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ABSTRACT

Background: Hepatitis B virus (HBV) is often associated with chronic hepatitis, cirrhosis and hepatocellular carcinoma. The World Health Organization (WHO) rolled out guidelines for treatment of HBV globally in 2015 and the Kenyan Ministry of Health started implementing these guidelines in 2018. About one thousand patients have been receiving the WHO-recommended treatment across the country. However, their treatment outcome has not been evaluated. Baringo County is one of the counties with high number of patients on treatment. The aim of this study, therefore, was to evaluate the treatment outcome of these patients.

Methods: Forty-one HBV positive patients attending comprehensive care clinic in Marigat, Baringo County were randomly selected. Five millilitres of blood were drawn from each patient and processed into serum. Presence of hepatitis B surface antigen (HBsAg), surface antibodies (HBsAb), e-antigen (HBeAg), e-Antibodies (HBeAb) and core antibodies (HBcAb) were tested using lumiquick HBV-5 panel test kit (Diagnostic, Inc, USA). HBV DNA extraction was done using Qiagen extraction kit, amplified and visualized on 2% agarose gel. All positive extracts were sequenced. HBV viral load was done using TaqMan™. HBV sequences obtained were aligned and phylogenetic trees developed. Genotypes were determined using geno2pheno software and mutations on the surface antigen and polymerase coding regions were determined from the aligned sequences.

Findings: Out of 41 serum samples collected, 2.4% patient was HBsAg negative and the remaining (97.6%) positive. HBsAb positivity was 2.4%, all samples were negative for hepatitis B core antibodies (HBcAb), 14.6% tested positive and 35(85.6%) tested negative for HBeAg. The mean age (±SD) of patients was 33.59 ± 2.307 (8-80). Ten (24.3%) samples were detectable in qPCR and their viral load ranged from 4.6x10⁴ to 1.04x10¹ IU/ml with majority of them (85.6%) having HBeAg negative and 80.4% having HBeAb positive. Two genotypes, genotype D (50%) and genotype A (50%) were identified among the HBV isolates. Putative mutations: rtM129L, rtW153R, rtP237T, rtN238T, and rtN248H were detected in the RT domain and have been identified to be associated with drug resistance, but not yet been confirmed experimentally in vitro.

Keywords: Hepatitis B virus, treatment outcome, genotypes, antiretroviral drug resistance
1. INTRODUCTION

Hepatitis B virus (HBV) is an enveloped DNA virus often associated with chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). In 2015 alone, 720,000 deaths and 470,000 deaths were due to liver cirrhosis and hepatocellular carcinoma respectively (Byambasuren et al., 2019). Hepatitis B virus is prevalent in sub-Saharan Africa and Western Pacific Regions (WHO, 2017). Globally, HBV has infected two billion people of which 257 million are chronic carriers (McLachan & Cowie, 2015). Kenya is categorized as HBV moderate risk zone with estimated prevalence of 5-7% (Ly et al., 2016). A survey conducted the ministry of Health through National sexually transmitted infection Program (NASCOP) found the national prevalence of HBV to be 3.4% with some areas having a prevalence as high as 13.3% (Malungu Ngaira et al., 2016). Baringo County, located in the former Rift valley province, has a notably much higher HBV disease incidence (O’Hara et al., 2017). In general there is also lack of accurate prevalence data in the general population (Ngaira et al., 2016).

Serological markers are indispensable in the diagnosis of HBV infection. HBsAg, is the main diagnostic marker of HBV infection and HBeAg provide information about active virus replication. HBV DNA amplification and quantitation assays continue to serve as valuable tools to monitor progression of HBV infection. The three viral markers are important for treatment initiation and the efficacy of treatment in chronic HBV infection (Heimbach et al., 2018; Liu et al., 2017). But given the dynamic nature of chronic HBV infection, regular monitoring of HBV DNA is done along the liver function tests and platelets count so as to accurately characterize the phase of infection. Detection of HBV DNA that is >2000IU is a reliable marker of active chronic HBV in patients with HBeAg-negative disease.

Lamivudine, a nucleoside analogue, was the first drug approved in 1998 to suppress HBV replication through inhibition of the reverse-transcriptase and DNA polymerase activities. Later, others nucleotide analogues have been also approved: adefovir (in 2002), entecavir (in 2005), telbivudine (in 2006), and most recently, tenofovir disoproxil fumarate (TDF) (in 2008) (Lai & Yuen, 2008). Lamivudine being the first nucleotide analogues has shown resistance; the main mutations responsible for lamivudine resistance include rtM204V and rtM204. rtA181V/T and rtN236T showed resistance after 5 years of treatment for adefovir (Fung et al., 2011). In addition to the rtM204V and rtL180M mutations that are responsible for resistance to lamivudine, mutation at rtI169T, rtT184G, rtS202I or rtM250V are required for entecavir resistance (Baldick et al., 2008). For telbivudine, rtM204I has been identified as the main mutation conferring primary resistance. Other mutations associated with telbivudine resistance include mutations at rtA181T/V and rtL229W/V. To date, tenofovir disoproxil fumarate (TDF) is the first line nucleoside analogue used to treat HBV chronic (Fung et al., 2011). High cost of medical care and antiviral drugs, frequent unpleasant side effects responsible for discontinuation of the therapy in some patients are the most critical challenge in treating CHB (Nwokediuko, 2011). The WHO rolled out guidelines for treatment and management of HBV globally in 2015 (World Health Organisation, 2015) and many countries in sub-Saharan Africa are now in the process of adopting these guidelines and strategic plans to achieve hepatitis elimination (Heimbach et al., 2018). The WHO guidelines have been implemented in Kenya since 2018, taking over the locally formulated and published protocols by the Kenyan Ministry of Health in 2014 (Gastroenterology Society of Kenya, 2014). Over 1000 patients have been enrolled into the treatment program across the country (WHO, 2017).
Baringo country was among the first counties to roll out the HBV treatment program and has the highest number of patients attending Marigat Sub-county hospital comprehensive care clinic (CCC). To date no study that has been conducted to establish the outcome of this treatment within the three-year period. The objective of this study, therefore, was to determine the molecular outcomes of the treatment in HBV-positive patients attending Marigat comprehensive care clinic in Baringo County of Kenya, where prevalence of HBV is known to be high (Muchiri et al., 2012).

2. MATERIALS AND METHODS

2.1 Study design and participants

This was a cohort randomised experimental study that involved HBV-positive patients attending a comprehensive care clinic (CCC) in Marigat subcounty Hospital, Baringo County. These patients were on HBV anti-retroviral therapy (HBV-ART) according to WHO 2015-guided treatment. The patient’s CCC list was used to select patients to participate in the study, every third patient in the list was randomly selected. Only those who consented the study to use of their blood specimen and clinical data were allowed to participate. Consent was sought from parents or guardians for minors who participated in the study. The study protocol was approved by Jomo Kenyatta University of Agriculture and Technology Scientific Ethics Review Committee (approval number: JUK/IERC/02316/0055) and experiments done at the Kenya Medical Research Institute (KEMRI) laboratories.

2.3 Sample Collection and transportation

Five millilitres of venous blood were drawn from the patients and serum obtained by centrifugation of the blood at 3500 rpm (Hittich Centrifuge D-7200®) for 10 minutes at the site of collection. The serum was aliquoted into two 2ml vials and shipped in cold chain to the KEMRI for laboratory tests where it was stored at -80°C till use.

2.4 Serological profiling of HBV antibodies and antigens

Presence of HBsAg was detected using DiaSorin murex® EIA test kit version 3 (DiaSorin S.p.A, UK) according to the manufacturer’s instruction. The other serological profiling of antibodies and antigens were detected using Lumiquick HBV-5 panel Rapid test kit (Lumiquick Diagnostics, Inc., USA). Hepatitis B surface antibody (HBsAb), Hepatitis B envelop antigen (HBeAg), Hepatitis B envelop antibody (HBeAb) and Hepatitis B core IGM antibody (HBcAb-IGM) in the samples was detected following the manufacturer’s instructions. Briefly, eighty microlitres of serum sample was dropped into each individual kit sample well and the results read in twenty minutes. Presence of two lines indicated a positive result whereas presence of a single line indicated a negative result for three serological markers (HBsAb, HBeAg, HBcAb-IGM) contrary to HBeAb where presence of two lines indicated a negative result whereas presence of a single line indicated a positive result.

2.5 DNA extraction and amplification of the Surface Antigen gene

The other vial of serum samples was subjected to DNA extraction using QIAamp DNA Mini kit (Qiagen, Germany) according to the manufacturer’s instructions. The extracted DNA was eluted in 60µl. The part of the HBV genome encoding the Major Hydrophilic Region (which also includes the ‘a’ determinant) up to the last amino acid of surface gene and overlapping catalytic domains of Polymerase gene (nucleotide position 425 to 840) was amplified by nested polymerase chain reaction (PCR). Briefly, 5µl of the extracted DNA was used for amplification
by nested PCR in 20µl reaction mixture containing 4µl of 5x Fire Pol Master Mix (Solis BioDyne®), 0.2µl of each forward and reverse primers (10 µM), 10.6µl nuclease-free water. The first set of primers (S1F: TCCTGCTGGTGGCTCCAG, S1R: CGTTGACATACTTTCCAATC) was used for the amplification of complete surface gene using Applied biosystems Veriti 96 Well thermal cycler, USA. The cycling conditions were 40 cycles of denaturation at 94°C, annealing at 62°C, primer extension at 72°C and a final single cycle for primer extension at 72°C for 7 min. Samples that were negative for 1st round were subjected to second round of PCR amplification. The primers used for the second round were S2F: ACCCTGYRCCGAACATGGA and S2R: CAACTCCCAAATTACATARCC. The reaction conditions for amplification were as round one above. Five microlitres of nested PCR product was analysed on 2% agarose gel stained with Gel Red nucleic acid dye (Biotium®) and viewed by GelDoc-It Imager (Transilluminator., USA) at 265nm.

2.6 Sequencing

Ten (10) microliters of the amplified DNA were sent to Macrogen® Europe for sequencing using the second-round amplification. The PCR product was purified with ExoSAP-IT™ PCR Product Clean-up Reagent Kit and sequenced by Big Dye chain-terminator (sanger sequencing) method using ABI 3730xlDNA Analyzer (96 capillary type).

2.7. Phylogeny

The electropherograms were visualized, edited and assembled through the Bioedit software v7.2 (Tom Hall) and DNA Baser software v5.15.0. Mutations were identified using Gene2pheno v2.0 online tool. Assembled sequences in fasta format were imported to Mega X v10.1 software, multiple sequence alignment built, the aligned sequences saved, exported to mega format and finally the phylogeny tree constructed by Maximum (ML) Likelihood method.

2.8 HBV Viral loads (qPCR) assay

Quantitative Polymerase Chain Reaction (qPCR) was used to access the viral load in patient samples. The reaction mix was set up with TaqMan™ Universal PCR Master Mix Kit (Applied Biosystems) according to the manufacturer’s instructions. A 25ul reaction comprised of 2.5ul of nuclease-free water, 12.5µl of TaqMan Universal PCR Master Mix (2X), 2.5µl of each forward and reverse primers (10 µM), 2.5µl of TaqMan probe (2.5µM) and 2.5µl DNA. The denaturation was done at 95 ºC for 10 min, annealing at 95 ºC for 15 s and elongation at 55 ºC for 1 min for a total of 40cycles. The forward TaqMan PCR primer used was CAA CCT CCA ATC ACT CAC CAA C and the reverse ATA TGA TAA AAC GCC GCA GAC AC. The fluorogenic probe used (FAM-TCC TCC AAT TTG TCC TGG TTA TCG CT-TAMRA) was 5’-labelled with FAM (6-carboxyfluoresceine) and 3’-labelled with TAMRA (6-carboxy3trimethylrhodamine). The 96 well plate was analysed using the Applied Biosystems 7500 fast real-time platform for one hour and forty-five minutes. The quantification cycle (Cq) also known CT value was recorded and the copy number of the samples calculated.

2.9 Data management and analysis

Patient demographic data was analysed using Statistical Program for Social Sciences (SPSS) version 22.0 and presented as percentages or as means ± (SD). The serological data was entered into SPSS software and variables such as: HBsAg, HBeAg, HBsAb, HBeAb, HBcAb tested by analysis of variance. The Student’s T test was used to analyze differences in viral load (mean). A P-value of >0.05 was considered not significant within 95% confidence limits. Treatment
outcomes of the patients were determined by comparing the current serological and virological data to the baseline data when the patient started the treatment. Changes in the viral loads level and seroconversion of serological markers (HBeAg and HBsAg) were considered significant. The loss of HBsAg among the patients on treatment was considered as having been cured. The loss of serum hepatitis B e antigen (HBeAg) and the development of anti-HBe antibodies (HBeAg seroconversion) marked a transition from the immune-active phase of disease to the inactive carrier state. HBsAg loss was considered a functional cure of chronic hepatitis B. Whereas undetectable HBsAg and HBV viral genomes was regarded as the ideal endpoint of the therapy. A drop of viral load from <2000IU/ml at the start of the treatment to undetectable levels was considered virus suppression.

Mega X v10.1 software was used to align the Fasta format of the sequences in mega format and the latter used to draw a phylogenetic tree using Maximum Likelihood method. On the aligned sequences, mutation on the HBsAg gene and polymerase gene for identification and drug resistance analysis was done using Gene2pheno v2.0 online tool.

3. RESULTS

3.1 Patient Demographic profile

A total of 41 out of 78 patients’ blood samples were collected from hepatitis B positives patients undertaking treatment from the comprehensive care clinic at Marigat sub-county, Baringo County, Kenya. The number of females was slightly more than that of males; 23(56.1%):18(43.9%) respectively. Of the 23 women 1(4%) was pregnant and 2(87%) were currently breastfeeding and the treatment ranged from a duration of less than 1year to 4 years. The longest period the patient was on treatment was 4 years; 7.3%, 3 years on treatment were 29.4%, 2 years were 14.6 %, one year 31.7% and less than a year were 17. % (Table 1).

Table 1: Patient demographics in terms of gender and duration of treatment of viral hepatitis.

<table>
<thead>
<tr>
<th>Gender; % (proportion)</th>
<th>Male 18 (43.9%)</th>
<th>Female 23 (56.1 %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological status; % (proportion) and frequency: Female</td>
<td>Pregnant 1 (4%)</td>
<td>Breastfeeding 2(87%)</td>
</tr>
<tr>
<td>Duration of treatment in years; % (proportion)</td>
<td>&lt;1 yr 7 (17%)</td>
<td>1 yr 13 (31.7%)</td>
</tr>
<tr>
<td></td>
<td>2 yrs 6 (14.6 %)</td>
<td>3 yrs 12 (29.4%)</td>
</tr>
<tr>
<td></td>
<td>4 yrs 3 (7.3%)</td>
<td></td>
</tr>
</tbody>
</table>

The age of the participants ranged from 8-80 years with a mean age of 33.6±14.8 years, and a majority number of patients aged 30-34 years (19.5%) and almost a quarter (19.7%) of the participants were less than 20 years of age. Kenya introduced national Hepatitis B vaccine for
infants in 2001 (Ndiritu et al., 2006) which intended to reduce HBV among infant through prevention of horizontal transmission, however, in this study population (19.7%) were participants born after 2001 (age below 20 years) (Figure 1).

Figure 1: Age distribution of hepatitis B patients attending Marigat Comprehensive Care Clinic.

Tenofevir disoproxil fumarate was only one drug of choice. Patients collected the treatment regimen monthly from the hospital CCC. Among the 41 none had failed to collect their medicine since they registered to the time the sample was collected. From the patient’s medical records, all patients met the WHO treatment criteria at enrollment: (a) patients with confirmed HBsAg positive results for over 6 months, (b) Viral load higher than 2000U/ml, with HBeAg + or -. Data on liver cirrhosis or biopsy was not available for all the patients.

3.2 Serological profiling

The samples collected were tested for the presence or absence of five serological markers (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb-IGM) in order to establish the patients’ serological status. Out of 41 serum samples, 40 (97.6%) tested positive and 1(2.4%) tested negative for HBV surface antigen (HBsAg) using EIA. The negative case, was a male aged 35 years and used the TDF for almost 2 years. Similarly, he was the only one with HBsAb+. His medical record showed he was having viral loads above 2000IU/ml at the time he registered for treatment. The current viral load was undetectable.

Among the 40 HBsAg positive samples, 23(57.5%) were female patients and 17 (42.5%) were male patients and the sample tested negative was from a male patient. Table 2 summarizes the prevalence of all the serological markers vis a vis their gender and also the viral load detection in HBV positive patients attending Marigat Comprehensive Care Clinic in Baringo County. All (100%) participants had immunoglobulin G antibodies to hepatitis B core antigen (HBcAb-IgG) indicating a viral infection, on the other hand none was recently infected with hepatitis as shown by negative results of immunoglobulin M antibodies to hepatitis B core antigen (HBcAb-IgM).
Table 2: Prevalence of serological markers by gender and viral load in HBV positive patients attending Comprehensive Care Clinic in Baringo County

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HBeAg</th>
<th>HBeAb</th>
<th>HBsAg</th>
<th>HBsAb</th>
<th>HBcAg-IgG</th>
<th>HBcAg-IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>Positive 6(14.6%)</td>
<td>Negative 35(85.6%)</td>
<td>Positive 33(80.4%)</td>
<td>Negative 8(19.6%)</td>
<td>Positive 40(97.6%)</td>
<td>Negative 1(2.4%)</td>
</tr>
<tr>
<td></td>
<td>Male Positive 16</td>
<td>Male Negative 16</td>
<td>Male Positive 2</td>
<td>Male Negative 17</td>
<td>Male Positive 1</td>
<td>Male Negative 0</td>
</tr>
<tr>
<td>Male</td>
<td>11.1%</td>
<td>88.9%</td>
<td>88.9%</td>
<td>11.1%</td>
<td>94.4%</td>
<td>5.6%</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0%</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Positive 4</td>
<td>Negative 19</td>
<td>Positive 18</td>
<td>Negative 5</td>
<td>Positive 23</td>
<td>Negative 0</td>
</tr>
<tr>
<td></td>
<td>Female Positive 19</td>
<td>Female Negative 18</td>
<td>Female Positive 5</td>
<td>Female Negative 23</td>
<td>Female Positive 0</td>
<td>Female Negative 1</td>
</tr>
<tr>
<td></td>
<td>17.4%</td>
<td>82.6%</td>
<td>78.3%</td>
<td>21.7%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>4.3%</td>
<td>95.7%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>VL detected</td>
<td>Positive 2</td>
<td>Negative 8</td>
<td>Positive 2</td>
<td>Negative 10</td>
<td>Positive 1</td>
<td>Negative 0</td>
</tr>
<tr>
<td></td>
<td>Male Positive 8</td>
<td>Male Negative 8</td>
<td>Male Positive 2</td>
<td>Male Negative 10</td>
<td>Male Positive 1</td>
<td>Male Negative 0</td>
</tr>
<tr>
<td></td>
<td>20.0%</td>
<td>80.0%</td>
<td>80.0%</td>
<td>20.0%</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>90%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>VL undetectable</td>
<td>Positive 4</td>
<td>Negative 27</td>
<td>Positive 26</td>
<td>Negative 5</td>
<td>Positive 30</td>
<td>Negative 1</td>
</tr>
<tr>
<td></td>
<td>Male Positive 26</td>
<td>Male Negative 27</td>
<td>Male Positive 5</td>
<td>Male Negative 30</td>
<td>Male Positive 1</td>
<td>Male Negative 0</td>
</tr>
<tr>
<td></td>
<td>12.9%</td>
<td>87.1%</td>
<td>83.9%</td>
<td>16.1%</td>
<td>96.8%</td>
<td>3.2%</td>
</tr>
<tr>
<td></td>
<td>0%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Note: VL = viral load

3.3 Characteristic of HBeAg and HBeAb

Hepatitis e antigen has been a key marker for viral replication and is an integral baseline serological marker for HBV treatment. Among the 41 samples collected 6(14.6%) were HBeAg+ whereas 35(85.6%) were HBeAg-. HBeAb was positive in 33(80.4%) and negative in 8(19.6%). The presence of HBeAb was directly associated with the duration of treatment (Fisher Test p= 0.037), with those who had taken the drugs for a long period developing the antibodies: all who had taken the drug for two and 4 years had HBeAb+ while 83.3% of those who had taken for three years had HBeAb+. Majority of the patients (85.4%) were HBeAg negative at the time of sample collection and the proportion varied across the duration of treatment (Figure 2).
3.3 Treatment outcomes: Viral load and HBeAg

All patients had viral loads >2000IU/ml at the initiation of treatment, by the time we collected samples three quarters 75.6% of the patients had undetectable viral loads. Of those with detectable viral loads, the viral load ranged from $4.6 \times 10^4$ to $1.04 \times 10^5$ IU/ml with majority of them (85.6%) having HBeAg negative and 80.4% having HBeAb positive. There was a strong association between drug and viral load (fisher Test $p=0.001$) however among those with detectable viral loads, the quantity was not associated with the duration of treatment (Fisher exact $p=0.12$). Not all patients who had undetectable viral load were HBeAb positive; 16.1% were negative and 12.9% were HBeAg positive. One male patient (2.4%) cleared HBsAg to attain a functional cure, the patient had undetectable viral loads, HBsAb positive and HBeAg negative. The patient was on TDF treatment for three years. Viral suppression state was observed among 31 (75.6%) who had undetectable viral loads. After taking TDF treatment, viral inactive state was observed in 35 (85.6%) of the patients who had antibodies to e-antigen.

3.4 Genotypes and drug resistance

DNA was isolated from all samples, the complete Hepatitis B surface region and Polymerase regions were amplified. Out of the 10 samples that had detectable viral loads only six isolates were successfully sequenced which was 14.6% of the total population. Half (50%) of the isolate were genotype A and 50% of genotype D (Figure 3). All genotype A isolate clustered with sub-genotype A1 from Africa and those previously isolated from Kenya (Ochwoto et al 2016). Genotype D isolates clustered into two different branches. One branch had two isolates that clustered with sub-genotype D6 previously referred as D/E recombinant and the other branch clustered with Sub-genotype D7 also referred as D/E recombinant.

The mean age of the six patients was 23.67 + 12.64 as compared to 29.3+ 8.4 years for genotype D and 18+ 5.5 for genotype A. Out of the six, the ratio of male to female was 1:1. Treatment duration of genotype A was a contrast of genotype D, among the genotype D patients 66.6% were on treatment for 3 years and the rest were on treatment for less than a year. When the genotypes and viral load levels were compared there was no significant difference between them (Fisher Test $p= 0.14$)
Figure 3: A phylogenetic tree based on Hepatitis B S-proteins from Baringo samples. The tree was built by Maximum (ML) Likelihood method based on the JTT matrix-based model. The phylogram was constructed from the alignment of nucleotide sequences and the node value given as percentages represent the measure of support for the node where 100 represents maximal support, that is, sequences to the right node cluster together to the exclusion of any other.

3.5 Drug resistance

Analysis of the Retro Transcriptase (RT) protein had concomitant amino acid changes at different positions (Table 3). All the isolates had rtR153W mutation had rtM129L. Other putative mutations reported on the Retro Transcriptase (RT) proteins include rtN248H, rtP237T, rtN238H (Table 3). Further analysis from RT and S region using Gene2pheno v2.0 software showed that none of the recorded mutations affected the susceptibility of HBV to the drug Tenofovir taken by HBV patients -in combination drugs.

Table 3: Amino acid substitutions in the reverse transcriptase (RT) domain and the surface hepatitis B antigen (SHB) protein of HBV.

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Genotypes</th>
<th>Age in years</th>
<th>Treatment Duration</th>
<th>Mutations on Retro Transcriptase (RT)</th>
<th>Mutations on surface</th>
<th>Resistance to TDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB03</td>
<td>M</td>
<td>A</td>
<td>19</td>
<td>3 yrs</td>
<td>rtM129L, rtW153R</td>
<td></td>
<td>unknown</td>
</tr>
</tbody>
</table>

Key: Tenofovir (TDF), S (susceptible).
3.6 Discussion

The aim of the present study was to characterize the outcome of anti-HBV treatment in HBV patients attending a selected CCC of Baringo County of Kenya. A number of studies have shown that chronic HBV infection is prevalent among males (Ochwoto et al., 2016) however, the population included in this study constituted majorly of females (56%). This situation does not necessarily mean that female is more infected than male but rather female have a better health seeking behavior as compared to male (Galdas et al 2005, and Phiri et al 2021). As the samples came from the remote setting of Baringo County, the higher number of HBV positive patients on treatment as compared to other counties could be attributed cultural factors and access to quality education, hence ignorance on HBV transmission and prevention strategies in the county.

However, there is need for in-depth research to identify the main transmission drivers of HBV infection in the region for similar situation is observed in the North rift valley region of Kenya (Ly et al 2016).

The age of HBV-infected patients ranged from 8-80 years old, with majority being the youthful age between 30-34 years. This agrees with the study of Weintock and others, which attributed the high prevalence of HBV in the middle aged to active sexual behaviour and risk taking, as the main route of HBV transmission is sexual (Weintock et al., 2004). A number (19.7%) of those on treatment were children born after the introduction of national Hepatitis B vaccine for infants in 2001 (Ndiritu et al., 2006), whose main purpose was to reduce HBV horizontal transmission. From the medical record it was not possible to tell whether the 19.7% were vaccinated or not.

Kenya is one of the countries that has not introduced Birth-dose HBV vaccine. In this study, 2 women were breastfeeding and one was expectant. Most infant transmission in developing countries and sub-Sahara Africa occur early in life through horizontal transmission (Zampino et al, 2015). Serological markers are indispensable in the diagnosis of HBV infection. All patient tested had HBV core antibodies positive for IgM and IgG an indication of chronic infection. One (2.4%) patient had acquired functional cure as evidenced by absence of HBeAg and presence of both HbsAb and undetectable viral load during treatment. The remaining patients tested HbsAg positive and tested negative for HbsAb. The The overall rate of HbsAg clearance was low but significant, similar results have been reported among HIV/HBV coinfection (Gantner et al 2019)(Song & Kim, 2016). Majority of the patients were negative for HBeAg and also another high number were positive for HBeAb, indicating seroconversion from immune-active to inactive status (Terrault et al., 2016). HBeAg disappearance and HBeAb appearance was associated with the duration of treatment, HBeAg-negative patients is predictive of spontaneous HBsAg seroclearance (Chun et al 2011).

HBV DNA amplification and quantitation assays continue to serve as valuable tools to monitor progression of disease and the efficacy of treatment in chronic HBV infection (Heimbach et al., 2018; Liu et al., 2017). As part of criterial to start treatment all patients must have detectable viral loads at the beginning of treatment. The study found 24.3% patients with detectable HBV DNA had viral loads at the time of sample collection. This drop was an important clinical observation, to indicate low rates of viral transmission, and progression to severe chronic infection (Nguyen et al., 2020). It was also an indication that the WHO-guided treatment was effective in suppressing virus replication in patients. Among those with detectable viral loads none had high viral loads and 30% had <10 IU/ml. According to the clinical management guidelines of the European Association for the Study of Liver Diseases for chronic hepatitis B, a highly sensitive HBV DNA test should be lower than the lowest detection limit of real-time
quantitative PCR (10-15 IU/mL). The low (<10 IU/ml) HBV DNA loads detected in this study meant that the kit used was highly sensitive and therefore reliable. These low viral loads accompanied by presence of anti-HBe in a majority of patients was an indication that they were in the inactive chronic hepatitis B phase. Kimura et al., (2002) showed a good correlation (100%) between circulating HBV DNA and replicative HBV disease. In cases as such where viral load is low and HBeAg is detectable, treatment should continue to prevent reactivation of the virus and seroconversion of patients back to immune-active status. Given the dynamic nature of chronic HBV infection, regular monitoring of HBV DNA is done along the determination liver function parameters and platelets so as to accurately characterize the phase of infection. Efforts by the study team to have these tests done proved to be impossible as all processes at health facilities has shifted to fight against COVID-19 pandemic. Detection of HBV DNA is also the only reliable marker of active HBV replication in patients with HBeAg-negative disease. In this study, 6/41 (14.6%) of samples were seropositive for HBeAg.

Genotype A and D are the most dominant in Eastern, central and southern African countries including Tanzania, Uganda, Somalia, Congo, Malawi and South Africa, (MacLachlan & Cowie, 2015.) (Ochwoto et al 2013, Kramvis 2021 distribution of HBV in Africa HBV ) This study identified two HBV genotypes (A and D) circulating in Baringo County. Fifty percent of the genotypes found in our study were of the genotype A subtype A1, information that correlates well with previous studies showing genotypes.

The open reading frame (ORF) of HBV surface protein gene is composed of preS1, preS2 and S regions. Due to the overlapping nature of the S and RT ORFs, mutations arising in the RT domain cause the appearance of mutations in the preS/S ORF in escape mutants (Caligiuri et al., 2016). Mutations in these regions could lead to liver disease and are relevant in providing knowledge about the pathogenesis of HBV and reactivation of immune-escape mutants. Antibody or immune escape strains of HBV carry mutations that affect antibody response, and might lead to an alteration in the antigenicity of hepatitis B surface antigen (Kaymakoglu et al., 2014). Recurrent and relevant mutations usually obtained on the S ORF of hepatitis B virus include I84T/M/L/V and A90T/V in PreS1, F141L in PreS2, and I68T and L213I/S in S (Jeong et al., 2021). None of these important mutations were identified in our study. Rather, putative mutations have been identified rtR153W, rtM129L rtN248H, rtP237T, and rtN238H on the reverse transcriptase domain.

The reverse transcriptase (RT) domain ORF of HBV has been used as the target of antiviral agents, namely nucleoside/nucleotide analogs and acyclic nucleotide analogs (NAs). Mutations occurring in this region during the virus replicative cycles may confer resistance to NAs (Clark & Hu, 2015). Further analysis revealed that the mutations had no effect on anti-HBV drug action but have the potential to confer resistance. Many studies have shown drug resistance in patients on monotherapy, especially with Lamivudine and Tenofovir (Torresi, 2002). The patients participating this study were on Tenofovir (TDF) therapy for between 1 yr and 4 years. The use of combination therapies with high potency therefore seems useful in averting drug resistance and should be applied in chronic Hepatitis B therapy. Our study concurs with other authorships which proposed that patients with an undetectable HBV DNA level after antiviral therapy could have high HBeAg seroconversion rates and low drug resistance (Fu et al., 2017).
CONCLUSION

The current study showed that the WHO-recommended treatment for CHB is successful if there is adherence to therapy as evidenced by favourable serological profiles and viral suppression in the study subjects. There was one case of a patient having attained functional cure after adhering to treatment for 2 years. No resistance to the combination therapies recommended by WHO was identified despite identification of putative mutations related to lamivudine and Adefovir monotherapies. Genotypes A and D still remain dominant in the region. This study, being the first in Kenya, provides valuable information to health practitioners and policy makers aiming to expand access to antiviral treatment of CHB.

Limitation of the study

The data was collected at the peak of COVID 19 pandemic and this affected the sample collection because some participants were not willing having their samples taken. The study did not do closed covalent circular DNA (cccDNA) and sequence the Basal Core promoter (BCP) sites that could have given much more insight to the treatment outcomes. Use of HBV DNA in monitoring Chronic HBV infection, may require determination liver function parameters and platelets so as to accurately characterize the phase of infection, this was hard to achieve in this study as the sample were collected at the peak of COVID-19 pandemic.

REFERENCES


