORS ASSOCIATED AMONG SELECTED YEMENI PATIENTS WITH CHRONIC HEPATITIS B INFECTION

Eman Mohammad Al-Shawkany¹, Abdul-Al-Raof Mohammad AlShawkany², Saleh S Bahaj¹,
 Arwa Mohammed Othman¹, Hassan A Al-Shamahy¹, Azhar Azher Mohammed Al-Ankoshy³

¹Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University.

²Molecular Genetics Department, College of Veterinary Medicine, Sana'a University, Yemen.

³Departement of physiology, Jabir Ibn Hayyan Medical University, Faculty of Medicine/Iraq.

ABSTRACT

Background and aims: Hepatitis B virus infection is a significant public health crisis global. Hepatitis B virus genotyping is an important tool in epidemiological studies to determine the category and extent of treatment and to predict the outcome of chronic infections, for instance hepatocellular carcinoma and cirrhosis. The study designed to determine the prevalence of hepatitis B virus genotypes among Yemeni patients with chronic hepatitis B (CHB) and to evaluate some of the associated risk factors.

Methods: Fifty patients (38 males, 12 females) with chronic hepatitis B from Al-Thawra Modern General Hospital, Al-Kuwait University Hospital, and AL-Gomhoria Hospital were included. HBV DNA was first detected by conventional PCR then HBV genotypes were determined using nested and multiplex PCR.

Results: Mixed HBV genotypes (A+B+C+D+E), (A+B+C+D+E+F), and (A+B+C+D) were found to be the most prevalent (60 %), it is followed by genotype D (16 %), genotype B (16%) and genotype A (8%), whereas C, E, and F genotype were not found individually among the study population. Blood transfusion was associated with mixed infection ($\chi^2=13.06$; $p=0.005$).

Conclusions: In assumption, this study demonstrates the general prevalence of hepatitis B virus genotypes among HBV-infected Yemeni hepatitis B patients who request medical consideration in a hospital. In mono-genotype HBV infection, genotype B and D were the most prevalent genotypes. In HBV mixed genotype infection, the A/B/C/D/E genotype was the most prevalent in the study area. In the future, based on genotype, clinical trials and treatment regimens must be individually assumed to efficiently manage chronic HBV infection. To this end, a prospective nationwide population study of HBV genotype spreading and clinical outcomes is suggested.

Keywords: chronic hepatitis B, HBV genotype, nested-PCR, prevalence, Yemen.

Article Info: Received 25 April 2021; Revised 23 May; Accepted 30 June, Available online 15 July 2021

Cite this article-

Al-Shawkany EM, AlShawkany AM, Bahaj SS, Othman AM, Al-Shamahy HA, Al-Ankoshy AM. Prevalence of different Hepatitis B virus genotypes and risk factors associated among selected Yemeni patients with chronic Hepatitis B infection. Universal Journal of Pharmaceutical Research 2021; 6(3):24-29.

DOI: <https://doi.org/10.22270/ujpr.v6i3.603>

Address for Correspondence:

Prof. Hassan A. Al-Shamahy, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 775 Sana'a, Yemen., Tel: +967-770299847; E-mail: shmahe@yemen.net.ye

INTRODUCTION

One of the global public health problem is Hepatitis B virus (HBV) infection. HBV belongs to the Hepadnaviridae family with a partially double-stranded DNA¹. It has been expected that about two billion people global have a proof of past or present infection with HBV and more than 358 million people have chronic lifelong infection and about 887 000 people die every year due to the outcomes of hepatitis B^{2,3}. The endemicity of hepatitis B virus was expected in Yemen, where the prevalence of positive HBsAg in the

general population and HCWs ranged from 8% to 20%, among infants, it was 4.1%, and up to 50% of health workers and populations usually had prior serological evidence of Hepatitis B virus infection in old reports. On the other hand, recent studies indicated that the rate of HBsAg, which ranges from 0.7-2% among the general population and to 4% among risk groups such as HCWs, as well as HBV decreased more among children⁴⁻¹¹. Though HBV contains DNA genome, it replicates via an RNA intermediate and due to lacking of proofreading activity for spontaneous error of viral reverse transcriptase, nucleotide mutations of HBV

genome lead to the occurrence of various genotypes and subtypes⁴. Currently, ten HBV genotypes (A-J) and twenty-four sub-genotypes (A1-A3, B1-B5, C1-C6, D1-D6 and F1-F4) are reported¹³. HBV genotypes show a distinct geographic and ethnic distribution. Genotype A is the most commonly distributed genotype in Europe, USA, Canada, Brazil, India, Central African countries, Tunisia and Benin¹⁴⁻¹⁶. Genotype B is predominant in Taiwan, Philippines, Japan, Hong Kong, China, Thailand, Indonesia, Vietnam, and USA¹⁵⁻¹⁸. Genotype C is prevalent in Australia, Melanesia, Micronesia, Polynesia, Indonesia, China, Hong Kong, Korea, Taiwan, Vietnam, Thailand, Japan, India, Solomon Islands, Brazil and USA. Genotype D is widespread in Mediterranean region, Spain, Czech Republic, Russia, Turkey, Albania, Afghanistan, South Asia, Middle East, Tunisia, Iran, Solomon Islands, Polynesia, Melanesia, Micronesia, Brazil and USA¹⁸⁻²⁰. Genotype E is found endemically in Western Africa while genotype F is widely distributed in new world countries. Genotypes G has been reported from France, and Germany and North America. Genotype H is recorded from Central America, South America and Mexico while Genotype I was isolated in Vietnam and Laos²¹⁻²³.

Different HBV genotypes are also related with dissimilar clinical phenotypes and prognosis. The rate of chronicity following acute genotypes A and D infection were reported to be high compared with genotypes B and C^{1, 14-26}. Genotype C infection alone has been found to be associated with a significantly higher risk of cirrhosis and hepatocellular carcinoma than genotype B infection^{27, 28}. In contrast, Genotype B infection has a slower progression to liver cirrhosis than genotype C²⁹. In addition, the response to antiviral therapy, mainly to interferon, is related to HBV genotypes. Patients with genotype A have been reported to be more sensitive for treatment by interferon α as compared to those infected with genotype D³⁰. HBV genotype B develops antiviral resistance more than genotype C³¹. This study aimed to determine the prevalence of different genotypes of hepatitis B virus among selected Yemeni patients with chronic hepatitis B (CHB) and to study the associated risk factors of contracting HBV infection.

SUBJECTS AND METHODS

Study population

Fifty patients with CHB were enrolled in this cross-sectional study. According to a random sampling descriptive study, the design and group effect was left equal to one, and the CHB population size equaled 5640 patients who were admitted to the main hospitals in Sana'a city^{32,33}. Since the expected frequency of genotype A or D is 20%, and an acceptable margin of error for the prevalence of different HBV genotypes is 11% according to previous studies in region^{1,14-26}; With a confidence level of 95% we need at least 50 randomly selected samples. The sample size was calculated using Epi Info version 6 (CDC). Patients from Al-Thawra General Modern Hospital, Kuwait University Hospital and Al-Jumhuri Hospital in

Sana'a-Yemen from December 2016 to June 2017 were selected from the diagnostic patient lists by systematic random selection (all 10 in the list are from the hospital records).

Inclusion criteria: Both males and Females infected with HBV for more than six months. Their HBs Ag test was positive but their HBc IgM test was negative.

Exclusion patients: Excluded patients were acute hepatitis B (Anti-HBc IgM test was positive), have mixed infected with HBV and HCV, any patient treated with antiviral of HBV, and patients who had liver cirrhosis or hepatocellular carcinoma.

Risks assessment

Demographic data were collected at the time of sample collection using a predesigned questionnaire. Also questionnaire included risk factors determinants of HBV infections. Then all statistical analyzes of the data were performed using the Statistical Package for Social Sciences (SPSS) version 24 and Excel 2007. Quantitative data were presented as means and stander divisions whereas nominal data was presented as numbers and percentages. Chi-square test was used for verifying existence of associations. P values ≤ 0.05 were considered statistically significant.

Ethical approval

Ethical approval was obtained from the Medical Research and Ethics Committee of the College of Medicine and Health Sciences, Sana'a University with reference number (11) on 14/08/2015. All data, including patient identification, was also kept confidential. A brief explanation of the purpose and importance of the study was given to each participant in order to obtain verbal consent and obtain signature to prevent misunderstanding.

Specimens' collection: Five ml venous blood were collected from each patient by the first author. Laboratory work was carried out at National Center of Public Laboratories (NCPHL). Two ml of whole blood was collected in an EDTA tube for detection of HBV-DNA while three ml was put in a plain tube for detection of Hepatitis B surface Ag (HBsAg) and liver enzymes AST and ALT. Demographic data were collected at the time of sample collection using a predesigned questionnaire.

Detection of Hepatitis B surface antigen and anti-HBc-IgM: HBsAg and anti-HBc IgM in patient serum were detected by ELISA method (Closed system-Abbott diagnostic). Samples that were positive for HBsAg and anti-HBc IgM negative were enrolled in this study.

Estimation of the serum levels of alanine and aspartate transaminase: Serum level of AST and ALT were measured using Enzyme kinetics method (kit -AGAPPE, spectrophotometer-Bayer Diagnostic RA-50 Clinical chemistry-Ireland).

Determination of HBV- DNA by conventional PCR: a. Virus DNA Extraction and PCR amplification

DNA of HBV was extracted using AccuPrep® Genomic DNA Extraction Kit (Bioneer, Korea) in accordance with the manufacturer's instructions. Extracted DNA was stored at -20°C for later analysis. HBV-DNA was then amplified using 1508 bp of P through S genes using universal primers, (FA2F) sense

primer was reported by (S1-2) antisense primer that was described by Naito *et al.*, 2001³⁴, shown in Table 1 using AccuPower® ProFi Taq PCR PreMix (bioneer Korea-Bio meter system, Germany)³⁴. The PCR program was run for one cycle as: initial denaturation

at 94°C for 5 minutes, 35 cycles consisted of denaturation at 94°C for 30 sec, annealing at 57°C for one minute followed by extension at 72°C for 1.5 minutes. The final extension was 72 °C for 5 minutes.

Table 1: Universal Primer sequences used for HBV detection.

Name primer	Sequences	Position
FA2F	5'- GCGTCG CAGAAGATCTCAAT -3'	2413–2432
S1-2-R	5'-CGA ACC ACT GAA CAA ATG GC-3'	685–704

b. Determination of HBV genotypes

Genotyping system was based on nested PCR, using type specific primers for determination of six genotypes A through F of HBV, using (AccuPower® Gold Multiplex PCR PreMix from Bioneer-Bio meter system, Germany). The nested PCR primers were designed based on the conserved nature of the nucleotide sequences in regions of the P through S genes. The genotypes can be determined according to differences in the sizes of amplified DNA, in respective of the six HBV genotypes Table 2. Two nested PCRs were performed in different mixtures for each sample: mix 1 (sit 1) applied for identification of genotypes A,

B, C with B2 sense universal primer and mix 2 (set 2) for genotypes D, E, F by B2R antisense universal primer. The nested PCR mixture made of 1μL aliquot of the first-round of PCR product in each of mix A and mix B, 1μL of each type specific primers (10 p mole) and 11μL ddH₂O. The nested PCRs were amplified for 40 cycles with the following parameters: initial denaturation at 95°C for 10 minutes, 20 cycles of amplification at 94°C for 20 seconds, 58°C for 20 seconds, and 72°C for 30 seconds, and 20 cycles of 94°C for 20 seconds, 60°C for 20 seconds, and 72°C for 30 seconds. Amplicon products were electrophoresed on a 3% agarose gel, stained with ethidium bromide, and evaluated under UV light.

Table 2: Primer sequences used for HBV genotyping by nested PCR.

Name primer		Sequences	Position
B2- sense	Set 1	5'-GGC TCM AGT TCM GGA ACA GT-3	67–86
A- antisense	(A,B,C)	5'-CTC GCG GAG ATT GAC GAG ATG T-3	113–134
B- antisense		5'-CAG GTT GGT GAG TGA CTG GAG A-3'	324–345
C- antisense		5'-GGT CCT AGG AAT CCT GAT GTT G-3'	165–186
D- sense	Set 2	5'-GCC AAC AAG GTA GGA GCT-3'	2979–2996
E- sense	(D,E,F)	5'-CAC CAG AAA TCC AGA TTG GGA CCA-3'	2955–2978
F- sense		5'-GYT ACG GTC CAG GGT TAC CA-3'	3032–3051
B2R		5'-GGA GGC GGA TYT GCT GGC AA-3'	3078–3097

RESULTS

The mean age of HBV patients was 32.64± 7.67 years. Most of CHB patients (24, 48%) were at age group 20-30 years old and only two (4%) CHB patients at age group >50 years old. Majority of CHB patients were males (38, 76 %) table (3). Distribution of HBV genotypes among study population is shown in table (4). Out of 50 patients, 30 patients (60%) had mixed genotypes, followed equally by genotype B and genotype D (8, 16%) and finally genotype A (4, 8%). Genotypes C, E, and F were not found alone but found in combinations with other genotypes. Mixed genotypes included A+B+C+D+E (20, 66.67%) followed by A+B+C+D+E+F genotypes (6, 20%) and finally genotypes A+B+C+D (4, 13.33%). The association between HBV genotypes and certain risk factors is shown in table (5). The association between the blood transfusion and HBV genotypes was found to be statistically significant ($\chi^2=13.06$; $p=0.005$). However, the surgical and dental procedures had no association with HBV genotypes in study groups ($\chi^2=3.96$; $p=0.27$; $\chi^2=1.39$; $p=0.71$, respectively).

DISCUSSION

HBV infection is a significant health problem in Yemen with intermediate to high endemicity of hepatitis B³⁵. HBV genotypes have attracted more attention as they may influence disease progression and outcome of HBV-associated chronic liver disease, in addition to patient's response to antiviral treatments³⁶. Therefore, this study focuses on evaluating the prevalence of the HBV genotype in Yemen. This molecular genotyping of hepatitis B virus was the first of its kind in Yemen using a polymerase chain reaction (PCR)-based method, and no data on hepatitis B virus genotypes and mutations in hepatitis patients have been previously reported. However, there was a previous study in genotyping of HCV conducted in Yemen³⁷. The small sample size may be an important limitation of this study, but we can justify this for two reasons: first by calculating the sample size using previous data from Yemen and the region, this calculation confirmed that 50 samples could be sufficient to achieve significant results similar to those that might be obtained of a larger sample size; Secondly, the cost of genetic testing was high for Sana'a University, which approved funds to conduct only 50 genetic tests.

Table 3: Characteristics of chronic hepatitis B patients.

		No.	%
Gender	Males	38	76
	Females	12	24
	Total	50	100
Age group (year)	Age mean±SD=32.64±7.67		
	20 – 30	24	48
	31 – 40	21	42
	41 – 50	3	6
	>50	2	4
	Total	50	100
AST	Normal	20	40
	High	30	60
ALA	Normal	20	40
	High	30	60

The mean age of the studied group is 32.64±7.67 years, which means that the registered patients were born before the implementation of the national program for neonatal hepatitis B vaccination in Yemen. The majority of CHB patients were predominantly men (38, 76%) versus women (12, 24%). The tendency of hepatitis B infection to be more common in males than females may be because males are exposed to risk factors more frequently than females. Other studies from Yemen⁷⁻¹⁰, Saudi Arabia³⁸, Bahrain²⁰, Rwanda³⁹ and Pakistan⁴⁰ reported that hepatitis B infection is more prevalent among males than females. The current study revealed that the majority of Yemeni patients

with CHB are infected with multiple HBV genotypes. CHB patients with mixed infection had four (A/B/C/D) to six (A/B/C/D/E/F) different HBV genotypes which might indicate co-infection or superinfection with different genotypes. The most common mixed genotypes were A+B+C+D+E while the least common mixed genotypes were A+B+C+D+E+F. Genotypes B, D and A were found to be mono-infection among CHB patients while Genotypes C, E, and F were only found in combinations with other genotypes. This result was similar to that described by Rashid and Saleh⁴¹, who found all Iraqi patients in their study had mixed infections⁴².

Table 4: Distribution of HBV genotypes in CHB* patients.

Genotype	Frequency	%
A	4	8
B	8	16
D	8	16
Mix	30	60
Total	50	100
Mix		
A+B+C+D	4	13.33
A+B+C+D+E	20	66.67
A+B+C+D+E+F	6	20
Total	30	100

However, our result differs from that reported from different countries around the world which reported that many patients are affected mainly by one genotype^{16,42-46}. For example, a study from Saudi Arabia, the country bordering Yemen, found genotype D to be the most common genotype among Saudi patients with CHB⁴⁷. It also differs from that reported

in the UAE which reported that many Emirati patients with viral hepatitis are commonly infected with either genotype D or A⁴⁸. Moreover, the Egyptian study revealed that all CHB patients had genotype D in which sub-D1 genotype was dominant⁴⁹.

Table 5: Association between HBV genotypes and risk factors among CHB patients.

Table 3: Association between HbA1c genotypes and risk factors among CHD patients													
Risk factor		Type of genotypes								Total		χ^2 *	P**
		A		B		D		Mix					
		No	%	No	%	No	%	No	%	No	%		
Blood transfusion	Yes	4	20	2	0	2	10	8	40	20	40	13.0	0.005
	No	0	0	6	20	6	20	22	73.3	30	60		
	Total	4	8	8	16	8	16	30	60	50	100		
Surgical procedure	Yes	0	0	2	20	0	0	8	80	10	20	3.96	0.27
	No	4	10	6	15	8	20	22	55	40	80		
	Total	4	8	8	16	8	16	30	60	50	100		
Dental procedure	Yes	0	0	0	0	0	0	2	100	2	4	1.39	0.71
	No	4	8.3	8	16.6	8	16.6	28	58.3	48	96		
	Total	4	8	8	16	8	16	30	60	50	100		

χ^2 *: Fisher exact

** P-value: probability value $p < 0.05$ (Significant)

With regard to risk factors, blood transfusion was found to be significantly associated with transmission of HBV genotypes in which patients may be exposed to co-infection or super-infection by transfusion of contaminated blood. No surgical history or dental operation was found to be significantly associated with HBV genotypes infection. A blood transfusion may result in mixed infection in recipients if blood from donors who are carriers of hepatitis B virus are not tested or tested using low-sensitivity laboratory techniques⁸⁻¹⁰. Shortcoming of this study was the fairly small sample size. Only 50 patients were tested for HBV genotypes, which may not represent the accurate picture of HBV genotypes among HBV patients.

CONCLUSION

In conclusion, this study demonstrates the general prevalence of hepatitis B virus genotypes among HBV-infected Yemeni hepatitis B patients who seek medical attention in a hospital. In mono-genotype HBV infection, genotype B and D were the most prevalent genotypes. In HBV mixed genotype infection, the A/B/C/D/E genotype was the most prevalent in the study area. Clinical trials and treatment regimens should be hypothesized individually based on genotype to effectively manage chronic HBV infection in the future. To this end, a prospective national population study of HBV genotype distribution and clinical outcomes is recommended. Testing blood donors with highly sensitive tests is also essential to avoid cross-infection and severe infection with hepatitis B virus.

AUTHORS' CONTRIBUTIONS

EMA, AMA, AMO, HAA, AA and SSB contributed equally to the design, implementation, statistical analysis and manuscript drafting. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

Authors are grateful to all patients who participated in this study.

CONFLICT OF INTEREST

There is no conflict of interest with this research.

REFERENCES

- Shen T, Yan XM. Hepatitis B virus genetic mutations and evolution in liver diseases. *World J Gastroenterol* 2014; 20(18):5435-5441. <https://doi.org/10.3748/wjg.v20.i18.5435>
- CDC. Hepatitis B. accessed on 1 January 2019.
- WHO. Hepatitis B. 18 July 2019.
- Al-kadassy AM, Al-Ashiry AFS, Al-Shamahy HA. Sero-epidemiological study of hepatitis B, C, HIV and *Treponema pallidum* among blood donors in Hodeida city-Yemen. *Universal J Pharm Res* 2019; 4(2):1-6. <https://doi.org/10.22270/ujpr.v4i2.256>
- AL-Marrani WHM and Al-Shamahy HA. Prevalence of HBV and HCV; and their associated risk factors among

- public health center cleaners at selected public health centers in Sana'a city-Yemen. *Universal J Pharm Res* 2018; 3(5):1-8. <https://doi.org/10.22270/ujpr.v3i5.204>
- Murad EA, Babiker SM, Gasim GI, Rayis DI, Adam I. Epidemiology of hepatitis B and hepatitis C virus infections in pregnant women in Sana'a, Yemen. *BMC Pregnancy Childbirth* 2013; 13: 127.
- Al-Shamahy HA, Ajrah MA, Al-Madhaji AG, et al. Prevalence and potential risk factors of hepatitis B virus in a sample of children in two selected areas in Yemen. *Universal J Pharm Res* 2019; 4(3): 1-5. <https://doi.org/10.22270/ujpr.v4i3.269>
- AL-Shamahy HA. Prevalence of Hepatitis B surface antigen and Risk factors of HBV infection in samples of healthy mothers and their infants in Sana'a, Yemen. *Ann Saudi Med* 2000; 20: 464-467. <https://doi.org/10.5144/0256-4947.2000.464>
- Al-Shamahy HA, Rabbad IA, Al-Hababy A. Hepatitis B virus serum markers among pregnant women in Sana'a, Yemen. *Ann Saudi Med* 2003; 23:87-89. <https://doi.org/10.5144/0256-4947.2003.87>
- Al-Shamahy HA, Hanash SH, Rabbad IA, Al-Madhaji NM. Hepatitis B vaccine coverage and the immune response in children under 10 years old in Sana'a Yemen. *SQU Med J* 2011; 11(1):77-82. PMID: 21509212
- Amran OAA, Al-Shamahy HA, Al Hadad AM, Jaadan BM. Explosion of hepatitis B and C viruses among hemodialysis patients as a result of hemodialysis crisis in Yemen. *Universal J Pharm Res* 2019; 4(5):1-6. <https://doi.org/10.22270/ujpr.v4i5.311>
- Kramvis A. Molecular characteristics and clinical relevance of African genotypes and sub-genotypes of hepatitis B virus. *South Afr Med J* 2018; S.I: 17-21. <https://doi.org/10.7196/SAMJ.2018.v108i8b.13495>
- Yin Y, He K, Wu B, et al. A systematic genotype and sub-genotype re-ranking of hepatitis B virus under a novel classification standard. *Heliyon* 2019; 5(10): e02556. <https://doi.org/10.1016/j.heliyon.2019.e02556>
- Janahi EM, Ilyas Z, Al-Othman S, Darwish A, et al.. Hepatitis B virus genotypes in the Kingdom of Bahrain: Prevalence, gender distribution and impact on hepatic biomarkers. *Medicina* 2019; 55, 622. <https://doi.org/10.3390/medicina55100622>
- Vachon A, Osiowy C. Novel biomarkers of Hepatitis B virus and their use in chronic Hepatitis B patient management. *Viruses* 2021; 13, 951. <https://doi.org/10.3390/v13060951>
- Mahmood M, Anwar MA, Khanum A, Zaman N, Raza A. Distribution and clinical significance of hepatitis B virus genotypes in Pakistan. *BMC Gastroenterol* 2016; 16: 104. <https://doi.org/10.1186/s12876-016-0513-5>
- Datta S. An overview of molecular epidemiology of hepatitis B virus (HBV) in India. *Virol J* 2008; 5: 156. <https://doi.org/10.1186/1743-422X-5-156>
- Awan Z, Idrees M, Amin I, Butt S, Afzal S. Pattern and molecular epidemiology of hepatitis B virus genotypes circulating in Pakistan. *Infect Genet Evol* 2010; 10: 1242–1246. <https://doi.org/10.1016/j.meegid.2010.08.006>
- Patel NH, Meier-Stephenson V, Genetu M, et al. Prevalence and genetic variability of occult hepatitis B virus in a human immunodeficiency virus positive patient cohort in Gondar, Ethiopia. *PLoS ONE* 2020; 15(11): e0242577. <https://doi.org/10.1371/journal.pone.0242577>
- Janahi EM, Ilyas Z, Al-Othman S, et al. Hepatitis B virus genotypes in the Kingdom of Bahrain: prevalence, gender distribution and impact on hepatic biomarkers. *Medicina* 2019; 55: 622. <https://doi.org/10.3390/medicina55100622>
- Tran TT, Trinh TN, Abe K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. *J Virol* 2008; 82: 5657–5663. <https://doi.org/10.1128/JVI.02556-07>
- Phung TB, Alestig E, Nguyen TL, Hannoun C, Lindh M. Genotype X/C recombinant (putative genotype I) of

- hepatitis B virus is rare in Hanoi, Vietnam-Genotypes B4 and C1 predominate. *J Med Virol* 2010; 82: 1327-1333. <https://doi.org/10.1002/jmv.21775>
23. Mahmood M. Hepatitis B Virus Genotypes in Pakistan. *Adv Res Gastroentero Hepatol* 2017; 5(5): 00104-00107. <https://doi.org/10.19080/ARGH.2017.05.555673>
 24. Kramvis A. Genotypes and genetic variability of Hepatitis B Virus. *Intervirolog* 2014; 57:141-150. <https://doi.org/10.1159/000360947>
 25. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterol* 2002; 123: 1848-56. <https://doi.org/10.1053/gast.2002.37041>
 26. Ito K, Yotsuyanagi H, Sugiyama M, Yatsuhashi H, *et al.* Geographic distribution and characteristics of genotype A hepatitis B virus infection in acute and chronic hepatitis B patients in Japan. *J Gastroenterol Hepatol* 2016; 31(1): 180-9. <https://doi.org/10.1111/jgh.13030>
 27. Chan HL, Hui AY, Wong ML, Tse AM, Hung LC, Wong VW, Sung JJ. Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; 53(10): 1494-1498. <https://doi.org/10.1136/gut.2003.033324>
 28. Chan HL, Wong ML, Hui AY, *et al.* Hepatitis B virus genotype C is associated with more severe liver fibrosis than genotype B. *Clinical gastroenterology and hepatology* 2009; 7: 1361-1366. <https://doi.org/10.1016/j.cgh.2009.08.004>
 29. Sumi H, Yokosuka O, Seki N, *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatol* 2003; 37(1): 19-26. <https://doi.org/10.1053/jhep.2003.50036>
 30. Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, *et al.* Response to interferon alpha is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 2005; 54: 1009-13. <https://doi.org/10.1136/gut.2004.060327>
 31. Lin CL, Kao JH. Hepatitis B virus genotypes and variants. *Cold Spring Harb Perspect Med* 2015; 2015 (5):a021436. <https://doi.org/10.1101/cshperspect.a021436>
 32. Al-Nabehe BA, Al-Shamahy H, Saeed WS, *et al.* Sero-molecular epidemiology and risk factors of viral hepatitis in Urban Yemen. *Int J Virol* 2015; 11(3):133-138. <https://doi.org/10.3923/ijv.2015.133.138>
 33. Ministry of Public Health and Population (Yemen) | Facts Sheet.
 34. 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):362-364. <https://doi.org/10.1128/JCM.39.1.362-364.2001>
 35. Al-Kasem MAA, Abbas MAI-K, Ebtihal MM, Al-shamahy HA. Hepatitis B virus among dental clinic workers and the risk factors contributing for its infection. *On J Dent & Oral Health*. 1(2): 2018. <https://doi.org/10.33552/OJDOH.2018.01.000509>
 36. Tufon KA, Meriki, HD, Anon DN, Mbunkah HN, Nkuo AG. Diversity, viraemic and aminotransferases levels in chronic infected hepatitis B patients from Cameroon. *BMC Res Notes* 2016; 9(117): 1-7. <https://doi.org/10.1186/s13104-016-1916-7>
 37. Al-Shamahy HA, Sultan Ahmed Abdu S. Genotyping of Hepatitis C Virus (HCV) in Infected Patients from Yemen. *Eur J Basic Med Sci* 2013; 3(4): 78-82. [PMID: 18626432](https://pubmed.ncbi.nlm.nih.gov/2646432/)
 38. Abdullah SM. Prevalence of Hepatitis B and C virus infection and their co-relation with hematological and hepatic parameters in subjects undergoing Premarital Screening in the Jazan Region, Kingdom of Saudi Arabia. *Pak J Med Sci* 2018; 34(2): 316-321. <https://doi.org/10.12669/pjms.342.14278>
 39. Makuza JD, Rwema JOT, Ntiabose CK, *et al.* Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. *BMC Infect Dis* 2019; 19(1): 381. <https://doi.org/10.1186/s12879-019-4013-4>
 40. Khan F, Shams S, Qureshi ID, *et al.* Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. *Virol J* 2011; 8: 225. <https://doi.org/10.1186/1743-422X-8-225>
 41. Rashid PMA, Salih GF. Identification and genotyping of hepatitis B virus by PCR assay using genotype specific primers. *European Sci J* 2014; 10(9).
 42. Matsuura K, Tanaka Y, Hige S, *et al.* Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. *J Clin Microbiol* 2009; 47(5): 1476-83. <https://doi.org/10.1128/JCM.02081-08>
 43. Świdorska M, Pawłowska M, Mazur W, *et al.* Distribution of HBV genotypes in Poland. *Clin Exp Hepatol* 2015; 1(1):1-4. <https://doi.org/10.5114/ceh.2015.51372>
 44. Hundie GB, Raj VS, Michael DG, *et al.* Molecular epidemiology and genetic diversity of hepatitis B virus in Ethiopia. *J Med Virol* 2016; 88(6): 1035-1043. <https://doi.org/10.1002/jmv.24437>. Epub 2015 Dec 22
 45. Rahman MA, Hakim F, Ahmed M, Ahsan CR, Nessa J, Yasmin M. Prevalence of genotypes and subtypes of hepatitis B viruses in Bangladeshi population. *Springerplus* 2016; 5: 278. <https://doi.org/10.1186/s40064-016-1840-2>. eCollection 2016
 46. Lampe E, Mello FCA, do Espírito-Santo MP, *et al.* Nationwide overview of the distribution of hepatitis B virus genotypes in Brazil: a 1000-sample multicentre study. *J Gen Virol* 2017; 98(6):1389-1398. <https://doi.org/10.1099/jgv.0.000789>
 47. Al-Qahtani AA, Pourkarim MR, Trovão NS, *et al.* Molecular epidemiology, phylogenetic analysis and genotype distribution of hepatitis B virus in Saudi Arabia: Predominance of genotype D1. *Infect Genet Evol* 2020;77:104051. <https://doi.org/10.1016/j.meegid.2019.104051>
 48. Alfaresi MS. Molecular epidemiological study of Hepatitis B virus in the United Arab Emirates based on the analysis of Pre-S gene. *J Med Microb Diagn* 2012; 1:4
 49. El-Mowafy M, Elgaml A, El-Mesery M, Elegezy M. Molecular analysis of Hepatitis B virus sub-genotypes and incidence of preS1/preS2 region mutations in HBV-infected Egyptian patients from Mansoura. *J Med Virol* 2017; 89(9):1559-1566. <https://doi.org/10.1002/jmv.24828>. Epub 2017 May 23