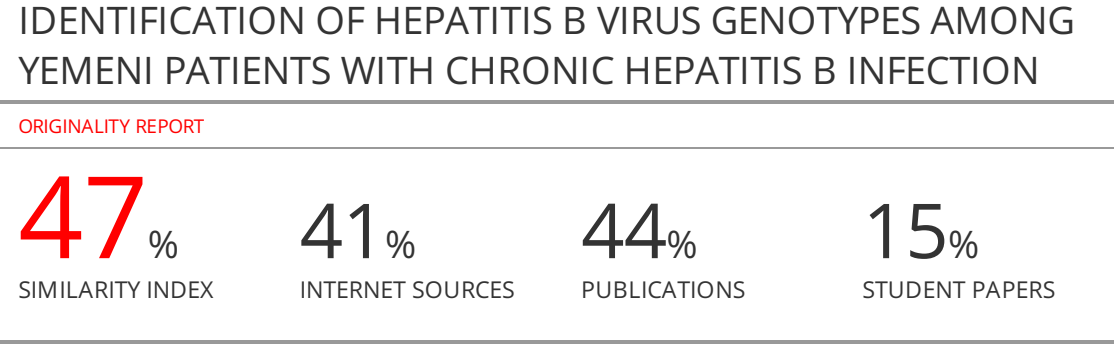
**Reviewer’s Comments**

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**IDENTIFICATION OF HEPATITIS B VIRUS GENOTYPES AMONG YEMENI PATIENTS WITH CHRONIC HEPATITIS B INFECTION**

**ABSTRACT**

**Background and aims:** Hepatitis B virus (HBV) infection is a serious public health problem worldwide. Hepatitis B virus genotyping is an important tool in epidemiological studies to determine the type and duration of treatment and to predict the outcome of chronic infections, such as cirrhosis and hepatocellular carcinoma. This study aimed 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 significantly associated with mixed infection (χ2 = 13.06; *p* = 0.005). **Conclusions:** 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. In the future, clinical trials and treatment regimens should be assumed individually based on genotype to effectively manage chronic HBV infection. To this end, a prospective nationwide population study of HBV genotype distribution and clinical outcomes is recommended.

**Keyword:** chronic hepatitis B, HBV genotype, nested-PCR, prevalence , Yemen

**INTRODUCTION**

Hepatitis B virus (HBV) infection is a global public health problem. HBV is a partially double-stranded DNA virus that belongs to the Hepadnaviridae family, in the Orthohepadnavirus genus1. It has been estimated that about two billion people worldwide 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 consequences of hepatitis B2,3. The endemicity of hepatitis B virus was estimated 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 children 4-11.

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 reported 13.

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 Benin 14-16. 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, Iran, Solomon Islands, Tunisia, Micronesia, Polynesia, Melanesia, Brazil and USA 18-20. 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 associated with distinct 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]. Infection with genotype C alone was found to be associated with significantly higher risks of liver cirrhosis and hepatocellular carcinoma compared to genotype B infection 27,28. In contrast, Genotype B infection has a slower progression to liver cirrhosis than genotype C 29. In addition, the response to antiviral therapy, particularly 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 D 30. HBV genotype B develops antiviral resistance more than genotype C31. This study aimed 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.

**Materials and Methods**

**Study population**

Fifty patients with CHB were enrolled in this cross-sectional study. Sample size was calculated by using Epi Info version 6 based on population size 1000000 and 2% prevalence of CHB 32. Patients were randomly selected from Al-Thawrah Modern General Hospital, Al-kuwait University Hospital and AL-Jomhori Hospital in Sana'a- Yemen from December 2016 to June 2017.

**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.

### Ethical Consideration

**Specimens collection:** Five ml venous blood were collected from each patient. 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-50Clinical 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*, 2001, shown in table (1) using AccuPower® ProFi Taq PCR PreMix (bioneer Korea-Bio metar system, Germany)33. 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`- GCGTCGCAGAAGATCTCAAT -3` | 2413–2432 |
| S1-2-R | 5`-CGA ACC ACT GAA CAA ATG GC-3` | 685–704 |

PCR amplicons were electrophoresed on a 1% agarose gel, stained with ethidium bromide to visualized viral DNA under UV light.

**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 metar 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  (A,B,C) | 5`-GGC TCM AGT TCM GGA ACA GT-3 | 67–86 |
| A- antisense | 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 (D,E,F) | 5`-GCC AAC AAG GTA GGA GCT-3 ` | 2979–2996 |
| E- sense | 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 |

**STATISTICAL METHOD**

Data analysis was done using SPSS program version 20 (SPSS Inc., Chicago, IL, USA). 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.

**RESULTS**

The mean age of HBV patients was 32.64± 7.67years. 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 (χ 2= 13.06; *p* = 0.005). However, the surgical and dental procedures had no association with HBV genotypes in study groups (χ 2= 3.96; p = 0.27; χ 2= 1.39; p = 0.71, respectively).

**DISCUSSION**

HBV infection is a major health problem in Yemen with intermediate to high endemicity of hepatitis B34. HBV genotypes have attracted more attention since they may affect the disease progression and outcomes of HBV-related chronic liver disease, in addition to patient's response to antiviral treatments 35. 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 Yemen 36.

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 Arabia 37, Bahrain20, Rwanda 38 and Pakistan39 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 Saleh, 2014 40, who found all Iraqi patients in their study had mixed infections40. However, our result differs from that reported from different countries around the world which reported that many patients are affected mainly by one genotype 16,41-45. For example, a study from Saudi Arabia, the country bordering Yemen, found genotype D to be the most common genotype among Saudi patients with CHB46. 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 A47. Moreover, the Egyptian study revealed that all CHB patients had genotype D in which sub-D1 genotype was dominant48.

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 superinfection 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.

Limitation of this study was the relatively small sample size. Only 50 patients were tested for HBV genotypes, which may not represent the accurate picture of HBV genotypes among HBV patients.

**CONCLUSIONS**

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. In the future, clinical trials and treatment regimens should be assumed individually based on genotype to effectively manage chronic HBV infection. To this end, a prospective nationwide population study of HBV genotype distribution and clinical outcomes is recommended. Also testing blood donors with highly sensitive tests is necessary to avoid co-infection and super-infection with HBV.

**AVAILABILITY OF DATA AND MATERIALS**

The data that support the findings of this study are available. Anyone interested can get upon reasonable request from corresponding author.

**FUNDING**

Authors didn’t take any fund for this study

**AUTHORS’ CONTRIBUTIONS**

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

**ACKNOWLEDGEMENTS**

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**CONFLICT OF INTEREST**

No conflict of interest associated with this work.

**REFERENCE**

1- Shen T, Yan XM. Hepatitis B virus genetic mutations and evolution in liver diseases. World J Gastroenterol. 2014; 20(18):5435‐5441.

2. CDC. Hepatitis B. Available online: <https://www.cdc.gov/hepatitis/hbv/bfaq.htm#overview> (accessed on 1 January 2019).

3. WHO. Hepatitis B. 18 July 2019. <https://www.who.int/news-room/fact-sheets/detail/hepatitis-B>.

4-Al-kadassy, A. M., A. F. S. Al-Ashiry, and H. A. Al-Shamahy. “Sero-epidemiological study of hepatitis B, C, HIV and treponema pallidum among blood donors in Hodeida city- Yemen”. Universal Journal of Pharmaceutical Research, 2019; 4(2):1-6. doi:https://doi.org/10.22270/ujpr.v4i2.256.

5-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 Journal of Pharmaceutical Research 2018; 3(5):1-8. doi:https://doi.org/10.22270/ujpr.v3i5.204.

6-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.

7-Al-Shamahy H A, 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 Journal of Pharmaceutical Research 2019; 4(3): 1-5. doi:https://doi.org/10.22270/ujpr.v4i3.269.

8-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 Medicine 2000; 20: 464-467.

9-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.

10-Al-Shamahy HA, Samira H Hanash, Iqbal A Rabbad, Nameem M Al-Madhaji, 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.

11-Amran O A A, Al-Shamahy HA, Al Hadad AM, and Jaadan BM. “Explosion of hepatitis B and C viruses among hemodialysis patients as a result of hemodialysis crisis in Yemen”. Universal Journal of Pharmaceutical Research 2019;. 4(5):1-6. doi:https://doi.org/10.22270/ujpr.v4i5.311.

12- Kao JH. Hepatitis B viral genotypes: Clinical relevance and molecular characteristics. J Gastroenterol Hepatol 2002; 17: 643–650.

13- Yin Y, He K, Wu B, Xu M, Du L, Liu W, Liao P, Liu Y, He M. A systematic genotype and sub-genotype re-ranking of hepatitis B virus under a novel classification standard. Heliyon. 2019; 5(10): e02556.

14- Kao JH, Chen DS. HBV Genotypes: Epidemiology and implications regarding natural history. Current Hepatitis Reports 2006; 5: 5–13.

15- Bonino F, Teerha P, Brunetto MR, Yun-Fan L. Diagnostic markers of chronic hepatitis B infection and disease. Antivir Ther. 2010;15 Suppl 3: 35–44.

16- 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.

17- Datta S. An overview of molecular epidemiology of hepatitis B virus (HBV) in India. Virol J. 2008; 5: 156.

18-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.

19- Ljunggre K. Genetic variability in hepatitis B viruses. J General Virol. 2002;83: 1267–1280.

20- 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.

21-Tran TT, Trinh TN, Abe K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. J Virol 2008; 82: 5657–5663.

22- 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.

23- Mahmood M. Hebatitis B Virus Genotypes in Pakistan. Adv Res Gastroentero Hepatol 2017; 5(5): 00104-00107.

24- Mayerat C, Mantegani A, Frei PC. Does hepatitis B virus (HBV) genotype influence the clinical outcome of HBV infection? J Viral Hepat 1999;6(4):299-304.

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. Gastroenterology 2002; 123: 1848-56.

26- Ito K, Yotsuyanagi H, Sugiyama M, Yatsuhashi H, Karino Y, Takikawa Y, 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.

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.

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.

29- Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, Kanda T, Fukai K, Kato M, Saisho H. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003; 37(1): 19-26.

30- Erhardt A, Blondin D, Hauck K, Sagir A, Kohnle T, et al. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. Gut. 2005; 54: 1009–13.

31-Hsieh TH, Tseng TC, Liu CJ, Lai MY, Chen PJ, Hsieh HL, et al. Hepatitis B virus genotype B has an earlier emergence of lamivudine resistance than genotype C. Antivir Ther 2009; 14: 1157–1163.

32-Al-Nabehi BA, Al-Shamahy H, Saeed WS, Khalil EA, Musa AM, ElHasssan AM. Sero-Molecular Epidemiology and Risk Factors of Viral Hepatitis in Urban Yemen. International Journal of Virology 2015; 11(3):133-138.

33- Naito, H., Hayashi, S & Abe, K. (2001). Rapid and Specific Genotyping System for Hepatitis B Virus Corresponding to Six Major Genotypes by PCR Using Type-Specific Primers. Journal of Clinical Microbiology, 39(1), pp. 362–364.

34- Al Kasem MA A, Abbas M Al-K, Ebtihal M M, 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. OJDOH.MS.ID.000509.

35- Tufon KA, Meriki, HD, Anon DN, Mbunkah HN, Nkuo AG. Diversity, viraemic and aminotransferases levels in chronic infected hepatitis B patients from Cameroon, BMC Research Notes 2016; 9(117): 1-7.

36- 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.

337-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.

38- Makuza JD, Rwema JOT, Ntihabose CK, Dushimiyimana D, Umutesi J, Nisingizwe MP, Serumondo J, Semakula M, Riedel DJ, Nsanzimana S. Prevalence of hepatitis B surface antigen (HBsAg) positivity and its associated factors in Rwanda. BMC Infect Dis 2019; 19(1): 381.

39- Khan F, Shams S, Qureshi ID, Israr M, Khan H, Sarwar MT, Ilyas M. Hepatitis B virus infection among different sex and age groups in Pakistani Punjab. Virol J. 2011; 8: 225.

40- Rashid, P.M.A & Salih, G.F. Identification and genotyping of hepatitis B virus by PCR assay using genotype specific primers. European Scientific Journal 2014;10(9).

41-Matsuura K, Tanaka Y, Hige S, Yamada G, Murawaki Y, Komatsu M, 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.

42- Świderska M, Pawłowska M, Mazur W, Tomasiewicz K, Simon K, Piekarska A, et al. Distribution of HBV genotypes in Poland. Clin Exp Hepatol 2015;1(1):1-4.

43- Hundie GB, Raj VS, Michael DG, Pas SD, Osterhaus AD, Koopmans MP, Smits SL, Haagmans B. Molecular epidemiology and genetic diversity of hepatitis B virus in Ethiopia. J Med Virol. 2016; 88(6): 1035-1043.

44- 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.

45- 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.

46- Al-Qahtani AA, Pourkarim MR, Trovão NS, Vergote V, Li G, Thijssen M, Abdo AA, 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.

47- 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

48- 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.

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 |

\*SD: standard deviation

**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** |

**Table 5: Association between HBV genotypes and risk factors among CHB 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 | 13.06 | 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)