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Research Article

Viral Hepatitis B Genotypes among Outpatient Clinic Attendees in North Rift, Kenya

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1. Abstract

1.1. Objective: Most hospital outpatients unaware of their Hepatitis B Virus (HBV) status could be seeking treatment for other different ailments in Kenya. To evaluate the HBV prevalence and the genetic diversity, the genetic analysis of the partial HBV S gene was conducted.

1.2. Methods: Two hundred blood samples were collected from consenting outpatients who were unaware of their HBV status at Moi Teaching and Referral Hospital (MTRH), Kenya, between September 2015 and October 2016. The serum was tested for the HBV surface antigen (HBsAg) using the ELISA test. DNA was extracted from HBsAg positive samples, amplified and sequenced for HBV S gene. The sequences were then compared with reference sequences retrieved from the GenBank.

1.3. Results: Ten percent of subjects (20/200) were positive for HBsAg. Thirteen of the 14 isolates (92.9%) belonged to HBV sub genotype A1 (HBV/A1). In all the 14 isolates either one or more mutation was detected within Major Hydrophilic Region (MHR). The most occurring mutation was S114T which appeared in 13 of 14 isolates. No known mutations associated with occult HBV infection or vaccine escape were observed.

1.4. Conclusion: Ten percent of outpatients at MTRH could be a source of unaware HBV transmission in the community. HBV/A1 remains the most predominant genotype. The findings that the HBsAg mutations in MHR were observed in all isolates revealed the importance of monitoring the MHR mutations in this country. The development of an optimized HBV screening, vaccine program and a monitoring system of MHR mutation are urgently needed in North Rift Kenya.

2. Keywords: HBV genotypes; MHR mutations; Outpatient attendees; Kenya

3. Introduction

Global estimates in the year 2015 suggest that quite 2 billion people are infected with Hepatitis B Virus (HBV) and 250 million of those people are chronically infected, of which 65million live in Africa [1, 2, 3].

The Prevalence of HBV among the rural population in Kenya stood at 8.8% in the year 2011 [4] while that of pregnant women attending antenatal clinic at Moi Teaching and Referral Hospital (MTRH) in Eldoret, North Rift Kenya stood as 9.3% in the year 2006 [5].

HBV exhibits genetic variability with an estimated rate of 1.4-3.2 x 10^{-5} nucleotide substitution per site per annum [6]. The genetic variability has resulted in emergence of ten HBV genotypes (A-J) that differ in more than 8% of the genome and their sub genotypes [7, 8]. HBV genotypes play a role in both course of infection and treatment management [9]. Genotypes exhibit structural and functional differences which can have an effect on how an HBV-infected person responds to treatment against the virus. It can also have an influence on the manifestation, severity and vaccination against the virus [7, 10]. In addition to HBV genotype diversity, genomic variation on HBV surface antigen (HBsAg) has led to description of mutations with considerable effect. Mutations within the HBsAg central region namely Major Hydrophilic Region (MHR), amino acid (a.a.) substitutions at 99-169; have been shown to be related with failure of HBsAg detection, antiviral resistance and vaccine escape [11, 12].

Previous studies have shown a high prevalence of HBV among populations in North Rift Kenya [13]. There is limited information on prevalent genotypes in many African countries including Kenya [3]. There could be more genetic diversity expected in Kenya since the presence of various HBV genotypes have been reported in Africa [14, 15, 16]. The aim of this study was to establish the HBV genotypes, associated mutations and HBV/HIV co-infections among outpatient attendees at MTRH in North Rift Kenya.

4. Materials and Methods

This was a cross-sectional study involving 200 outpatients who were unaware of their HBV or HIV status seeking treatment at MTRH in Eldoret, Kenya during the period between September 2015 and October 2016. The recruited study subjects were aged between 18 and 82 years. Majority of the outpatients (53%) were female. The median age of study participants was 35years, with a range of 18 to 82 years. After informed consent and ethical clearance from Scientific and Ethical Review Unit of Kenya Medical Research Institute (KEMRI), blood samples were collected from consenting participants.

4.1. Serological Assays

The serum obtained from the blood samples was tested for the presence of HBV surface antigen (HBsAg) using Hepanostika® ultra II HBsAg (Netherlands) kit according to the manufacturer instruction. For anti-HIV antibody (Ab) screening, Vironostika® HIV Uni-Form II Ag/Ab Enzyme Linked Immune Sorbent Assay (ELISA) kit (Bio-Merieux®, Boxtel, Netherlands) was used as instructed by manufacturer.

4.2. Deoxyribonucleic Acid (DNA) Extraction and Amplification of HBV S-Gene

Viral DNA was extracted from 200µL of serum using the QIAamp® DNA Blood Mini Kit (QIAGEN® GmbH, Hilden, Germany) according to the manufacturer's instructions, and eluted in 100µl buffer. Eluted DNA samples were stored at -40^{0}C until use.

Nested Polymerase Chain Reaction (PCR) was performed to amplify part of HBV S-gene. The first PCR was done using primers S1F (5'-TCC TGC TGG TGG CTC CAG-3') and S1R (5'-CGT TGA CAT ACT TTC CAA TCA A-3'). Second PCR was carried out with primers S2F (5'-ACC CTG YRC CGA ACA TGG A-3') and S2R (5'-CAA CTC CCA ATT ACA TAR CCC A-3'). Amplification conditions were initial denaturation at 95^{0}C for 7min, followed by 35 cycles of 45sec at 94^{0}C denaturation, 30sec at 46^{0}C annealing, and 2min at 72^{0}C extension, followed by a final extension of 7min at 72^{0}C; using Applied Bio systems® Thermal Cycler (Bio-Rad Lab, Singapore). Cycling parameters for the second PCR remained the same as in the first one except that the annealing temperature was increased to 50^{0}C. In order to prevent PCR carryover contamination strict care and procedures was implemented as previously mentioned [17].

4.3. DNA Sequencing

Big Dye Terminator v3.1 Cycle Sequencing Ready Reaction Kit was then used for bi-directional sequencing with ABI PRISM® 3730 DNA Analyzer (Applied Bio systems®, CA, USA). The second pair of primer (S2F and S2R) was used for sequencing.

4.4. Sequence Analysis and Genotyping

Multiple sequence alignment was conducted using ClustalW implemented in MEGA-7 (Molecular Evolutionary Genetics Analysis version 7) [18]. HBV genotypes were determined with phylogenetic analysis of aligned sequences in comparison to HBV reference sequences retrieved from GenBank. Phylogenetic tree construction was done basing on Neighbor-Joining method using Kimura-2 parameter model and estimation of the tree reliability using bootstrap method of 1000 replicates. All of these processes were done using MEGA-7 [19]. Visualization of the tree was done using Tree View program. The accession numbers of HBV isolates sequenced in this study have been deposited in GenBank as MK177878-MK177891.

4.5. Mutation Analysis

Amino-acid sequences obtained from translation of DNA sequences were compared with known wild type (ALG02604.1), to analyze presence of mutations within the MHR. The mutation pattern was assessed based on published reports [20, 21] using GENETYX® *ver.*9.

4.6. Statistical Analysis

SPSS® (Statistical Package for the Social Sciences) *ver.* 22 (IBM Corporation, Armonk, NY, USA) was used as tool for statistical analysis.

Categorical data were analyzed using the Chi-square test. A *p*-value of <0.05 was considered statistically significant.

5. Results

Out of the 200 samples, 20 (10%) were HBsAg positive. The anti HIV-Ab was found in 10% (2/20) of the HBsAg positive samples.

5.1. HBV Genotype

HBV partial S-gene was successfully amplified and sequenced from 14 of 20 HBsAg positive samples. Based on phylogenetic analysis of the 14 isolate sequences with reference sequences obtained from GenBank (Figure 1), 13 (92.9%) of the isolates classified into sub genotype A1 (HBV/A1), while 1 (7.1%) of isolates categorized into sub genotype D1 (HBV/D1). None of the sequence isolates from this study classified with the recombinant strains sequences.

5.2. Mutation in HBV S-region

In all the 14 isolates either one or more mutation was detected within the MHR (Figure 2). The most occurring amino acid substitution within the MHR was S114T which appears in 13/14 isolates (Table 1). No known mutations associated with occult HBV infection or vaccine escape were observed.

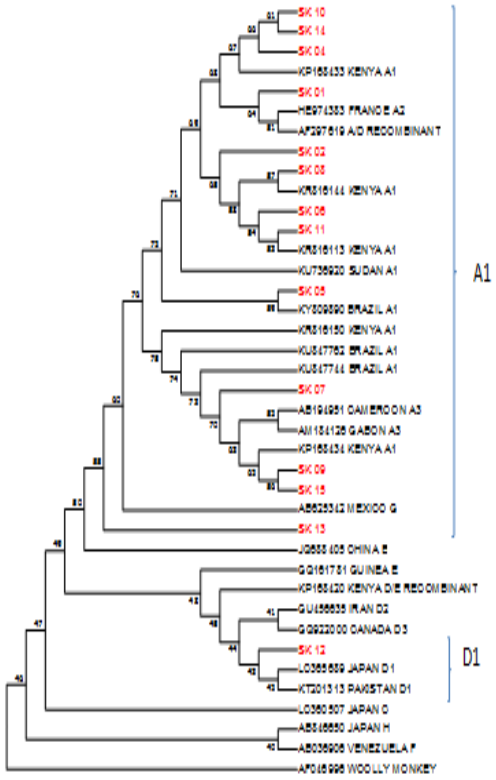

Figure 1: Phylogenetic analysis of HBV S-gene region among outpatients at MTRH in North Rift Kenya. The samples obtained from this study are written in red.

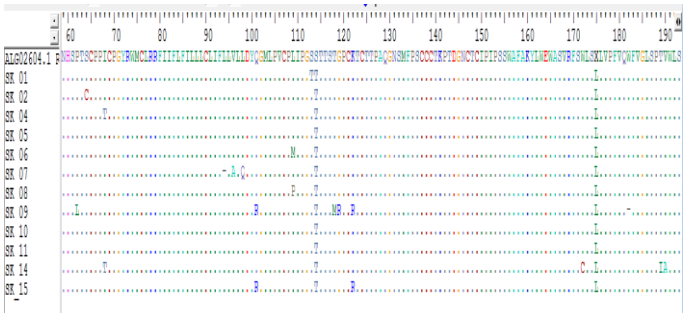

Figure 2: Distribution of amino acid substitutions (mutations) detected within the major hydrophilic region (MHR) of HBV S-gene among 12 genotype A1 isolates aligned with a reference sequence (ALG02604.1) retrieved from GenBank.

Table 1: Distribution of mutations in partial HBV S-gene among isolates from outpatients at MTRH.

HBV S-gene Region	Mutation	Sample/Sequence Identification
MHR (a.a positions 99-169)	Q101R	SK.09,15
	L109M	SK.06
	L109P	SK.08
	S113T	SK.01
	S114T	SK.01,02,04,05,06,07,08,09,10,11,12, 14,15
	T118M	SK.09
	G119R	SK.09
	K122R	SK.09,15

6. Discussion

Kenya is a country with high HBV carrier among the blood donors [22] and neighboured by countries with variety of HBV genotypes distribution. However, there is still limited report regarding the molecular study of HBV among outpatient attendees in different hospitals in Kenya available. Therefore, in this study we determined the prevalence, genotype distribution and mutational patterns of HBV isolates from outpatients at MTRH in North Rift Kenya.

In this study, 10% of the samples were HBsAg positive and the anti-HIV Ab was found in 10% of the HBsAg positive samples. This finding agrees with previous studies that have shown a high prevalence of HBV among populations in North Rift Kenya [13]. Chronic HBV infection affects 10% of individuals living with HIV worldwide and 6% in Kenya with the majority of these individuals living in low- and middle-income countries [23-25].

Our study demonstrated the presence of genotype A sub genotype A1 (92.9%) and genotype D sub genotype D1 (7.1%) among MTRH outpatients. The predominance of genotype A is consistent with recent HBV genotype distribution studies in other parts of Kenya like Nairobi, Kericho and Mombasa [13, 26, 27]. The HBV genotypes show a definite geographical distribution in Africa, with genotype A predominating within the South-East, genotype D within the North and genotype E within the West [7]. HBV genotype A has been re-

ported to be predominant in Kenya [26, 28], Uganda [29] and Tanzania [30], while genotype D is prevalent in Sudan [31, 32] and Egypt [33]. Knowing about the predominant HBV molecular variants present at specific area is significant for the assessment of diagnostic capabilities and vaccine efficacy [34].

Amino-acid substitutions at MHR are associated with immunological pressure resulting from both natural and HBV vaccination [11, 21]. Mutations at MHR are shown to be connected with diagnostic problems, emergence of vaccine-escape mutants, and HBV immune globulin medical care failure [11-12, 21]. The mutation analysis of our study showed that all 14 isolates harboured amino-acid substitutions within the MHR. Another study conducted on HBV/HIV mono-or co-infected Ethiopians reported prevalence of immune-escape mutants at totally different amino-acid positions [35]. G145R has been found in various studies shown to contribute to occult HBV infection [29]. There was no G145R mutation found in the samples sequenced and this may be a rare mutation in Kenya. These findings of mutations in the MHR in our study which represents immune escape variants should be taken into account as there is a possibility for these variants to unfold more and consequently influence immunogenic efficaciousness and treatment strategy within the country. The present study suggests that the outpatients at MTRH may develop chronic sequelae of HBV infection and could be a source of unaware HBV transmission in the community.

The participants of this study were outpatients who were seeking treatment for other different ailments at the hospital and were not aware of their HBV or HIV status. Since testing for HBV and genotype determination is not routinely done in Kenya, the population could be exposed to a potential chronicity of infections due to the presence of asymptomatic HBV mono-and HBV/HIV co-infected people indicating a long standing transmission of these viruses circulating in the population without notice. This could consequently lead to development of advanced diseases due to HBV and HIV over time. Therefore HBV testing would be important especially in the implementation of diagnostic measures in the population. And there is a need for hospitals to advise patients on the importance of knowing their HBV status and getting vaccinated against HBV.

HBV genotyping that was previously considered a research tool has been suggested as an important element to guide the selection of therapy by several investigators and national professional associations [36]. Genotype A1 has been reported to be associated with high rates of hepatocellular carcinoma in sub-Saharan Africa [37]. Genotype information of chronic HBV infections enables practicing physicians to identify those patients at risk of disease progression and to determine the appropriate and optimal anti-viral therapy [38]. Thus our study will become an input in narrowing the existing gap of HBV molecular studies in Kenya.

7. Conclusion

Ten percent of outpatients at MTRH could be a source of unaware HBV transmission in the community. HBV/A1 remains the most predominant genotype. The findings that the HBsAg mutations in MHR were observed in all isolates revealed the importance of monitoring the MHR mutations in this country. The development of an optimized HBV screening and vaccine program and a monitoring system of MHR mutation are urgently needed in North Rift Kenya.

8. Acknowledgement

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References

1. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet, 2015; 386: 1546-55.

2. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med. 2004; 350: 1118-29.

3. Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes and clinical associations of genotypes. Hepatol Res. 2007; 37: S9-s19.

4. Mutuma GZ, Mbuchi MW, Zeyhle E, Fasana R, Okoth FA, Kabanga MJ et al. Prevalence of Hepatitis B Virus (HBV) surface antigen and HBV-associated hepatocellular carcinoma in Kenyans of various ages. Afr J Health Sci. 2011; 18: 53-61.

5. Okoth F, Mbuthia J, Gatheru Z, Murila F, Kanyingi F, Mugo F et al. Seroprevalence of hepatitis B markers in pregnant women in Kenya. East Afr Med J. 2006; 83: 485-93.

6. Fares MA, Holmes EC. A revised evolutionary history of hepatitis B virus (HBV). J Mol Evol. 2002; 54: 807-14.

7. Kramvis A, Kew M, Francois G. Hepatitis B virus genotypes. Vaccine. 2005; 23: 2409-23.

8. Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, et al. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. Intervirology. 2004; 47: 289-309.

9. Kramvis A, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. J Viral Hepat. 2005; 12: 456-64.

10. Kao JH, Chen PJ, Lai MY, Chen DS. Clinical and virological aspects of blood donors infected with hepatitis B virus genotypes B and C. J Clin Microbiol. 2002; 40: 22-5.

11. Coleman PF. Detecting hepatitis B surface antigen mutants. Emerg Infect Dis. 2006; 12: 198-203.

12. Wu C, Zhang X, Tian Y, Song J, Yang D, Roggendorf M et al. Biological significance of amino acid substitutions in hepatitis B surface antigen (HBsAg) for glycosylation, secretion, antigenicity and immunogenicity of HBsAg and hepatitis B virus replication. J Gen Virol. 2010; 91: 483-92.

13. Hannoun C, Soderström A, Norkrans G, Lindh M. Phylogeny of African complete genomes reveals a West African genotype A subtype of hepatitis B virus and relatedness between Somali and Asian A1 sequences. J Gen Virol. 2005; 86: 2163-7.

14. Hasegawa I, Tanaka Y, Kurbanov F, Yoshihara N, El-Gohary A, Lyamuya E, et al. Molecular epidemiology of hepatitis B virus in the United Republic of Tanzania. J Med Virol. 2006; 78: 1035-42.

15. Mwangi J, Nganga Z, Songok E, Kinyua J, Lagat N, Muriuki J, et al. Molecular genetic diversity of hepatitis B virus in Kenya. Intervirology. 2008; 51: 417-21.

16. Saudy N, Sugauchi F, Tanaka Y, Suzuki S, Aal AA, Zaid MA et al. Genotypes and phylogenetic characterization of hepatitis B and delta viruses in Egypt. J Med Virol. 2003; 70: 529-36.

17. Bell TG, Makondo E, Martinson NA, Kramvis A et al. B. Hepatitis virus infection in human immunodeficiency virus infected Southern African adults. PLoS One. 2012; 7: e45750.

18. Makondo E, Bell, A. Kramvis. Genotyping and molecular characterization of hepatitis B virus from human immunodeficiency virus-infected individuals in Southern Africa. PLoS One. 2012; 7: e46345.

19. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016; 33: 1870-74.

20. Missiani O, James H. Kimotho, Oyugin J, Okoth F, Kioko H, Mining S et al. 'Hepatitis B Infection Is Highly Prevalent among Patients Presenting with Jaundice in Kenya'. BMC Infectious Diseases. 2016; 16: 101.

21. Aslanzadeh J. Preventing PCR amplification carryover contamination in a clinical laboratory. Ann Clin Lab Sci. 2004; 34: 389-96.

22. Echevarria JM, Avellon A. (2006). Hepatitis B virus genetic diversity. J Med Virol, 78 (Suppl 1), S36-42.

23. Shah, Reena & Karuru, Jane & Nelson, Mark & Stebbing, Justin. HIV, hepatitis B and hepatitis C coinfection in Kenya. AIDS (London, England). 2008; 22: 1221-2.

24. Soriano V, Puoti M, Peters M, Benhamou Y, Sulkowski M, Zoulim F et al. Care of HIV patients with chronic hepatitis B: updated recommendations from the HIV-HBV international panel. AIDS. 2008; 22: 1399-1410.

25. Soriano V. Viral hepatitis and HIV co-infection. Antiviral Research. 2010; 85: 303-15.

26. Ma Q, Wang Y. Comprehensive analysis of the prevalence of hepatitis B virus escape mutations in the major hydrophilic region of surface antigen. J Med Virol. 2012; 84: 198-206.

27. World Health Organization (WHO). (2008 August). Fact Sheet N°204 on Hepatitis B Virus. Revised August 2008. http://www.who.int/mediacentre/factsheets/fs204/en/ September 10, 2009.

28. Kibaya MR, Lihana W, Kiptoo, R, Songok ME, Ng'ang'a Z, Osman S et al. Characterization of HBV among HBV/HIV-1 Co-Infected injecting drug users from Mombasa, Kenya. Current HIV research. 2015; 13: 292-9.

29. Nyairo Ednah K, James Kimotho, and Samoel A Khamadi. Mutations in the "a" Determinant Region of Hepatitis B Virus Genotype A among Voluntary Kenyan Blood Donors. Journal of Biology, Agriculture and Healthcare. 2016; 6: 1–8.

30. Kwange SO, Budambula NL, Kiptoo MK, Okoth F, Ochwoto M, Oduor M et al. Hepatitis B virus subgenotype A1, occurrence of subgenotype D4, and S gene mutations among voluntary blood donors in Kenya. Virus Genes. 2013; 47: 448-55.

31. Calisti G, Muhindo R, Boum Y, Wilson LA, Foster GM, Geretti AM et al. Epidemiology of HBV infection in a cohort of Ugandan HIV-in-

fected patients and rate and pattern of lamivudine-resistant HBV infection in patients receiving antiretroviral therapy. Trans R Soc Trop Med Hyg. 2015; 109: 723-9.

32. Yousif M, Mudawi H, Bakhiet S, Glebe D, Kramvis A. Molecular characterization of hepatitis B virus in liver disease patients and asymptomatic carriers of the virus in Sudan. BMC Infect Dis. 2013;328.

33. Yousif M, Mudawi H, Hussein W, Mukhtar M, Nemeri O, Glebe D et al. Genotyping and virological characteristics of hepatitis B virus in HIV-infected individuals in Sudan. Int J Infect Dis. 2014; 29:125-32.

34. Stanaway JD, Flaxman AD, Naghavi M, Fitzmaurice C, Vos T, Abubakar I et al. The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet. 2016; 388(10049): 1081-8.

35. Belyhun Y, Maier M, Liebert UG. HIV therapy with unknown HBV status is responsible for higher rate of HBV genome variability in Ethiopia. Antivir Ther. 2017; 22: 97-111.

36. Cooksley WGE. Do we need to determine viral genotype in treating chronic hepatitis B?. J Viral Hepatitis, 2010; 17: 601-10.

37. McMahon BJ. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. Hepatology international. 2009; 3: 334-42.

38. Tanwar S, Dusheiko G. Is there any value to hepatitis B virus genotype analysis?. Curr Gastroenterol Rep. 2012; 14: 37-46.