*Original Research Article*

Evaluation of Selected Biochemical Parameters, CRP and Viral Load in Hepatitis B Patients with Acyclic Phosphate-Resistant Genes at Rivers State University Teaching Hospital

ABSTRACT

|  |
| --- |
| **Aim:** To estimate the activities of aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), Gamma glutamyl transpeptidase (GGT) Lactate dehydrogenase (LDH) as well as concentrations of alpha fetoprotein, C reactive Protein and viral load in Hepatitis B positive subjects at Rivers State University Teaching Hospital  **Study design:** Analytical Cross-sectional study  **Place and Duration of Study:** Rivers State University Teaching hospital, between February and August 2024.  **Methodology:** This cross-sectional study compared 88 HBV-positive subjects (44 naïve, 44 on ART) with 50 HBV-negative controls. Liver enzymes (AST, ALT, ALP, and GGT), CRP, AFP, and viral load were measured. DNA was extracted using a commercial kit and analysed via nested PCR. Statistical significance (p < 0.05) was determined using t-tests/ANOVA and data analyzed using GraphPad Prism version 9.02.  **Results:** HBV-positive subjects showed significantly higher AST (46.75 vs. 37.04 U/L), ALT (55.17 vs. 38.10 U/L), ALP (237.1 vs. 175.4 U/L), GGT (103.1 vs. 48.54 U/L), CRP (5.33 vs. 4.09 ng/mL), and AFP (166.20 vs. 7.72 ng/mL) compared to controls (p < 0.05). Resistance genes A202C (7%), T184G (10%), and A181T (61–62%) were detected.  **Conclusion:** HBV infection is associated with elevated liver injury and inflammatory markers, alongside prevalent resistance genes. Regular monitoring of these parameters is critical for clinical management. |

*Keywords: Liver enzymes, Hepatitis B, acyclic phosphate resistant genes, Rivers State University Teaching hospital*

1. INTRODUCTION

“Hepatitis B virus (HBV) is an enveloped deoxyribonucleic acid (DNA) virus whose replication involves transcription to ribonucleic acid (RNA) intermediates that are then reverse transcribed back to DNA. It is a hepatotropic pathogen that directly causes Liver diseases of variable severity” [1,37]. Despite its DNA genome, HBV is thought to exist as a quasi-species; high levels of diversity are maintained through lack of proofreading during reverse transcription [2]. “This means that, like HIV and many RNA viruses, HBV infections maintain polymorphism at almost all nucleotide positions within a host at a given time” [3,4], “paving the way for resistance mutations to increase in frequency upon drug treatment. HBV is most treated with reverse transcriptase inhibitors, particularly lamivudine. However, several resistance mutations against lamivudine have been observed, many of which have genetic barriers” [5]. “Given this, a second reverse transcriptase inhibitor is often supplemented” [4]. “This method of drug-switching, if done within a single class, is potentially problematic: it encourages more robust resistance mutations that are either effective across many drugs and/or suffer lower fitness costs when drug treatment (selection) ceases” [4,6,7]. “Initial combination therapy is becoming more frequent in clinical trials but is not usually the standard treatment.

Although NAs are more convenient than IFN-based therapies and have fewer side effects, sustained viral suppression is usually not achieved after withdrawal of a 48week course of NA therapy, necessitating long, and in many cases, indeﬁnite treatment. Unfortunately, a long duration of NA treatment is associated with an increasing risk of development of drug resistance, aggravation of decompensated cirrhosis and auto-immune diseases” [8]. “Similarly, some medications such as Immuno-suppressive drugs, Antiretroviral and Anti-tuberculosis drugs are associated with hepatotoxicity and can cause different degree of liver injury ranging from slightly elevated liver enzyme to acute liver failure” [9]. “Hepatotoxicity is a foremost issue for clinicians and the primary reason for pharmaceutical produce recalls, and this can be classified according to severity and intensity of hepatic cell damage and the elevation of hepatic biomarkers. Hepatotoxicity is the major cause of hepatocellular, cholestatic or mixed hepatic damage which is caused by a 2-3 times higher increase in alanine aminotransferase (ALT) or alkaline phosphatase (ALP)” [10].

“Although the clinical picture of HBV and HCV in chronic cases is similar, aminotransferase activities are different. In HBV, ALT activity is generally constant, except increases are evident at seroconversion times. On the other hand, two-thirds of HCV cases experience fluctuation. Serum ALT activities of chronic hepatitis C cases range between one to four times the upper reference limit, reaching maximum less than seven times. On the other hand, 15-50% of cases with chronic hepatitis C have normal ALT activities” [38], “but this rate decreases with increasing number of measurements. From 43% of cases experiencing fluctuation between normal and abnormal, 16% of those with normal ALT on their first two visits and 11% of those with normal ALT on their first three visits subsequently develop increased ALT. Chronic hepatitis C cases with persistently normal ALT activities experience lower rates of progression to cirrhosis” [39].

Thus, there is need to assess liver function, inflammatory biomarkers and some genes in hepatitis B subjects on acyclic phosphate drugs at the Rivers State University Teaching Hospital. Results obtained from this study will be used as baseline information to Hepatitis B patients, clinicians and academics.

2. materialS and methods

**3.1** **Study Area**

This research was conducted in Rivers State University Teaching Hospital (RSUTH). RSUTH is a Teaching Hospital of Rivers State University. [11].

**3.2 Study Population**

All subjects comprised of hepatitis B positive and naïve patients sampled at the study population. They were randomly sampled with their consent obtained and questionnaire administered to them to ascertain their age, the use or non-use of ART is put into consideration, and type of antiretroviral therapy used. Information obtained from them was confidentially treated and maintained.

**3.3 Study Design**

This is an analytical cross-sectional study, which consist of 88 patients (test subjects) aged 35 – 70 years were used on this study out of which 44 subjects are HBV Positive (Naïve) who are not on drugs, 44 subjects are HBV Positive on antiretroviral therapy (ART) for Six months and 50 subjects who are HBV negative were used as control, making a total of 138 subjects

**3.4 Eligibility Criteria**

**3.4.1 Inclusion Criteria**

Subjects who are HBV positive, comprising those on ART, those who are not on ART (naïve) and those who are HBV negative during this research who gave their consent to participate were included.

**3.4.2 Exclusion Criteria**

Subjects with history of diabetes, kidney diseases, hypertension, those below or above the stipulated age and pregnant women were excluded.

**3.5 Sample Size**

The sample size was determined by Cochran formula [12].

**3.6 Sample Collection**

Venous Blood (10mls) was collected from the subjects and put into a plain sample bottle with the aid of 10mls syringe and needle, cotton wool and methylated spirit. The samples collected were put in a specimen rack and moved to the laboratory where they were separated by centrifugation at 3500 rpm for 10 minutes using 800D Centrifuge CE. The supernatant was collected using a pasture pipette into a new plain bottle and stored in a refrigerator at -20oc until the time of analysis.

**3.7 Laboratory Analysis**

**3.7.1** **Determination of Aspartate Aminotransferase (AST)** [13]

**Principle**: Kinetic determination of Aspartate aminotransferase is based upon the following reaction.

**3.7.3 Determination of Alkaline Phosphatase** [14].

**Principle**: Alkaline Phosphatase acts in highly alkaline pH in the presence of divalent Mg ions where it catalyzes the hydrolysis of (PNPP) which results in release of P – Nitrophenol and free phosphate group. Absorbance is proportional to the serum alkaline phosphatase at 450nm.

**3.7.4 Determination of Gamma-Glutamyltranspeptidase (GGT)** [15].

**Principle:** Kinetic determination of Gamma GT according to the following reaction.

**3.7.5 Determination of C – Reactive Protein (CRP)** [16].

**Principle**: CRP samples bind to specific anti-CRP antibodies which have been absorbed to latex particles and agglutinates. The agglutination is directly proportional to the quality of CRP in the sample.

**3.7.6 Determination of Lactate Dehydrogenase (LDH)** [17].

The ELISA Kits uses the sandwich ELISA

**3.7.7** **Determination of Alpha-Feto Protein (AFP)** [17].

**3.7.8 Determination of Viral Load**

**Extraction Process**

Viral load is done using HBV Viral load Quick-DNA/RNA extraction Kit.

DNA from the sera was extracted using quick– DNA mini-Kit supplied by Inqaba west Africa following the Zymo research instructions. Extracted genomic DNA was quantified using the Nanodrop 100 Spectrophotometer. The S gene of the virus was amplified using nested PCR approach with primer with on an ABI 9700 applied Biosystem thermal Cycler at a final volume of 30 microlitres for 35 cycles.

**3.8 Statistical Analysis**

The results obtained were analyzed using GraphPad Prism version 9.02. Descriptive statistic invoving the use of mean and standard deviation, and statistical significance was set at p 0.05. Inferential statistics involving the use of one-way ANOVA (PostHoc: Tukey’s multiple comparison test), Student t-test was used.

3. results and discussion

**Table 1: Biochemical analysis Result (Mean ± SD) of Hepatitis B Positive and Negative (Control)**

**Subjects**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Positive  (n=88) | Control  (n=50) | T value | P value | Remark |
| Age (Years) | 52.09±7.65 | 51.18±6.38 | 0.7144 | 0.4762 | NS |
| AST (U/L) | 46.75±16.76 | 37.04±10.42 | 3.707 | 0.0003 | S |
| ALT (U/L) | 55.17±22.75 | 38.10±9.89 | 5.036 | <0.0001 | S |
| ALP (U/L) | 237.1±81.34 | 175.4±55.10 | 4.770 | <0.0001 | S |
| GGT (U/L) | 103.1±12.11 | 48.54±16.56 | 3.372 | 0.0010 | S |
| CRP (ng/ml) | 5.33±1.48 | 4.09±1.31 | 5.045 | <0.0001 | S |
| LDH (U/L) | 186.40±89.14 | 183.70±46.54 | 0.2049 | 0.8380 | NS |
| AFP (ng/ml) | 166.20±53.59 | 7.72±5.96 | 20.80 | <0.0001 | S |

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05.

**Table 2: Biochemical analysis Result (Mean ± SD) of Hepatitis B Positive Naïve, Resistant and Negative (Control) Female Subjects**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters | Female (Naïve)  (n=22) | Female (Resistant)  (n=22) | Female (Control)  (n=25) | F value | P value | Remark |
| Age (Years) | 51.18±6.666 | 49.50±7.209 | 49.72±6.413 | 0.4082 | 0.6665 | NS |
| Viral Load (cells/ml) | 1961±443.8 | 21408±6473 | TND | t=14.06 | <0.0001 | S |
| AST (U/L) | 41.64±17.51a | 48.73±15.51a | 36.88±9.75b | 3.956 | 0.0238 | S |
| ALT (U/L) | 52.00±17.36a | 61.3±8.83b | 40.20±7.97c | 158.8 | <0.0001 | S |
| ALP (U/L) | 217.5±76.13a | 261.3±80.83a | 41.20±7.97b | 82.82 | <0.0001 | S |
| GGT (U/L) | 68.95±17.52a | 93.93±32.84b | 46.32±15.40c | 25.17 | <0.0001 | S |
| CRP (ng/ml) | 4.820±1.45a | 5.68±1.51a | 3.84±1.24b | 10.21 | 0.0001 | S |
| LDH (U/L) | 128.7±112.0a | 196.1±71.38b | 185.7±48.87b | 4.531 | 0.0143 | S |
| AFP (ng/ml) | 164.7±60.72a | 176.0±48.55a | 6.99±3.88b | 110.7 | <0.0001 | S |

**PostHoc:** Values in the same row with different superscripts differ significantly at p<0.05.

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05, TND=Target Not Detected.

**Table 3: Biochemical analysis Result (Mean ± SD) of Hepatitis B Positive Naïve, Resistant and Negative (Control) Male Subjects**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters | Male (Naïve)  (n=22) | Male (Resistant)  (n=22) | Male (Control)  (n=25) | F value | P value | Remark |
| Age (Years) | 53.45±8.210 | 54.23±7.994 | 52.64±6.013 | 0.2691 | 0.7649 | NS |
| Viral Load (cells/ml) | 2346±542.9 | 22246±7531 | TND | t=12.36 | <0.0001 | S |
| AST (U/L) | 45.86±17.42a | 50.77±16.23a | 37.20±11.24b | 4.940 | 0.0100 | S |
| ALT (U/L) | 50.05±18.29a | 58.73±15.61a | 36.00±11.27b | 13.51 | <0.0001 | S |
| ALP (U/L) | 214.6±80.04a | 254.8±82.48a | 172.6±55.20b | 7.457 | 0.0012 | S |
| GGT (U/L) | 158.2±215.4a | 91.48±33.27b | 50.76±17.68c | 4.479 | 0.0150 | S |
| CRP (ng/ml) | 5.154±1.35a | 5.670±1.30a | 4.345±1.35b | 5.910 | 0.0044 | S |
| LDH (U/L) | 219.9±73.64 | 201.1±69.80 | 181.6±45.01 | 2.135 | 0.1263 | NS |
| AFP (ng/ml) | 158.8±49.19a | 165.2±57.21a | 8.436±7.49b | 102.7 | <0.0001 | S |

**PostHoc:** Values in the same row with different superscripts differ significantly at p<0.05.

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05, TND=Target Not Detected.

**Table 4: Biochemical analysis Result of Hepatitis B Positive Naïve Female against Naïve Male Subjects**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Female (Naïve)  (n=22) | Male (Naive)  (n=22) | T value | P value | Remark |
| Age (Years) | 51.18±6.666 | 53.45±8.210 | 1.008 | 0.3192 | NS |
| Viral Load (cells/ml) | 1961±443.8 | 2346±542.9 | 2.575 | 0.0136 | S |
| AST (U/L) | 41.64±17.51 | 45.86±17.42 | 0.8027 | 0.4266 | NS |
| ALT (U/L) | 52.00±17.36 | 50.05±18.29 | 0.3636 | 0.7180 | NS |
| ALP (U/L) | 217.5±76.13 | 214.6±80.04 | 0.1216 | 0.9038 | NS |
| GGT (U/L) | 68.95±17.52 | 158.2±25.4 | 1.937 | 0.0495 | S |
| CRP (ng/ml) | 4.820±1.445 | 5.154±1.348 | 0.7931 | 0.4322 | S |
| LDH (U/L) | 128.7±112.0 | 219.9±73.64 | 3.192 | 0.0027 | S |
| AFP (ng/ml) | 164.7±60.72 | 158.8±49.19 | 0.3574 | 0.7226 | NS |

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05.

**Table 5: Biochemical analysis Result of Hepatitis B Positive Resistant Female against Resistant Male Subjects**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Female (Resistant)  (n=22) | Male  (Resistant)  (n=22) | T value | P value | Remark |
| Age (Years) | 49.50±7.209 | 54.23±7.994 | 2.060 | 0.0456 | S |
| Viral Load (cells/ml) | 21408±6473 | 22246±7531 | 0.3959 | 0.6942 | NS |
| AST (U/L) | 48.73±15.51 | 50.77±16.23 | 0.4273 | 0.6713 | NS |
| ALT (U/L) | 59.91±34.49 | 58.73±15.61 | 0.1464 | 0.8843 | NS |
| ALP (U/L) | 261.3±80.83 | 254.8±82.48 | 0.2621 | 0.7945 | NS |
| GGT (U/L) | 93.93±32.84 | 91.48±33.27 | 0.2459 | 0.8070 | NS |
| CRP (ng/ml) | 5.681±1.513 | 5.670±1.301 | 0.02671 | 0.9788 | NS |
| LDH (U/L) | 196.1±71.38 | 201.1±69.80 | 0.2328 | 0.8171 | NS |
| AFP (ng/ml) | 176.0±48.55 | 165.2±57.21 | 0.6762 | 0.5026 | NS |

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05.

**Table 6: Biochemical analysis Result of Hepatitis B Positive Resistant Subjects with Varying Resistant Genes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameters | A202C  Female  (n=3) | T184G Female  (n=6) | A181T Female  (n=13) | F value | P value | Remark |
| Age (Years) | 57.33±2.89a | 51.33±10.07a | 46.85±4.76b | 3.532 | 0.0496 | S |
| Viral Load (cells/ml) | 19911±8182 | 21024±6674 | 21930±6532 | 0.1220 | 0.8858 | NS |
| AST (U/L) | 46.67±17.10 | 46.00±11.12 | 50.46±17.69 | 0.1848 | 0.8327 | NS |
| ALT (U/L) | 68.00±3.00 | 79.33±58.79 | 49.08±17.56 | 1.803 | 0.1919 | NS |
| ALP (U/L) | 273.7±69.14 | 291.2±118.0 | 244.6±63.61 | 0.7011 | 0.5084 | NS |
| GGT (U/L) | 92.14±14.30 | 103.2±36.61 | 90.06±35.13 | 0.3127 | 0.7351 | NS |
| CRP (ng/ml) | 6.533±1.234 | 5.080±1.877 | 5.762±1.384 | 0.9647 | 0.3990 | NS |
| LDH (U/L) | 192.0±82.29 | 208.8±60.74 | 191.2±78.36 | 0.1197 | 0.8879 | NS |
| AFP (ng/ml) | 141.7±42.10 | 167.7±29.23 | 187.8±54.72 | 1.249 | 0.3092 | NS |

**PostHoc:** Values in the same row with different superscripts differ significantly at p<0.05.

KEYS: AST= Aspartate aminotransferase, ALT=Alanine aminotransferase, ALP=Alkaline Phosphatase, GGT=Glutamyl Transferase, CRP=C-Reactive Protein, LDH=Lactate Dehydrogenase, AFP= α-Feto Protein**,** S=Significant, NS=Not Significant at p<0.05.

The study showed significant increase (P<0.05) in activities of AST, ALT, ALP, GGT CRP and AFP in HBV positive subjects compared with their respective controls. This study is in consonance with the report of Rana et al. [18] and Domfeh et al. [19] which reported that the serum levels of liver injury markers (ALT and AST) and serum levels of ALP and GGT were significantly higher among the chronic HBV-infected patients compared to the healthy controls. Rana et al. [20] observed “significant increase in AST, ALT, ALP activities in HBV subjects compared with healthy controls ALT activity is consistently higher than aspartate aminotransferase (AST) with all causes of chronic hepatic injury except for alcoholic hepatitis” [21]. “These enzymes are associated with the liver parenchymal cells and are useful biomarkers of liver injury” [22]. “AST and ALT are often released into the blood stream once there is hepatocellular damage, so ALT serum level elevation correlates more with hepatic injury. Sometimes the ratio of ALT to AST can also help deﬁne the patterns of a disease” [23]. Abulude et al. [24] in their study reported that HBsAg seropositivity was signiﬁcantly associated with an abnormal ALP. The hepatitis B virus mainly affects the functions of the liver by multiplying in the liver cells leading to inflammation of the hepatocytes [25]. These inflammatory processes comprise tissue breakdown and repair mechanisms; therefore, the high tissue turnover in chronic active viral hepatitis B often leads to scarring and hepatocyte damage [25]. Subsequently, serum levels of aspartate and alanine transaminases are raised. These enzymes are associated with the liver parenchymal cells and are useful biomarkers of liver injury [26]. In addition, the hepatocytes also produce C-reactive protein (CRP), an acute phase protein [27], “which causes the release of pro-inflammatory cytokines, specifically interleukin-6 (IL-6), from macrophages and T cells; hence, C-reactive proteins and IL-6 are used to assess the degree of inflammation” [28]. Domfeh et al. [19] reported significant increase in the biomarkers of inflammation, C-reactive protein (CRP) and interleukin 6, and liver injury (liver transaminases, FIB-4 index, and APRI) among the drug-naive chronic HBV-infected patients.

In addition, the hepatocytes also produce C-reactive protein (CRP), an acute phase protein [27], which causes the release of pro-inflammatory cytokines, specifically interleukin-6 (IL-6), from macrophages and T cells; hence, C-reactive proteins and IL-6 are used to assess the degree of inflammation [28]. The C reactive protein (CRP) is an inflammatory marker that is synthesized by the liver. Shiyong et al. [29] also found that the serum CRP level was markedly increased in patients with HBV infections compared to the healthy control group. These results were consistent with the results of the cell-based experiments, demonstrating that HBV upregulates the synthesis and secretion of CRP at the protein level. In addition, to explore the relationship between the CRP level and disease progression, we compared the difference in the CRP serum levels between various groups of patients with HBV infections. The results showed that as the disease became aggravated, the CRP content gradually increased. This finding indicates that the CRP level may reflect disease progression to a certain extent. CRP is an evolutionarily highly conserved host defense molecule. It can bind to phosphocholine ligands and further activate the complement system, thereby exerting opsonic and anti-inflammatory effects [29]. The result of this study showed significant increase (P<0.05) in CRP of hepatitis B subjects which is similar to report of Shiyong et al. [29] and Domfeh et al. [19]. Elevated on- treatment AFP is a specific tumour marker for Hepatocellular carcinoma in patients with Chronic Hepatitis B Virus receiving entecavir [30]. There is little debate that AFP should not be used alone in HCC surveillance, but it has been debated whether AFP should be included in HCC surveillance due to its suboptimal sensitivity (39–65%) and specificity (76–97%) [30].

“AFP is the best characterized and most widely used serum biomarker for HCC surveillance. However, its effectiveness is limited as not all HCCs secrete AFP. In addition, AFP serum levels can be elevated in patients with chronic hepatitis or cirrhosis. However, with the advent of highly effective NUCs for the treatment of CHB, elevated on- treatment AFP levels were shown in a large retrospective- prospective study to be a specific marker for HCC because falsely elevated AFP levels in 1,531 patients receiving entecavir were minimized compared to 424 patients that received no treatment, suggesting that, in this group of patients, a lower AFP cut off value could be used” [30].

The study showed no significant changes(P>0.05) in viral load, AST, ALT, ALP, CRP, GGT and AFP of Males with A202C, T184G and A181T genes while there was significance difference (P<0.05) in the LDH activity of the studied population. The study further showed no significant changes (P>0.05) in viral load, AST, ALT, ALP, CRP, GGT, LDH and AFP of females with A202C, T184G and A181T genes. This is suggestive that the genes detected did not have any effect on the parameters. This corroborates the work of Kim et al. [31]. Kim et al. [31] in their study in Patients with Adefovir Resistant Chronic Hepatitis B with A181T/V Polymerase Mutations reported that only the ALT level was significantly lower in patients with the sA184V mutation (P=0.012) while in this study it was only LDH that showed significant change. Primary drug-resistant mutations cause an amino acid substitution that result in reduced susceptibility to an antiviral agent while secondary compensatory mutations cause amino acid substitutions that restore functional defects in viral polymerase activity (i.e., replication ﬁtness) associated with primary drug resistance [32]. Mokaya et al. [33] suggested that other explanations for incomplete suppression of HBV viraemia on therapy include a higher baseline HBV DNA level, positive baseline HBeAg status, history of 3TC exposure, a lower nadir CD4+ T cell count in the context of HIV coinfection, and high serum HBV RNA levels. Given that HBV DNA is inhibited in a dose dependent manner, it is also possible that insufficient drug delivery to the infected hepatocyte could be the cause of persistent viraemia even in the absence of specific RAMs. The study showed resistance gene A202C with 7, T184G with 10, and A181T with 27 in the subjects studied. Also, the study reported resistance gene A202C with males 4(19%) and females as 3(13%), T184G had 4(19%) males and 6(26%) females while A181T genes of 13 (62%) males and 14 (61%) females. This is suggestive of high level of resistance genes in the population studied with A181T genes as the most detected in the hepatitis B positive subjects studied. Zhou et al. [19] in their study in northern Henan Province of China, using 148 cases of HBV patients showed that drug resistance mutation sites of ETV were I169T (1 case, 0.95%), T184I (2 cases, 1.90%), S202G (2 cases, 1.90%) and T184I (1 case, 0.95%), and no mutation sites of T184A, T184F and T184G were found; the drug resistance mutation sites of ADV were A181T (8 cases, 7.6%). Previous study has reported two primary adefovir resistant mutations, the rtA181T and rtN236T substitutions in the viral polymerase [34]. TFV resistance has been linked to the rtA194T substitution, but only in association with changes that cause LMV resistance and typically in the setting of coinfection with HIV-1 [35]. It is known that TDF resistance mutation site is A194T in RT gene region [36].

4. Conclusion

The study has shown the presence of some resistant genes in Hepatitis B positive subjects on acyclic phosphonate drug, with elevated liver injury markers (ALT and AST), serum levels of ALP, GGT and inflammatory markers CRP and AFP among the studied population. This suggested that the Hepatitis B virus causes liver damage and inflammation of the liver.

Consent

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images.

Ethical approval

Ethical approved for the research was obtained from Rivers State Hospitals Management Board (RSHMB/RAHREC/2023/055).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

References

1. Lago, B. V., Portilho, M. M., Mello, V. M., Desousa, P. S. F., Angelice, G. P. Genetic Variability of Hepatitis B Virus in Acute and in Different Phases of Chronic Infection in Brazil. Scientific Report, 2024; 14: 10742. https://doi.org/10.1038/s41598-024-60900-2.

2. Farshadpour, F., Taherkhani, R. and Sabori, F. Molecular Evaluation of Hepatitis B Virus Infection and Predominant Mutations of Pre-core, Basal Core Promoter and S Regions in an Iranian Population with Type 2 Diabetes Mellitus: A case control study. BMC Infectious Diseases 2022; 22: 553. <https://doi.org/10.1186/s12879-022-07528>.

3. Khudyakov, Y. Coevolution and HBV Drug Resistance’, *AntiviralTherapy*, 2010; 15: 505–15.

4. Bang, K. B., and Kim, H. J. Management of Antiviral Drug Resistance in Chronic Hepatitis B’, *World Journal of Gastroenterology*, 2014; 20: 11641–9.

5. Özlük, S., Bayram, Y., Ozkagmaz, A., Parlak, M., Ozdemir, A. Antiviral Drug Resistance Rates among Patients with Chronic Hepatitis B Infection *European Journal of Clinical and Experimental Medicine,* 2023;21(2): 283-8. https/doi.org/10.15584/ejcem.2023.2.23

6. Locarnini S, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, Pawlotsky JM, Zoulim F. Management of antiviral resistance in patients with chronic hepatitis B. *Antivir Ther*., 2004; 9: 679– 93.

7. Shaw, T. *et al*. ‘HBV Drug Resistance: Mechanisms, Detection and Interpretation. *Journal of Hepatology*, 2006; 44:593–606

8. Kayaaslan, B. and Guner, R. Adverse Effects of Oral Antiviral Therapy in Chronic Hepatitis B. *World Journal of Hepatology,* 2017; 9(5): 227-41.

9. Sun, J., Yu, X., Weng, Z., Jin, L., Yang, J., Zhang, H., Gu, J. and Yang, J. The Impact of Hepatotoxic Drugs on the Outcome of Patients with Acute Deterioration of Hepatitis B virus Related Chronic Disease. *European Journal of Gastroenterology and Hepatology,* 2022;34: 782-90.

10. Thakur, S., Kumar, V., Das, R., Sharma, V. and Mehta, D. K. Biomarkers of Hepatic Toxicity: An Overview. *Current Therapeutic Research,* 2024; 100: 100737.https://doi/10.1016/j.curtheres.2024.100737.

11. Wekere, F.CC, Iwoh-Amah, R.S.,Kwosah, J.N., Bademosi, A. and Amadi, S.C. A Five-year review on Caesarean section at the Rivers State University Teaching Hospital, South-South, Nigeria. *Journal of Advances in Medicines & Medical research*, 2021; 33923: 159-67.

12. Cochran W.G. Sampling Techniques *3rd Edition*. John Wiley & Sons New York, 1977.

13 Thefeld W. *et al.,* Dtsch.medwschr. 99, 343. (1974).

14. Sclebusch, H, *et al.,* Determination of Alkaline Phosphatase. Dtsch. Med.wschr. 99, 765. (1974).

15. Szasz, G. Determination of Gamma-Glutamyltranspeptidase (GGT) *Clin. Chem*, 22, 1976; 7: 2051.

16. Zeigenhagen G, Drahovshy D, Klinische-Bedeutungdes. C-reactive protein. *Medklin* 1983;78: 45-50.

17. Engvall E.& Perlmann P. Enzyme-linked Immunonosorbant Assay. (ELISA). Quantitative assay of immunoglobin G. Iimmunochemistry, 1971; 8: 871-4. <http://dx>. doi.org/10.1016/0019-2791(71)9045-X

18 Rana M.T., Estabraq W.G., Raghad H.A., and Ali H.A. Correlation of chronic viral hepatitis B and liver function tests. *Journal of University of Anbar for Pure Science,* 2022; 16(1): 9-12

19. Domfeh, S.A., Sakyi, S.A., Anto E.O., Narkwa, P.W., Mutocheluh, M. & Frempong M.T. Evaluation of inflammatory and liver injury biomarkers among drug-naive viral hepatitis B patients: A study in a referral laboratory, Ghana. *African Journal of Gastroenterology and Hepatology*, 2023; 43: 64-73.

20. Mokaya J, McNaughton AL, Bester PA Goedhals D., Barnes E.J.,Marsden B.D.,Matthews P.C.Hepatitis B virus resistance to tenofovir: fact or fiction? A systematic literature review and structural analysis of drug resistance mechanisms. *Wellcome Open Research*, 2020; 5: 151-7.

21. Chang C, Ko Y, Liu H. Serum alanine aminotransferase levels in relation to hepatitis B and C viruss infections among drug abusers in an area hyperendemic for hepatitis B. *Dig Dis Sci* 2000; 45:1949-52.

22. Nyblom H, Björnsson E, Simrén M, Aldenborg F, Almer S, & Olsson R. The AST/ALT ratio as an indicator of cirrhosis in patients with PBC. *Liver International,* 2006; 26(7): 840-5.

23. Oh,R.C.;Hustead,T.R. Causes and Evaluation of Mildly Elevated Liver Transaminase Levels. *Am. Fam. Physicians,* 2011; 84: 1003–8.

24. Abulude, O.A., Ahmed, I. and Sadis, F.U. Assessment of Hepatitis B Viral Infection as a Predictor of Hepatic Enzymes and Compounds Alteration among Antenatal Patients. *Medical Science*, 2017; 5: 24-9.

25. Glebe D, & Urban S. Viral and cellular determinants involved in the hepadnaviral entry. *World Journal of Gastroenterology*., 2007; 3(1): 22-38.

26. Karatayli E, Karayalcin S, Karaaslan H, *et al*. A novel mutation pattern emerging duringlamivudine treatment shows cross-resistance to adefovir dipivoxil treatment. Antivir Ther, 2007; 12: 761–8.

27. Thompson D, Pepys M.B., & Wood S.P. The physiological structure of human C-reactive protein and its complex with phosphocholine. *Structure,* 1999; 7(2): 169-77.

28. Sproston N.R., & Ashworth J.J. Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology,* 2018; 9: 754-8.

29. Shiyong H.,,Yan W.,Guosheng G.,and Zhishan L. Hepatitis B Virus Upregulates the Expression of C-reactive Protein Both in vivo and in vitro. *Annals of Clinical & Laboratory Science*, 2017; 47(4): 432-5.

30. Kramvis A., Chang K-M., Dandri M., Farci P.,Glebe D,, Hu J., Janssen H.L.A., Lau D.T.Y., Penicaud C., Pollicino T., Testoni B,, Van Bömmel F., AndrisaniQ., Beumont- Mauviel M., Block T.M., Chan H.L.Y., Cloherty G.A., Delaney W.E., Geretti A.M., Gehring A., Jackson K., Lenz O., Maini M.K., Miller V., Protzer U., Yang J.C., Yuen M- F., Zoulim F. and Revill P.A. A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook. *Nature Reviews Gastroenterology & Hepatology,* 2022; 19: 727-46.

31. Kim, J.H. Jung, Y.K. Joo, M.K. Kim, J.H., Yim, H.J., Park, J-J., Kim,J.S. Bak, Y-T., Yeon, J. E & Byun K.S. Hepatitis B Viral Surface Mutations in Patients with Adefovir Resistant Chronic Hepatitis B with A181T/V Polymerase Mutations.J Korean Med Sci, 2010; 25: 257-64.

32. Lok, A. S., Zoulim, F., Locarnini, S., Bartholomeusz, A., Ghany, M. G., Jean-Michel P., Yun-Fan L., Mizokami, M., Kuiken, C., and the Hepatitis B Virus Drug Resistance Working Group. Antiviral Drug-Resistant HBV: Standardization of Nomenclature and Assays and Recommendations for Management*. Hepatology*, 2007; 46(1): 254-65.

33. Mokaya J, McNaughton AL, Bester PA Goedhals D., Barnes E.J.,Marsden B.D.,Matthews P.C.Hepatitis B virus resistance to tenofovir: fact or fiction? A systematic literature review and structural analysis of drug resistance mechanisms. *Wellcome Open Research*, 2020; 5: 151-7.

1. Zhou, Z., Chen, M., Li, P., & Yang J. Multilocus drug resistance mutation analysis in 148 HBV patients in northern Henan Province of China. *Med Rxiv*. 2021; https://doi.org/10.1101/2021.05.14.21257253.
2. Angus P., Vaughan R., Xiong S., Yang H., Delaney W., Gibbs C., Brosgart C., Colledge D., Edwards R., Ayres A, Bartholomeusz A, Locarnini S. Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology,* 2003; 125: 292–7.
3. Sheldon J, Camino N, Rodes B, Bartholomeusz A, Kuiper M, Tacke F, et al. Selection of hepatitis B virus polymerase mutations in HIV-coinfected patients treated with tenofovir. *Antiviral Therapy*, 2005; 10: 727-34.
4. Anthony, R., Brenyah, R. C., Darkwah, K. O., Egbule, B. C., Ninnoni, J. P. K., Okantey, C., & Ephraim, R. K. D. Hepatitis B Infection in People Living with HIV/AIDS; A Retrospective Study of the Effia Nkwanta Regional Hospital. Journal of Advances in Medicine and Medical Research, 2019; 29(8), 1–9. <https://doi.org/10.9734/jammr/2019/v29i830108>.
5. Inglesby TV, Rai R, Astemborski J, et al. A prospective community-based evaluation of liver enzymes in individuals with hepatitis C after drug use. Hepatology, 2019; 29: 590–6.
6. Mathurin P, Moussalli J, Cadranel J-F, et al. Slow progression rate of fibrosis in hepatitis C patients with persistently normal alanine aminotransferase activity. Hepatology, 2018; 27: 568–72.