Characterization of Hepatitis B Virus (HBV) Among Liver Patients in Kenya

By

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Introduction: Viral Hepatitis

- Hepatitis A Virus
- Hepatitis B Virus
- Hepatitis C Virus
- Hepatitis D Virus
- Hepatitis E Virus

- It is the most prevalent of all Hepatitis (>8% in Kenya)
- HBV Progress to either acute, asymptomatic carriers and chronic infection.
- The infection worsen if co-infected with HDV
- Transmitted through sexual intercourse, horizontal and thro’ blood products
- Have 10 genotypes (A-I) with several subtypes that are geographically distributed
Global distribution of HBV

HBV is a member of the *Hepadnaviridae family* and is known to be one of the smallest human DNA virus

- HBV has four open readings frames (ORF) and three antigens; namely **HBeAg**, **HBsAg** and **HBcAg**.

- Basal Core Promoter (BCP) and Pre-core (Pc) are responsible for expression of (HBeAg).

- The presence of HBeAg confers immunity

- **HBsAg**- used for detection and vaccination

- Polymerase gene - treatment - ARV
Genotypes among patients with jaundice

Among chronic patients

- Genotype A1 and outlier of the clade containing subgenotype D6 and the D/E recombinant
- HBeAg-negativity was a result of G1896A in HBV/D
- Mutations at positions A1762T and G1764A occurred more frequently in HCC patients (p < 0.05).
Genotypes and HBsAg mutations among Blood donors

- 85.7% - A1 and 14.3% - D4 in coastal region

- The HBsAg mutations included
  - F20S, P46A,
  - K122R, T189I
  - S55F, Y200F
  - S204N, S193L

- No occult detected or vaccine mutant strains

Abstract: Kenya is one of the high endemic zones for hepatitis B virus (HBV) infection. The consensus on prevalence of the HBV genotypes and the existence of their variants have not been fully established in Kenya. Hence, there is a need to further monitor the diversity of HBV. This study aimed to extend the current molecular and epidemiological information about the geographical distribution of HBV genotypes and subgenotypes, as well as to describe the hepatitis B surface antigen (HBsAg) variants circulating in different Regional Blood Transfusion Centres of Kenya. A total of 32 HBsAg positive blood units (65.6%) were DNA positive and were successfully sequenced. Eighteen out of the twenty-one isolates (85.7%) belonged to subgenotype A1 Afro-Asian; six were from Nairobi, four from Kisumu, two from Embu, and three each from Eldoret and Mombasa. The other three strains (14.3%, 3/21) belonged to subgenotype D4 from Mombasa. The HBsAg mutations were detected in nine isolates (42.9%, 9/21). The HBV/A1 and HBV/D4 are dominant among blood donors in Kenya. This demonstrates that continuous monitoring of the HBV diversity would help reveal circulating genotypes and subgenotypes.
Genotypes and HBsAg mutations among Blood donors

HBV/A1 (90.3 %) followed by D (9.7 %) among HBV DNA positive specimens. Full genome analysis showed HBV/D isolates having similarity to both D4 and D6 subgenotypes and D/E recombinant reference.
Main Objective

• This cross-sectional characterized the Hepatitis B virus among chronic patients enrolled at liver clinic in Kenya:

• Specific objectives was to Find out:
  • the HBV genotypes in circulation,
  • Mutations in full S-region
  • Basal Core Promoter (BCP) mutations
  • Mutation in the Polymerase gene
Serology and Molecular Methods

1) 7ml of blood was taken
2) Serological tests: HBsAg ELISA, HBeAg, Anti-Core IgM and IgG
3) Serology for HIV and Anti-HCV

HBV DNA extraction

BCP amplification

- BCP PCR -ve
- BCP PCR +ve

Full S- gene amplification

- S- gene PCR +ve
- S- gene PCR -ve

Sequencing

Bio-informatics and phlogenic analysis
HBV genotypes in Kenya

- **HBV +ve** 54/88 (61.4%)
- **HBV genotype A1** was prevalent 34/44 (77.2%)
- **HBV genotype D** 6/44 (13.6%) and
- **Genotype E** 1/44(2.2%).
- Genotype A1 clearly distinguished into A1 Africa and A1 Asia strains

(Mwangi 2008, kwange et al 2013 and ochwoto et al 2013, Ochwoto 2015 found similar results)
HBV/D and HBV/E Genotypes

- **HBV/D 4/6 were D6**
  - West Africa mainly Tunisia

- **HBV/D 2/6 were D1**
  - clustered with sequences from Asia, Iran, Lebanon and China

- **Genotype E**
  - 1/44 (2.2%)
  - clustered with sequences from Senegal and Côte d’Ivoire.
HBV Diversity in Kenya

- Genotype A is distributed throughout the country.
- Genotype D is common in the Western Strip.
- Recombinant D/E was from Eldoret.
- The clear distinctions of HBV genotypes/subgenotypes depict population migration.
HBV/D Recombinants and unique subtype

- Full genome analysis of HBV/D isolates showed that a 2 were (D/E recombinant) with 31 insertion nt
A/E Recombinant in circulation

• Previous study had a putative new Recombinant of D/E in circulation in the North Rift.

• Then current study identified the same Recombinants

• Recombination analysis of the full genome sequences showed it was a (D/E)

• The prevalent of this
HBV Mutations

Electrophorogram: nt.1862

Wild

G1862G

Nt. GTT
AA Valine

Mutant

G1862T

Nt. TTT
AA Phenylalanine
Pre-core and BCP Mutations

➢ HBV-core promoter mutations
  ➢ A1762T/G1764A - 11/44 (25%)
  ➢ pre-core G1896A - 4/44 (9.1%)
  ➢ G1862T – 2/44 (4.5%)

➢ A1762T/G1764A, G1862T and G1896A mutations are associated with HBeAg negative and HCC status
Mutations in the S-gene

- **PreS1** - T86S mutation was the most common 24/43 (55.8 %)
- **PreS2** - 20/43 (46.5%) had (P54S, G19D and A24V).

One patient had a combination of triple mutations was detected (Q10H, T31I, P35Q).

Other mutations detected in this region were at position Q2R, W3C, Y21N, I45T, R48N, and H9Q.
Immunodominant “α” determinant

- HBsAg gene:
- Within the antigenic determinant region (aa100-160), no mutations were found with HBV/D sequences
- whereas in HBV/A1; sG112R, sT114S, sF134V, sT143M, sM103I, sQ129R, sG130N, and sP135H mutations were observed.

Most diagnostic kits target (aa100-160) region the effect of such mutations on diagnostic remain unknown

Zhu HL, Li X, Li J, Zhang ZH. Genetic variation of occult hepatitis B virus infection. World J Gastroenterol 2016; 22(13): 3531-3546
Two drug resistance; **sM204N** and **rtS202I** mutations were detected among chronic patients. The patients were not on at the treatment.
Conclusion

❖ More than 80% of HCC are caused by hepatitis B infection (Muttuma et al., 2011).

❖ HBV infection is preventable with a vaccine, Vaccination decreases the incidence of HCC.

❖ S-gene mutations may lead to occult HBV. Combination therapy for Chronic HBV patients as initial strategy is required for patients with inadequate response to mono-therapy.

❖ Treatment of hepatitis B infection reduces the risk of developing HCC regardless of its high cost
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