

## RESEARCH PAPER

# Liver Molecular-biochemical Markers in Viral Hepatitis B patients

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### ABSTRACT:

The prevalence of liver disease has led to its emergence as a major challenge. This challenge is also attributed to factors such as the challenging diagnosis, complexity of pathogenesis, and the absence of established therapies. When Hepatitis B virus (HBV) infections occurs in patients that do not have detectable hepatitis B surface antigen (HBsAg), it is referred to as occult infections. Despite the fact that these kinds of infections have been found in patients with chronic hepatitis C liver disease, there is still no evidence on their clinical implication and prevalence. HBV is a small partial deoxy ribonucleic acid (DNA) virus with like retroviruses. The virus falls under the group of Hepadnavirus infections and family of orthohepadna virus. About 66% of patients with acute HBV infection have an asymptomatic, mild, and sub-clinical illness that typically goes undetected. Thus, in this work, hematological parameters were used in detecting the virus, and the results of the hematological parameters revealed significant changes in white blood cell (WBC), lymphocyte and platelet area under curve (AUC) (0.95), (0.66), and (0.82). The mean values for ALT and ALP in HBV-positive patients increased significantly as compared to the control. Similarly, there was no significant difference between the AUC, CI for HBV-positivity for ALT 0.91, (0.8485-0.9854) and ALP 0.89 (0.8123-0.9633). This study revealed a significant positive correlation between ALP level and ALT and each lymphocyte, granulocyte and WBCs. Thus, each of them may be considered as major factors of development of HBV level.

KEY WORDS: HBV, qPCR, Liver test.

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### INTRODUCTION:

Even with the availability of HBV vaccines and treatment drugs, it is still regarded as a major global health challenge, with over 350 million people having chronic infections worldwide, and almost 1 million people die from the complications associated with HBV [1]. The liver can be damaged to different extents as a result of persistent HBV infection, and this in turn results in cirrhosis, hepatitis, hepatocellular carcinoma (HCC), and fibrosis [7]. HBV belongs to the family of noncytopathic hepatic DNA virus, which can only infect the liver cells of orangutans and humans, and the possibility of HBV to infect other cells that are non-hepatocyte is yet to be proven [8].

Hepatocyte lesions are not directly caused by HBV infection, ways the host's immune responds to the infection determines it clears the virus or causes liver disease. While, transient and self-limiting hepatitis is developed in HBV-infected adults, and 95% of infections end with the elimination of the virus and establishment of protective antibodies, a large number of neonatal vertical transmission of HBV from mother to child leads to chronic infection [9]. Regardless of the fact that there are many factors that contribute to the pathogenesis of the virus, persistent and chronic HBV infection is a complex process which involves the interaction of the virus and the host immune system, thereby incapacitating the adaptive and innate immune response [8]. Chronic hepatitis B virus (HBV) is a frequently occurring human viral infection that affects millions of individuals globally, and is a major cause of morbidity and mortality [10]. Hepatitis B virus is a small partial deoxy ribonucleic acid (DNA) virus with like retroviruses [11]. The virus falls under the group

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of Hepadnavirus infections and family orthohepadna virus. About 66% of patients that are chronically infected with HBV infection have asymptomatic, slight, and sub-clinical illness that typically goes undetected. The disease has a compound history, where its development involves various stages or phases. More active liver disease and high level of HBV DNA in their serum are known to be predisposing factors to the development of cirrhosis and other liver diseases. These are the people who are mostly recommended for treatment.

Patients with Hepatitis will be easily identified due to their elevated serum aminotransferase level. More so, their liver biopsy findings will show a reading with stage 1-3 fibrosis and grade 1-3 inflammation. To some extent, patients with cirrhosis will quickly be pointed out, due the presence of chronic liver infection and a reduced number of platelet counts. The presence of the hepatitis B markers now makes the identification of patients with liver complications advanced to a greater extent. The Agency for Healthcare Research and Quality on the administration of drugs has continuously reported that patients at various clinical phases of Complete Heart Block (CHB) have been undeniably described since antiviral treatment guides for hepatitis B rely upon explicit classes of patients [12].

There is an interaction between HBV and receptors like TLR2/4 that are manifested on Kupffer cells for the production of a large number of inflammatory cytokines and chemokines (TNF, CCL2), which in turn cause damage to the liver [13,14]. By means of the inflammatory mediators, the peripheral monocytes are induced to permeate the liver, and then proliferate and differentiate into macrophages. This in turn, intensifies the production of inflammatory chemokines and cytokines, further inducing the development of liver inflammation and fibrosis [15,16]. Furthermore, immunomodulatory molecules (IL-10, TGF- $\beta$ , PD-L1/2) which inhibit the anti-fibrotic T cells and NK cells, are produced by HBV-induced suppressive monocytes/macrophage; the produced immunomodulatory molecules in turn secrete cytokines like PDGF and TGF- $\beta$  so that the HSC can be activated, thereby allowing the survival of HSC [17,18]. In recent times, it has been reported that patients with HCC and cirrhosis exhibit increase in the numbers of ascetic and peripheral

MDSC, but its effect on such pathology was not established [19]. Theoretically, the function of the NK cells and T cells are impaired by HBV-induced MDSCs which also inhibit the IFN- $\gamma$  secretion through CTLA-4 or PD-L1 [20]. Similarly, the cytotoxicity of NK cells are inhibited by the MDSC through TIGIT or PD-L1 [21,22], thereby preventing the HSC from being killed by the NK cell.

In this paper, the recurrence of these markers has been determined in various clinical phases of chronic hepatitis B infection as portrayed in this paper. The study participants were grouped into two groups, one consisting of normal (control group) and the other group consisting of hepatitis B patients (HBV positive). The control group (n = 40) had 20 males and 20 females, while the hepatitis B virus positive group (n = 40) had 25 males and 15 females.

## Liver Anatomy

The liver is the largest organ of the human body with a weight of around 1500 g, and is positioned in the upper right corner of the stomach. The organ is intently connected with the small digestive tract, setting up the supplement rich venous blood that leaves the body-stomach related tract. The liver performs over 500 metabolic tasks, achieving the synthesis of items that are released into the circulation system (for instance glucose got from glycogenesis, plasma proteins, coagulating parts and urea), or those that are discharged to the extensive intestinal tract (bile). In addition, the liver parenchyma serves as a storage for numerous items such as glycogen, fat-and fat-solvent supplements) [23].

A good number of researchers have attributed the hepatocellular damage resulting from chronic hepatitis B infection (CHB) to increase in oxidative stress [1-4]. Nevertheless, in majority of these works, only the individual antioxidants measurements of patients have been used for the evaluation of oxidative status, and there is limited information about the total antioxidant response (TAR) of subjects with cirrhosis and CHB caused by hepatitis B virus (HBV) [5]. Based on the review of literature done in this study, the literature is lacking in information about oxidants in subjects with cirrhosis resulting from HBV infection, as well as information on antioxidants and oxidants in inactive hepatitis B carriers.

## Liver Disease

One of the significant roles played by the liver is to process all ingested food substances. It can either convert the elements into a useful product or waste products which are eliminated at the end of the process [24]. The liver, just like other organs that comprise a complete human body, can be damaged in or inflamed as a result of abnormal conditions. Some of the complications associated with the liver are discussed below [25].

Hepatic steatosis is considered to be the most common liver disease, and it may be reversed. It often affects individuals who have Crohn's sickness, and ulcerative colitis. In this complication, fats become deposited in the liver affecting the accommodation of liver cells, hence affecting the performance and functioning of the liver in general. Such complications are likely to affect individuals with diabetes, obesity, and those who are pregnant as well. Individuals using steroids also stand a chance of suffering from the same complications. In most cases, these conditions do not require to be corrected as it bears minimal effects [24].

Another liver disease is Gallstone; the condition continues to affect individuals with Crohn's illness. The situation leads to cases whereby the bile is withheld until it is required for absorption. In such a fact, bile tends to become hard leading to the formation of stones in the gallbladder. Such a case leads to the blockage of the bile duct as a result of the need for the rocks to leave the gallbladder. Such an issue may, however, be resolved by the treatment of the gallbladder [26]. The bile channel may be affected through aggravation by primary sclerosing cholangitis (PSC). Such a condition often tends to change in patients who have ulcerative colitis, but rarely in those who have Crohn's malady. A small number of patients experiencing PSC tend to lack IBD. Complications of this kind leads to irregularities in the bile stream. The damage of the bile may lead to the development of jaundice and tingling. Such a condition is often difficult to control. Stents may be used in the control of bile development in order to enhance the bile streaming process [24]. Primary Sclerosing Cholangitis entanglement may cause bile contamination and excessive growth of bile channel. In such a case, the liver becomes excessively damaged, and transplantation may be

required. Colon malignant growth is yet another complication likely to affect patients with PSC. In such a case, patients are required to seek regular checkups [27].

### Liver cancer caused by HBV

Hepatitis B virus is the most common factor leading to liver cancer. Hepatitis virus link is associated with liver cancer in the sense that it causes an inflammation of the liver, and over time after it has become chronic hepatitis, it stops the liver from carrying out its important duties such as filtering toxins and controlling blood glucose. Out of the nine cancers, hepatocellular carcinoma is the one majorly caused by Hepatitis C and B viruses. Worldwide, 54% of people infected with liver cancer have Hepatitis B. Extra hazard factors for developing liver cancer growth include cirrhosis and smoking, exorbitant alcohol use, just as diabetes and obesity. Some inherited ailments that cause liver harm, also increase the possibility of developing liver cancer. Race or ethnicity and a family history of liver cancer growth are regarded as risk factors. Liver cancer growth is more typical among men than women, regardless of factors such as race or ethnicity [28].

### Hepatitis B Virus

The HBV is a small partial DNA virus with like retroviruses [11]. Hepatitis B virus falls under the group of Hepadnavirus infections and family of orthohepadna virus. About 66% of patients with acute HBV infection have asymptomatic, mild, asymptomatic and subclinical illness that typically goes undetected. HBV is a DNA-containing virus. However, the replication occurs by means of an RNA middle of the road, a feature that places hepadna viruses near retroviruses. The remarkable features of the HBV replication cycle show the ability of the virus to survive in infected cells. This virus has an impact on the liver, making some parts of the body not to function normally. In the case of constant hepatitis, liver cirrhosis. The infection spreads through the blood and body liquids, making it simple to spread [29].

### Molecular structure of HBV

HBV is a small, partly double stranded DNA virus, enveloped, and hepatotropic virus that is

spherical with filamentous structures and relaxed circular DNA genome [28].

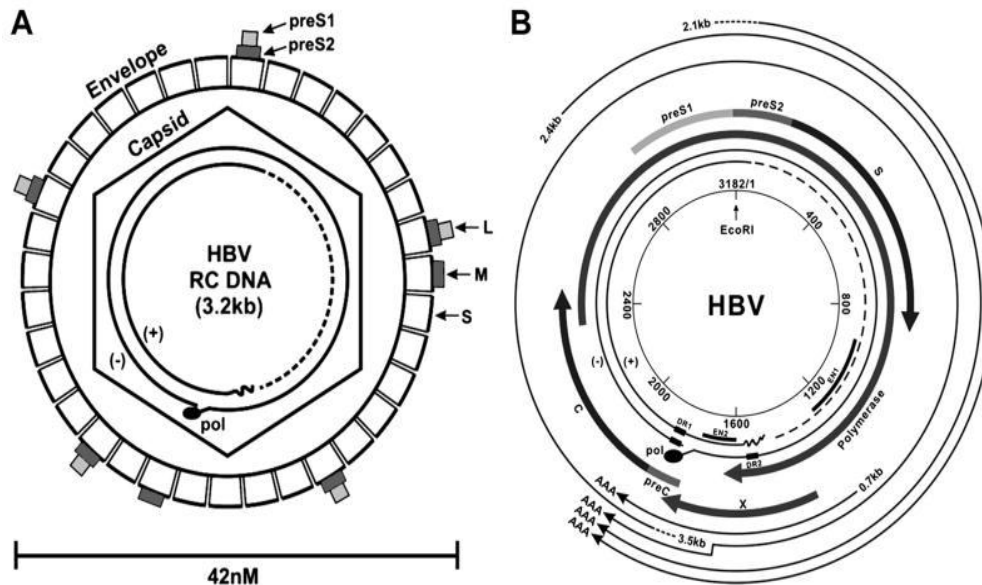


Figure 1: Molecular structure of HBV (Ghany M *et al.*, 2007)

Mostly, the HBV genome contained within the capsid appears in form of a double stranded and fragmented hover, with the polymerase protein combined with the “minus” strand in a covalent manner (figure1 A). the circular map of the HBV genome alongside nucleotides numbered from the single EcoRI site (by tradition), transcripts alongside their polypeptide products. It is regarded as unique because it consists of DNA that is not twofold stranded (figure1 B). [30].

### Mode of transmission and prevention

The most common mode of transmission is perinatal, whereby an infected mother passes on the virus to the child at birth. Another common mode of transmission is horizontal transmission, which is a transmission whereby the virus is transmitted to an uninfected person through the blood of an infected person, e.g. through accidental needle pricks. Another way is through sexual contact with an infected person, and in this mode a person gets infected through contact with the body fluid of the infected person. Therefore, efforts such as education and vaccines must be directed towards the avoidance of high-risk behaviors. Also, in several countries where the prevalence of HBV is low, there are infant

vaccination programs and adolescents are also vaccinated against HBV so that they do not get infected even if engaged in sexual behaviours. Researchers have suggested that people in high risk areas should be vaccinated, and people who have been exposed to the virus not after two weeks of the prophylaxis therapy [31].

### HBV genotypes and vaccines

Currently, there are ten known types of genotypes, and these also have mutants (A-J). They are classified according to geographical distribution as well as their response to antiretroviral therapy. The different genotypes contribute to the different characteristics in people with HBV worldwide as each their own characteristic. Recombinant hepatitis B immunizations are of the A2 genotype, which is one of the ten known genotypes whose circulation shifts internationally. Reports of uncommon HBV contaminations in blood donor with an imbalance of non-A2 genotype HBV in vaccinated subjects have brought up issues about the cross-insurance managed by HBV-A2 antibodies. Contaminations in HBV vaccines were asymptomatic and transient, demonstrating that immunization could result in clinical infection. Preclinical data demonstrates cross-reactivity and cross-assurance

by A2 antibodies against non-A2 HBV genotypes. Significant enhancements in HBV control have been exhibited in nations with differing genotype dispersion that have presented general youth HBV immunization programs. Available information shows that current HBV-A2 vaccination are of great importance in the prevention of infections and clinical disease caused by all the known HBV genotypes [32].

## Materials and Methods

### Participants

The study recruited n= 80 cases with a mean age of 47 years participants were grouped into two groups, (normal control ) and viral Hepatitis B (HBV-positive). The control group (n=40) had 20 males and 20 females, while HBV-positive group (n=40) had 25 males and 15 females.

The study was conducted at the public health laboratory of Erbil city / Kurdistan region /Iraq from the month of October 2018 to the month of February 2019.

### Sample Collection

The blood was collected from arm vein by using sterile disposable syringe. This, however, posed a danger because of the rupturing of the blood vessels. This means that, only sterile materials were used in this process so as to reduce the emergence of other health risks associated with puncturing of blood vessels such as spreading of germs and infections. Blood from the elbow bend, forearm and the back of the hand was used as the sample in the public health laboratory Erbil. The blood was extracted with standard specimen collection jell tubes. About 6ml of blood treated with EDTA was used to as the sample. The purpose of treating the blood with EDTA was to prevent the blood sample from clotting. The blood from veins was used because it contained all the wastes and diseases as it moved around the body, and it had not been purified in the liver, thereby making the concentration of the virus in the venous blood high. After the blood samples were taken, the centrifuge was fixed at the rpm of about 4000 because the serum was being worked on.

### DNA Extraction

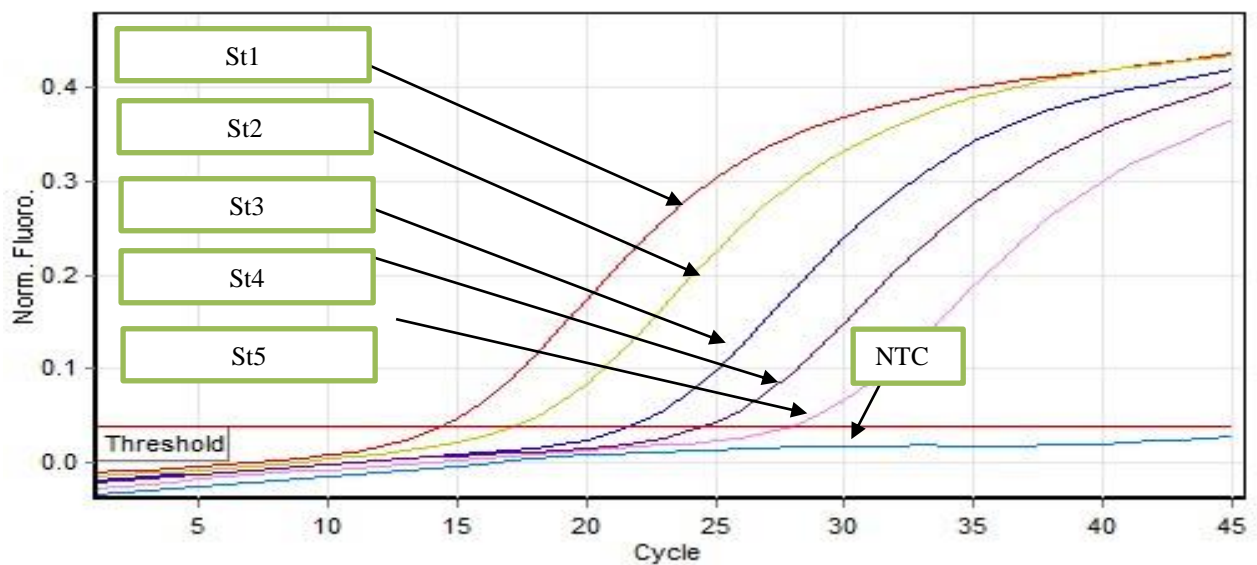
The EZ1 automated machine ,simultaneous purification of viral nucleic acids from serum samples using artus<sup>®</sup> HBV RG PCR kits were used in this work, and their contents are Elution tubes (1.5ml), QIAGEN<sup>®</sup> protease (lyophilized), Protease re -suspension buffer 6 ml, Carrier RNA 310 µg, Buffer AVE 2ml with adding 2ml of each samples.

### Real Time PCR

Real time PCR serves as a method of monitoring how the polymerase chain reaction progresses so that the replicas of the DNA region of interest can be generated (in this case, it is the DNA of Hepatitis B). With the use of this technique, the amount of Hepatitis B virus was determined. There are three main steps involved in the process, and they are given as follows:

Denaturing (95 °C for 15 secs): here, the DNA's double stranded template was heated so that it can be separated into two single strands. Annealing (55 °C for 30 secs): this involved lowering of temperature to enable the DNA primers to initiate the attachment to the DNA template. Extending (72°C for 15 secs) the temperature was again increased so as to make the new strand of DNA using the Taq polymerase enzyme, These three stages were repeated about 40 times so that the yield of the replicated DNA can be increased. Electrophoresis was then employed after the three processes were repeated so as to determine the size and quantity of the DNA fragment produced.

By means of the Real-time PCR, the amplification of PCR and location are consolidated into a solitary step. This highlights the relevance of using gel electrophoresis to detect items, and ultimately, it makes the strategy to be quantitative. With real-time PCR, the use of fluorescent dyes is employed in the labeling of PCR products during the process of thermal cycling. More so, using the real-time PCR instruments, the collection of fluorescent signals were measured during the exponential period of the reaction. This was aimed at achieving rapid and accurate evaluation of PCR products as well as objective data analysis by Rotor gene Q.



**Figure 2:** Five standard curve for quantitation detection of HBV standards No Template Control (NTC)

#### Data Analysis:

One-way ANOVA t-test was used for liver test parameter with chi-square. A P-value less than 0.05 was considered statistically significant. For PCR data interpreting using the software provided with the Rotor-Gene Q Series Software 2.1.0 this algorithm allows the direct comparison of the samples that have different starting fluorescence levels with the quantitation curves of samples. The Statistical Package for the Social Sciences program was used for data analysis GraphPad Prism 8.

## Results

#### Liver function parameters

In the multiple regression analysis performed in this study, ALT ( $p < 0.0001$ ) and ALP ( $p < 0.0001$ ) were identified as independent predictors of HBV ( $r^2 = 0.67$ ,  $p > 0.001$ ). The mean values for ALT

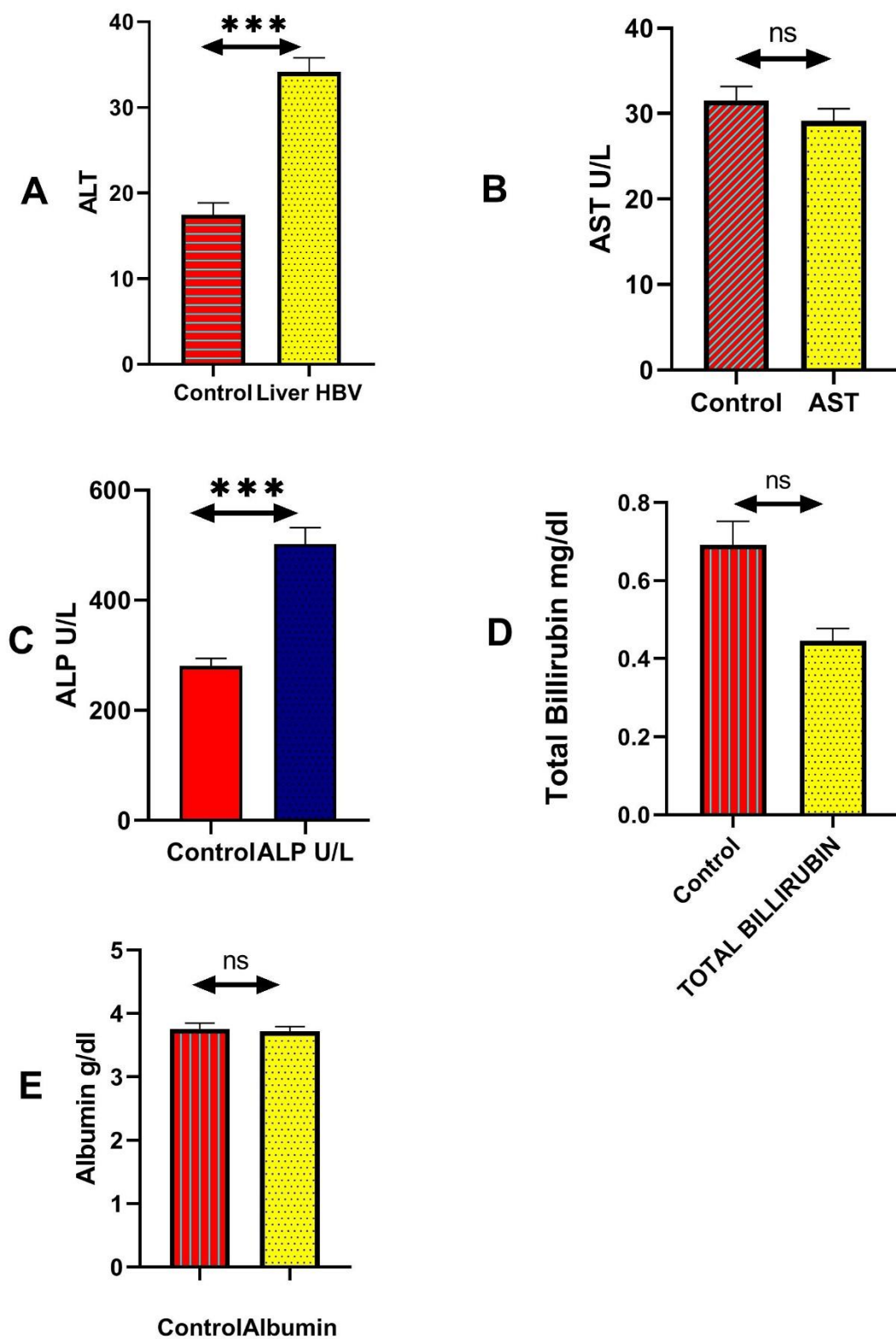
and ALP in HBV-positive patients increased significantly as compared to that of the control group. Similarly, there was no significant difference between the AUC (CI) for HBV-positivity for ALT 0.91 (0.8485-0.9854) and ALP 0.89 (0.8123-0.9633). On the other hand, total bilirubin, serum albumin and AST were not significantly associated with the HBV-positivity even after excluding AST from the model since it is associated with ALT.

Sensitivity test revealed that HBV-positivity did not affect the accuracy of AST, total bilirubin and serum albumin, However, their (Mean  $\pm$  SE) was found to be lower in HBV positive patients, normal control : AST  $31.54 \pm 1.63$  vs  $29.14 \pm 1.44$   $p = 0.21$ , Total bilirubin  $0.69 \pm 0.09$  vs  $0.44 \pm 0.03$   $p = 0.063$ , and serum albumin  $3.75 \pm 1.63$  vs  $3.72 \pm 0.07$ , but were not significantly ( $p = 0.073$ ) different from the control group.

**Table 1:** Linear Regression analysis for Viral load as dependent variable with ALT, AST, ALP, as independent Variables.

Parameters	AUC	95% CI	P value	Mean $\pm$ SE	
				Control	HBV+ve
ALT (UI/L)	0.91	0.8485 to 0.9854	<0.0001	17.48 $\pm$ 1.375	34.19 $\pm$ 1.604
AST (UI/L)	0.60	0.4591 to 0.7394	ns	31.54 $\pm$ 1.625	29.14 $\pm$ 1.441
ALP (UI/L)	0.89	0.8123 to 0.9633	<0.0001	280.2 $\pm$ 14.08	502.2 $\pm$ 29.63
Total bilirubin (mg/dL)	0.71	0.5857 to 0.8488	ns	0.6913 $\pm$ 0.06060	0.4454 $\pm$ 0.03213
Serum Albumin (g/dL)	0.51	0.3727 to 0.6424	ns	3.755 $\pm$ 0.09355	3.720 $\pm$ 0.07393

ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase  
, NS: not significant



**Figure 3:** Box plot show comparison between control and HBV patient for liver tests parameters: (A) ALT: Alanine transaminase, (B) AST: Aspartate transaminase,(C) ALP: Alkaline phosphatase, (D) Total bilirubin. (E) Serum albumin



### Correlation between liver function test and hepatitis B virus.

Linear regression analysis was used to determine the correlation between liver function levels (AST, ALT, ALP, total bilirubin, serum albumin) and hepatitis B viruses among the patients of the

**Table 2:** Linear regression analysis for viral load as dependent variable with alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, albumin as independent variables.

Stat. Variables	R	95% CI	P value
Liver Function tests			
ALT	-0.1509	-0.4452 to 0.1729	ns
AST	0.001875	-0.3223 to 0.3257	ns
ALP	-0.09158	-0.3919 to 0.2264	ns
Total bilirubin	0.06964	-0.2650 to 0.3893	ns
Serum Albumin	-0.3014	-0.5665 to 0.02023	ns
ALT	-0.1509	-0.4452 to 0.1729	ns

In order to obtain the CT value, quantitation information standard curve was run on software version 2.1.0.9. The CT value ( $CT = -3.287 \cdot \log(\text{conc}) + 34.370$ ) refers to the cycle number at the point that the amplification curve goes beyond a threshold (0.06182) of detection. In order to determine the CT value for each of the curves, a threshold line is set and the intersection between the curves is calculated. R2 value (correlation coefficient): the R square value (0.99971), or R2 value refers to the percentage of data that corresponds with the hypothesis that the standards form a standard curve. In an event of low R2 value, then it is difficult for standards to fit into a line of best fit. This may have a bearing on the reliability of the results, which are the calculated concentrations. A good R2 value is approximately 0.99. M and B. the calculations of the slope (M)

study samples, no correlation was found between ALT and HBV (-0.1509, p= ns), but a positive correlation was found between AST and HBV (0.0018), whereas it can be seen from table (4) which shows the liver function and hepatitis (ALP, serum albumin), there was no correlation between them except total bilirubin .

and the intercept (B) of the standard curve are done automatically through the use of the formula  $y = Mx + B$ , and shown in the “Standard Curve” Reaction. Efficiency Threshold: The “Reaction Efficiency Threshold” refers to an alternate approach that is used in eliminating noise from the analysis. In this normalizing algorithm, the reaction efficiency estimation techniques are employed in comparative quantitation. Any sample that lacks a reaction efficiency of at least this level are excluded and a flag “NEG (R.Eff)” will be shown in the “CT Comment” column. A level of 0% shows that at the exponential stage, there was no reaction. On the other hand, 100% is indicative that a totally efficient reaction occurred at the exponential phase. In addition, when the percentages are negative, it means that there was a decline in the signal during the exponential phase.

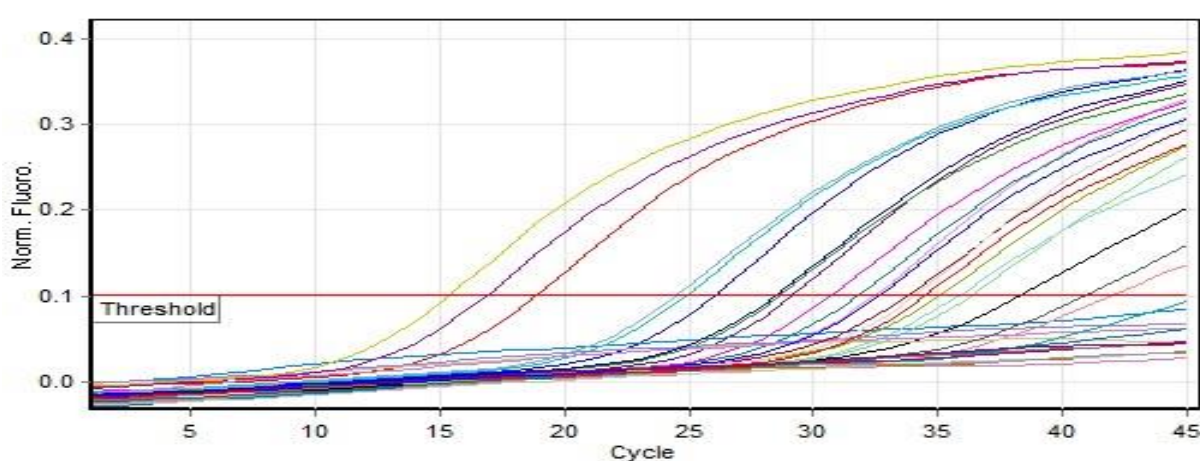


Figure 3: Quantitation data of Q PCR detection of hepatitis HBV virus

## Discussion

This study evaluated a cohort of 80 participants out of which 40 were patients on various stages of viral hepatitis B infection. More so, 40 of them were the normal case with no detectable HBV by PCR. All the infected patients had detectable HBV DNA by PCR, and some had elevated viral loads of up to 106 virions/ml. Acute or chronic hepatitis can be caused by HBV infection. In both stages, the hepatitis B antigen (HBsAg) is usually detected in serum first before the diagnosis is performed [36].

The research showed that there was a significant increase in the (ALP), 5' nucleotidase (5-NT), gamma glutamyl transferase (GGT), prothrombin time, (PT), alanine aminotransferase, (ALT) aspartate aminotransferase (AST), and Bilirubin in patients with viral hepatitis in comparison to the control group. Additionally, there was a decrease in the activity of serum, albumin and TSP concentration. From the results, it can be concluded that a peculiar way to diagnose viral hepatitis and to distinguish it from other types may be GGT, ALP, AST, ALT, 5-NT, and PON.

The infection results in the production of a substantial amount of HBsAg that is easily detected in some portions of HBsAg antigens representing the virions that are intact in serum. This is because a significant proportion of HBsAg consists of the antigen particles that are usually produced in excess of the intact particles [33]. The detection of HBsAg is therefore challenged by the absence of a simple tissue for culturing the virus.

Originally, HBV was detected through the recognition of the serum surface antigen (HBsAg) in individuals having HBV [34]. However, viral nucleic acids and other forms of antigenic

constituents have been identified and categorized [35]. The uses of both immunometric and molecular methods have facilitated the diagnosis of HBV [36]. It is worth noting that during the selection of the right assay, there should be a close diagnostic relationship to the biological properties of the particular marker in question. Additionally, some body enzymes particularly those found in the liver and other fluids such as bilirubin make it easy for the identification of HBV based on various factors.

The superiority of PCR over other techniques such as hybridization assays has been demonstrated before. Moreover, PCR is said to detect HBV DNA in acute infections and in HBsAg-negative patients. The specificity of PCR however, may be hampered on by contamination with HBV DNA, which may arise during sample preparation, thereby resulting in false positive. Thus, extra precautions are required during sample collection and processing. In this study, all the 40 patients had detectable HBV, while none of the healthy individuals was found with HBV DNA. These findings are consistent with those of previous, which showed that HBV DNA was not detectable in normal cases. It is worth noting that HBV DNA is also not usually detectable in serum of patients who are recovering from acute HBV infection.

results of the molecular analysis were compared and correlated with the biochemical parameters including serum alkaline phosphatase, aminotransferase, albumin and bilirubin and also demographic data to evaluate the role of viral load in HBV infection. The HBV virus which belongs to the family of Hepadnaviral contains minute amounts of double-stranded DNA. Hepatitis virus is thought to have a negative effect on the liver

cells either directly or indirectly, but this effect remain unclear. In addition, the viral DNA can have a direct effect on the liver by integrating into the liver cell genome, and resulting in chromosomal instability. Chromosomal instability is associated with deletions or rearrangements of the chromosome. This integration of HBV DNA into the genome of hepatocytes can also result in dysfunction of the tumour suppressor genes and oncogenes which are involved in the survival of the cell. Furthermore, the direct effect is also linked with the 16.5kDa HBX protein that is made up of 154 amino acid. HBX protein transactivates genes that regulate cell proliferation and cell cycle as well as apoptosis and DNA repair. Management of liver cirrhosis basically involves the treatment of chronic viral hepatitis and abstinence from etiological parameters such as alcohol.

## Conclusion

This study assessed the biochemical markers of the liver with specific emphasis on the significance of HBV viral load as a diagnosis parameter. Serum total bilirubin is among the first known markers for liver functioning, but still not adequate for diagnosis despite its increased activity in some liver disorders. In this study, liver damage has been attributed to high amount of bilirubin. Damaged hepatocytes produce large amounts of bilirubin resulting in high levels of bilirubin in serum. The degree of damage is dependent on the ratio of direct and indirect bilirubin. The findings indicate that most of the patients had elevated levels of bilirubin in serum suggesting necrotic tissue necrosis [37].

Despite being insufficient for a diagnosis of liver disorder, bilirubin possess a significant diagnostic value. The present study revealed high values of enzymes ALT (74-1049U/L) and AST (30-436.6U/L) in HBV patients, while normal values were detected in the normal control group. Compared with the previous findings, the results of this study are higher significantly [38]. Elevated enzyme levels occur due to increased enzyme activity which occurs when the hepatocytes are damaged. An elevated level of serum ALT is the best-known indicator of liver destruction. It has been shown that necrosis of the liver cells does not necessarily result in high levels of AST, and this explains the weak correlation

that was observed between AST and HBV DNA. Previous findings have also shown that normal ALT levels and detectable HBV DNA in patients may be associated with liver damage. This was evidently shown in the present study. This study found a strong correlation between albumin, bilirubin, ALT and AST. This is in line with the findings of previous studies which showed a correlation between high levels of these parameters with liver damage, while their levels correlated well with the viral load of HCV RNA [38]. Interestingly, in the current study a strong correlation was found between ALT, AST, bilirubin and other demographic parameters with HBV DNA. High levels of serum albumin bilirubin ALT and AST has also been found in elderly individuals who are infected with the hepatitis B virus. This was evidently shown by the strong correlation that was observed between age and the biochemical parameters, as well as the HBV DNA. This reveals the role of immunity in the pathogenesis of the chronic liver disease. Molecular techniques detected very low quantities per specimen, but very low titers of serum HBV antigen in the circulation could still lead to a negative result for HBV DNA. This is because not all patients with positive HBsAg possess detectable HBV DNA. In normal cases (patients with HBV antigen but no HBV infection), the DNA is usually undetectable in serum indicating that there is no liver disease present. This study indicates that all the infected patients had detectable HBV DNA by PCR, while some had elevated viral loads. Polymerase chain reaction was able to detect extremely low amounts of HBV DNA in the circulation; all the healthy individuals had no detectable HBV DNA in their serum. However, prevailing theory behind the presence of HBs Ag with no detectable DNA is still unclear since the virions could be present in levels that are below the detection limit of PCR. Finally, more findings are required to determine whether the patients in a particular stage is in the transition to another phase or not. This is needed specifically to assess the severity of liver disease; the distinct phenotypes of HBV infection should be determined as there is a great need for antiviral therapy.

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