Hepatitis B genotypic and serologic characteristics in Jordan

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Objective: To identify the most common hepatitis B virus (HBV) genotype in Jordan

Methods: Patients were recruited from the Central Blood Bank and hepatology clinic in Al-Bashir Hospital from 2010 to 2013 and tested at the Central Laboratory Directorate. Demographic data, HBeAg status, ALT and HBV DNA were collected for all patients.

491 blood samples were examined between 2010 and 2013, 262 (53.3%) were of male gender and 229 (46.6%) were female. Individuals were categorized after clinical and serological testing in chronic hepatitis B patients 72 (14.6%) and healthy inactive HBsAg carriers 419 (85.3%).

All patients with acceptable titer were tested for HBV genotype.

Results: From the 491 examined individuals, seventy two had HBV DNA values more than 2000 IU/ml (14.6%) while 419 had HBV DNA less than 2000 IU/ml (85.3%). Fifty four patients (10.9%) were HBeAg positive while 379 (89.1%) were HBeAg negative.

Genotype D was the only genotype detected in our study group (100%).

Conclusion: Our study showed that the predominant genotype in Jordan is genotype D which is in concordance with genotypes found in Middle East.

In addition, most of our Jordanian individuals infected with HBV are healthy carriers, with majority of them having HBeAg negative serologic marker. On the other hand, the majority of patients with active hepatitis B, showed HBeAg positive serological marker.

Key words: HBV, genotype, serotype, Jordan.

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Introduction

The World Health Organization (WHO) estimated the burden of HBV infection to be around 2 billion cases with more than 350 million known to be chronically infected.[1] More than half a million of patients worldwide die annually as a result of HBV-related liver diseases such as decompensated liver cirrhosis, end-stage liver disease or hepatocellular carcinomas (HCCs).[2,3]According to WHO reports Jordan is considered to fall into the 'high endemicity' categoryaccording to the studies which were conducted in the mid-eighties of the last century which showed that the prevalence of hepatitis B in Jordan is around 10% ,[3-6] but now, experts sustain that this prevalence had dropped to 2.5 -3.5% because of the introduction of HBV vaccination in the mid-nineties and other infection control measurements,[7,8] although no statistical studies were conducted to confirm this drop in prevalence. Prevalence of HBV in patients on hemodialysis was found to be 5.9%.[9]The overall HBV prevalence among Jordanian blood donors was around 1.4% depending on the population studied. [38] The only published study regarding HBV genotypes in Jordan by H. Masaadeh et al sustain that HBV genotype D appears to be the only circulating type in Jordanian patients. [38] Classification of HBV has changed from serologic subtype

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classification to more precise genotype genetic classification. HBV genotypes represent naturally occurring strains of HBV that have evolved over the years and reflect the geographical distribution of HBV throughout the world. Up to now, eight different HBV genotypes have been identified and shown to cluster in different areas of the world.[10,11]

Genotype D is the most prevalent genotype concentration in the Middle East and the regions around as Turkey, Egypt, and Gulf region. Recent studies have found that genotype D accounts for 81%-85% of all genotypes in Saudi Arabia and almost all genotypes in Egypt.[12,13] This finding might have potential impact on selection of antiviral drugs, prediction of disease courses and clinical responses.

Methods

Patients were consecutively recruited from the Central Blood Bank (screened blood donors and found to have HBsAg by ELISA) and all were referred to the hepatology clinic of the largest tertiary care referral center in Jordan from 2010 to 2013. All blood samples were tested at the Central Laboratory Directorate at the Ministry of Health . Demographic data (Age, sex, anti HBV vaccination and mode of transmission), clinical evaluation, serological markers including HBeAg status, ALT and HBV DNA by PCR were collected for all patients. There were no patients with hepatocellular carcinoma or cirrhosis in this study. All patients with acceptable titer that permits genotyping were tested for HBV genotype.

491 blood samples from the patients were examined between 2010 and 2013, 262 (53.3%) were of male gender and 229 (46.6%) were female.

HBV markers were tested using TKA Billini System (ELISA) with lower detection limit of 1.

HBV DNA quantification were tested using real time amplification PCR type COBAS® AmpliPrep/COBAS® TaqMan® HBV Test, v2.0 with low detection limit of 20 IU/ml. Roche diagnostics. HBV genotyping were tested using Line probe genotyping assay (INNO-LiPA HBV Genotyping assay; Innogenetics, Ghent, Belgium) with lower detection limit of 1000 IU/ml. Serum Alanine aminotransferase (ALT) normal limits was between 0-41 IU/ml.

Inactive HBV carrier patients were defined as patients with HBsAg positivity for more than 6 months, with persistently normal ALT/AST levels with serum HBV DNA less than 2000 IU/ml, while chronic hepatitis B patients were diagnosed if they had HBsAg more than 6 months, serum HBV DNA more than 2000 IU/ml and persistently elevated ALT/AST levels[22, 23, 24]

Results

Investigated individuals were categorized after clinical, chemical and serological testing in chronic hepatitis B patients 72 (14.6%) and inactive HBsAg carriers 419 (85.3%). Fifty four patients (10.9%) were HBeAg positive while 437 (89.1%) were HBeAg negative Table I.

HBV DNA with values more than 2000 IU/ml were seen in patients with active disease (72/491), while healthy carriers had values of less than 2000 IU/ml (419/491). From individuals with HBV values of less than 2000 IU/ml, 379 (90.4%) had undetectable values or low titer for genotyping and 40 (9.5%) were of genotype D, Table II.

Patients with HBV DNA values of more than 2000 IU/ml (72/491) were all of genotype D.

The total number of blood samples which were genotyped was 112 sample and all were of genotype D, 54 (48.2%) were HBeAg positive and 58 (51.7%) were HBeAg negative. Detailed, patients with genotype D and active disease and HBV DNA of more than 2000 IU/ml counted 72 patients, 38 (52.7%) were HBeAg positive and 34 (47.2%) were HBeAg negative, Table III. While individuals with genotype D and HBV DNA less than 2000 IU/ml counted 40 patients, 16 (40%) were HBeAg positive and 24 (60%) were HBeAg negative Table IV.

Ethical Issues: Data collected were treated confidentially, and all participants provided informed consent for this study. This study was approved by the Ministry of Health Ethics Committee (MOH/EC/11633/2014).

Statistical Issues: Quantitative variables are expressed as mean ± SD. Qualitative variables are expressed as percentage with range.

Statistical analyses were performed using SPSS version 15. A value of $P < 0.05$ was considered to be statistically significant.

Discussion

Eight genotypes have been identified worldwide labeled A through H.[14,15] Genotype A occurs in Africa, Europe and India, genotype B occurs in east and southeast Asia, genotype C occurs in East Asia and the Pacific Islands and genotype D occurs in the Mediterranean region, Middle East, central Asia and India and can be used to study anthropological migration patterns in the past.[16,18] Genotype E occurs in West Africa and genotypes F and H in central and South America. The genotype G appears partially defective and invariably occurs together with another genotype.[17] Hybrids of B and C are found in Asian countries, A and D in Italy and C and D in Tibet and China.[19] Recent data suggest that HBV genotypes may play an important role in the progression of HBV related liver disease as well as response to Interferon therapy.[14] Studies from Asia found that HBV genotype B is associated with HBeAg seroconversion at an earlier age, more sustained remission after HBeAg seroconversion, less active hepatic necroinflammation, a lower rate of progression to cirrhosis, and a lower rate of HCC development compared to genotype C.[20,21] It is also known that that genotype D has a higher likelihood of developing advanced cirrhosis compared to genotype A.[29]Furthermore, there is evidence that the rate of resistance to lamivudine is lower in patients infected with genotype D than in patients with genotype A. [28] The guidelines for management of chronic hepatitis B published by the American Association for the study of Liver Diseases (AASLD), [22] the European Association for the study of Liver (EASL) [23]and the Asian Pacific Association for the study of the Liver (APASL) [24] suggest that genotyping the virus is not a recommended part of the management and is best regarded as a research tool. In contrast, individual reviews [25,26] cite the same evidence and recommended that therapy must be based on genotyping. The German guidelines[27] for the management of hepatitis B virus infection strongly support this position. The Dutch guidelines[30] mention pegylated interferon should be considered for initial therapy particularly with genotypes A and B, and the Swedish guidelines[31] recommend pegylated interferon as first line treatment in particular for genotypes A and B. Several studies were done to investigate the relationship between response to immune modulation therapy and hepatitis B genotypes , Lau *et al.*,[32] 814 patients participated worldwide, genotypes B and C were predominant, and there were also 56 patients with genotype A and 37 patients with genotype D. The HBe Ag seroconversion rate in those receiving pegylated interferon plus placebo was 52% (12/23) in genotype A and 22% (2/9) in genotype D. According to Janssen *et al.*, 34% of patients were genotype A and 39% genotype D, 9% had genotype B and 15% had genotype C and utilized 100 μg for 52 weeks. Response rates (HBe antigen loss) varied by HBV genotype using univariate analysis ($P = 0.01$): genotype A n = 42 patients (response rate 47%); B n = 10 (44%); C n = 11 (28%); and D n = 26 (25%) (32). Both of the studies (Lau and Janssen) with genotypes A, B, C and D had shown the best response in genotype A and the lowest response in genotype D. Marcellin P *et al.*[34] in HBe antigen-negative patients using pegylated interferon or pegylated interferon with lamivudine or lamivudine monotherapy involving 530 patients in Europe and Asia. Patients received pegylated interferon alfa 2a 180 μg for 48 weeks. Genotyping was available for 346 patients in the two arms containing PEG interferon, and the largest group was genotype C, then genotypes D and B. Only 6% had genotype A. The combined response (normalization of ALT and DNA <20 000 copies/mL) was 44% for genotype B, 49% for genotype C and 16% for genotype D in patients receiving PEG interferon only. These studies concluded that treatment response to nucleoside analogues was not significantly influenced by HBV genotype in HBeAg-positive or HBeAg-negative individuals. In contrast, HBV genotypes were informative concerning responses to interferon treatment in all patients with genotype A *vs.* D and in HBeAg-positive patients with genotype B *vs.* C. They

concluded that if no contraindications are present, interferon may be considered as first-line therapy in all patients with genotype A and in patients with genotype B who are HBe antigen positive.

A study conducted by Hakan S. *et al*. evaluated long-term outcomes of interferon alpha treatment in initially HBeAg positive chronic hepatitis B patients in Egypt, where HBV genotype D accounts for almost all cases and concluded that sustained response to interferon treatment is low in HBeAg positive chronic hepatitis B patients with genotype D.[34] Pietro L. *et al*.[36] assessed the influence of treatment duration on response rates among HBeAg negative chronic hepatitis B patients with genotype D virus and concluded that genotype D has been shown to be less responsive to interferon compared with genotypes A, B, or C, but In HBeAg negative genotype D patients with chronic hepatitis B, 2 year treatment with pegylated interferon alfa-2a was safe and improved significantly the rates of post-treatment virological and serological response

As a conclusion, the outcome of genotyping if carried out in HBe antigen-positive patients prior to treatment; for those patients with genotype A and B will be offered pegylated interferon as first line of therapy as it offers the likelihood of HBeAg seroconversion with a finite course of therapy.

In HBe antigen-negative patients, the same recommendations with respect to genotypes A and B at least will apply. For genotype D (the Jordanian genotype), patients with positive HBeAg have a small chance of response to treatment with pegylated interferon, higher chances of success could be seen in patients with negative HBeAg especially if longer duration of treatment is granted.

Surprisingly, our study had showed a number of 16 patients which had low HBV DNA values (< 2000 IU/ml) and persistently normal liver enzymes but with HBeAg positivity, although those patients underwent repetition of the above mentioned tests but they showed the same results, which made us label them as inactive HBV carriers with close monitoring, actually nine patients (who accepted the procedure) underwent liver biopsy and showed no or mild inflammation in their specimens.

Table I: Characteristics of 491 Chronic Hepatitis B patients

	No/%
Age (mean/ year)	42.1
sex	
Female	229 (46.6)
Male	262 (53.3)
HBV vaccination	0
Unknown mode of transmission	242 (49.2)
Vertical transmission	163 (33.1)
Horizontal transmission	86 (17.5)
ALT (mean IU/ml)	29.2
HBeAg positive	54 (10.9)
HBeAg negative	437 (89.1)
HBV DNA > 2000 IU	72/491 (14.6)
HBV DNA < 2000 IU	419/491 (85.3)

Table II: Genotype D serological characteristics

	No/%
Total number	112
HBeAg positive	54 (48)
HBeAg negative	58 (51.7)
ALT (mean IU/ml)	37.9
HBV DNA > 2000	72 (64.2)
HBV DNA < 2000	40 (35.7)

Table III: Genotype D/ HBV healthy carrier characteristics

	No/%
HBV genotype D	40
ALT (mean IU/ml)	16.9
HBeAg positive	16 (40)
HBeAg negative	24 (60)

Table IV: Genotype D/HBV chronic hepatitis B patient's characteristics

	No/%
HBV genotype D	72
ALT (mean IU/ml)	71.6
HBeAg positive	38 (52.7%)
HBeAg negative	34 (47.2%)

Conclusion

Our study showed that the predominant genotype in Jordan is genotype D which is in concordance with genotypes found in Middle East.

In addition, most of our Jordanian individuals infected with HBV are healthy carriers, with majority of them having HBeAg negative serologic marker. On the other hand, the majority of patients with active hepatitis B, showed HBeAg positive serological marker.

Awareness of these serologic and genotypic viral markers might help in the formulation of management plans and in predicting clinical outcomes.

References

1. **Harkisoen S, Arends JE, Van Erpecum KJ, Van den Hoek A, Hoepelman AI.** Hepatitis B viral load and risk of HBV-related liver disease: from East to West? *Ann Hepatol* 2012; 11:164–71.
2. World Health Organization. Hepatitis B. World Health Organization Fact Sheet 204; 2008.
3. World Health Organization. Sixty third world health assembly 25. World Health Organization 2011.
4. **ToukanAU, SharaihaZK, Abu-el-Rub OA, *et al.*** The epidemiology of hepatitis B virus among family members in the Middle East. *Am J Epidemiol* 1990 Aug;132(2):220-32
5. Toukan Au et al. Hepatitis B in the Middle East: aspects of epidemiology and liver disease after infection. Gut. 1996;38 Suppl 2:S2-4
6. **N. Qirbi and A.J. Hall.** Epidemiology of hepatitis B virus infection in the Middle East. *Eastern Mediterranean Health Journal* Volume 7, No. 6, November 2001, 1034-1045
7. **W.Hamoudi .** Jordan and hepatitis B virus. Do we have to worry? World Gastroenterology Organization – Viral hepatitis 2007:11
8. **Belbisi A, Hadadin A, Toukan A, Hamoudi W, Ghazzawi I, Khatib MA, *et al.*** Jordan National Strategy for Viral Hepatitis; 2010.www.moh
9. **Al Hijazat M, Ajlouni YM.** Hepatitis B infection among patients receiving chronic hemodialysis at the royal medical services in Jordan. *Saudi J Kidney Dis Transplant* 2008; 19:260–7.
10. **Norder H, CourouceAM, Magnius LO.** Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes.*Virology*1994; **198**: 489-503
11. **Naito H, Hayashi S, Abe K.** Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specifi c primers. *J ClinMicrobiol*2001; **39**: 362-364
12. **Al Ashgar HI, Imambaccus H, Peedikayil MC, *et al.*** Prevalence of hepatitis B virus genotype in Saudi Arabia: a preliminary report. *Indian J Gastroenterol*2008; **27**: 81-82
13. **Saudy N, Sugauchi F, Tanaka Y, *et al.*** Genotypes and phylogenetic characterization of hepatitis B and delta viruses in Egypt. *JMed Virol*2003; **70**: 529-536
14. **Fung SK, Lok AS.** Hepatitis B virus genotypes: do they play a role in the outcome of HBV infection? *Hepatology* 2004; 40(4):790-792.
15. **Norder H, Courouce AM, Coursaget P, *et al.*** Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; 47(6):289-309.
16. **Sugauchi F, Mizokami M, Orito E *et al.*** A novel variant genotype C of hepatitis B virus identified in isolates from Australian Aborigines: complete genome sequence and phylogenetic relatedness. *J Gen Virol*, 2001; 82: 883–892.
17. **Kato H, Orito E, Gish RG *et al.*** Characteristics of hepatitis B virus isolates of genotypeG and their phylogenetic differences from the other six genotypes (A through F). *J Virol*2002; 76: 6131–6137.
18. **Jazayeri MS, Basuni AA, Cooksley G, Locarnini S, Carman WF.** HBV genotypes, core gene variability and ethnicity in the Pacific region. *J Hepatol* 2004; 41: 139–146.
19. **Kao JH,WuNH, Chen PJ, Lai MY, Chen DS.** Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; 33(6):998-1002.
20. **Chan HL, Hui AY, Wong ML, *et al.*** Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. Gut 2004; 53(10):1494-1498.
21. **Yu MW, Yeh SH, Chen PJ, *et al.*** Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; 97(4):265-272+
22. **Lok ASF, Mc Mahon BJ.** AASLD practice guidelines. Chronic hepatitis B. *Hepatology* 2007; 45: 507–538.
23. European Association for the study of the liver. EASL clinical practice guidelines: management of chronic hepatitis B. *J Hepatol*2009; 50: 227–242.
24. **Liaw YF, Leung N, Kao JH *et al.*** Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hep Intl* 2008; 2: 263–283
25. **Perrillo R.** Benefits and risks of interferon therapy for hepatitis B. *Hepatology* 2009; 49: S103–S111.
26. **Buster EHCJ, Schalm SW, Janssen HLA.** Peginterferon for the treatment of chronic hepatitis B in the era of nucleos(t)ide analogues. *Best Pract Res ClinGastroenterol* 2008; 22: 1093–1108.
27. **Cornberg M, Protzer U, Dollinger MM *et al.*** The German guideline for the management of hepatitis B virus infection: short version. *J Viral Hepat*2008; 15(Suppl. 1): 1–21.
28. **Palumbo E.** Hepatitis B genotypes and response to antiviral therapy: a review. *Am J Ther*2007; 14: 306-309
29. **Thakur V, Guptan RC, Kazim SN, Malhotra V, Sarin SK.** Profile, spectrum and

significance of HBV genotypes in chronic liver disease patients in the Indian subcontinent. *J GastroenterolHepatol*2002; 17: 165-170

30. **Buster EHCJ, van Erpecum KJ, Schalm SW *et al.*** Treatment of chronic hepatitis B virus infection – Dutch national guidelines. *Neth J Med* 2008; 66: 292–305.

31. **Lindh M, Uhnoo I, Blackberg J *et al.*** Treatment of chronic hepatitis B infection: an update of Swedish recommendations. *Scand J Infect Dis* 2008; 40: 436–450.

32. **Lau GKK, Piratvisuth T, Luo KX *et al.*** Peginterferonalfa 2-a as monotherapy and in combination with lamivudine versus lamivudine monotherapy in patients with HBeAg positive chronic hepatitis B. *N Engl J Med* 2005; 352: 2682–2695.

33. **Janssen HLA, van Sonneveld M, Senturk H *et al.*** Pegylated interferon alfa 2b alone or in combination with lamivudine for HBe antigen positive chronic hepatitis B: a randomized trial. *Lancet* 2005; 365: 123–129

34. **Marcellin P, Lau GKK, Bonino F.** Peginterferonalfa 2a alone, lamivudine alone, and the two in combination in patients with HBe antigen negative chronic hepatitis B. *New Engl J Med* 2004; 351: 1206–1217.

35. **HakanSenturk, BirolBaysal, VeyselTahan, *et al.*** Long-Term Effect of Interferon Therapy in Patients with HBeAg Positive Chronic Hepatitis B Infection. *Digestive Diseases and Sciences January* 2011, Volume 56, Issue 1, pp 208-212

36. **P Lampertico, M Vigano, G Di Costanzo, *et al.*** Extended treatment with peginterferon alfa-2a improves sustained response rates in genotype D patients with HBeAg negative chronic hepatitis B. 45th Annual Meeting of the European Association for the Study of the Liver (EASL 2010). Vienna, Austria. April 14-18, 2010

37. **FathiAbed Al-Gani.** Prevalence of HBV, HCV and HIV-1, 2 infections among blood donors in Prince Rashed Ben Al-Hassan hospital in North region of Jordan. *Int J Biol Med Res* 2011; 2:912–6.

38. **Hani A Masaadeh, Wail AHayajneh,** Enayat A Alqudah hepatitis B virus genotypes and lamuvidine resistance mutations in Jordan. *World J Gastroenterology* 21, 2008 Volume 14 Number 47.

39.