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Assessment of Haematological, Cytokines, and Hepatitis B Virus Genotypes among Individuals Infected by Hepatitis B Virus and Liver Dysfunction in Abakaliki, Ebonyi State, Nigeria.

Chibueze A. Okereke[1], Prof. Silas A. Ufelle[2]

[1,2]University of Nigeria, College
Of Medicine, Enugu Campus
Medical Laboratory Sciences
[1]Afogugodswill204@gmail.com,[2]silas.ufelleunn.edu.ng

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ABSTRACT

Background: Hepatitis B virus (HBV) infection remains a major public health challenge in Nigeria, contributing significantly to chronic liver disease and mortality. The interaction between HBV genotypes, host immune response, and haematological alterations determine the clinical outcome of infection and progression to liver dysfunction. **Objectives:** This study was designed to assess the haematological parameters, cytokine profiles, and HBV genotypes among individuals infected by HBV with liver dysfunction. Ethical clearance was obtained from Directorate of Health Research and Ethical Committee of the Ebonyi State Ministry of Health, Abakaliki. **Methods:** One hundred and twelve individuals with HBV infection were drawn from Alex Ekwueme Federal Teaching Hospital and Ebonyi State University Teaching Hospital, Abakaliki consisting of 78 males and 34 females. Informed consent were obtained from them after pretest counsel. Blood sample (10.0ml) was collected aseptically from each subject using a sterile syringe and needle. 4.0ml was placed in ethylene diamine tetra-acetic acid and 6.0ml in plain container. The analysis of the samples were done in Marylmas Research and Diagnostic Laboratory, Abakaliki, Ebonyi State, Safety Molecular Laboratory, Warri, Delta State and DNA LABS Molecular Diagnostics and Research, Ungwan rimi, Kaduna State using standard operating procedure and automation using automated machine, Zybio Z3 (China) for full blood count and Thermal Cycler Polymerase Chain Reaction (PCR) machine (Germany) for HBV genotyping. **Results:** The significant ($p < 0.001$) results of HBV infected subjects were increased in white blood cells
(WBC) (9.11 ± 3.71x10^9/L), red blood cells (RBC) (5.59 ± 1.50x10^{12}/L), mean platelet volume (MPV) (11.42 ± 1.73fl), and decreased in haemoglobin (Hb) (11.51 ± 2.07g/dL), haematocrit (Hct) (34.66 ± 6.05%), mean cell volume (MCV) (86.66 ± 5.60fl), red cell distribution width – standard deviation (RDW-SD) (40.38 ± 3.45fl) and platelet (Plt) (141.58 ± 39.98x10^9/L) when compared to control, WBC (5.06 ± 0.92x10^9/L), RBC (4.18 ± 0.52x10^{12}/L), MPV (8.32 ± 1.51fl), Hb (12.83 ± 0.78g/dL), Hct (39.27 ±

1.67%), MCV (86.93 ± 4.11fl), RDW-SD (42.80 ± 4.14fl) and Platelet (185.00 ± 24.05x10^9/L). The significant ($p < 0.001$) results of Liver enzyme markers in HBV infected subjects were increased in alanine aminotransferase (ALT) (23.25 ± 13.54U/L), aspartate aminotransferase (AST) (14.82 ± 7.91U/L), total bilirubin (Tbil) (11.34 ± 6.54Umol/l) and direct bilirubin (Dbil) (8.05 ± 3.61Umol/l) when compared to control, ALT (6.16 ± 2.72U/L), AST (8.09 ± 3.20U/L), total bilirubin (8.92 ± 2.61Umol/l) and direct bilirubin (1.57 ± 1.12Umol/l). The significant ($p < 0.001$) results of cytokines changes in HBV infected subjects increased in interleukin-10 (IL-10) (198.68 ± 217.96pg/mL) and tumour necrotic factor-alpha (TNF-α) (85.93 ± 89.04pg/mL) when compared to control, IL-10 (46.93 ± 13.86pg/mL) and TNF-α (23.73 ± 7.27pg/mL). The genotype distribution of HBV infected subjects showed that 13.3% were of mixed genotype B and E, 36.7% were genotype E, 13.3% were genotype B and 36.7% indicated no amplification. **Conclusions:** The study have proven that unified assessment of haematological variables, cytokine profiles, liver enzyme markers and hepatitis B virus genotypes contribute thorough knowledge on the pathophysiology, variations in clinical manifestations and treatment outcomes of HBV infection and has also revealed the HBV genotypes circulating in Abakaliki, Ebonyi State.

1. INTRODUCTION

1.1 Background of the study

Hepatitis B virus (HBV) infection remains a major global public health concern, with an estimated 254 million people living with chronic HBV infection as of 2025 (WHO, 2025), and chronic viral hepatitis causes 1.3 million deaths every year, mostly from liver cancer and cirrhosis (WHO, 2025). The impact of HBV infection is devastating for individuals, families, communities, and the world at large (Ade-Ojo *et al*., 2023). "Chronic HBV infection leads to activation of the compensatory mechanism for liver cell death by triggering a sustained inflammatory response, which gradually worsens to cirrhosis and eventually to hepatocellular carcinoma" (Capone *et al*., 2015; Rico *et al*., 2021). Hepatitis B virus (HBV) is a hepatotropic DNA enveloped virus (Hoan *et al*., 2021), and it is a member of the family *Hepadnaviridae* and under the genus *Orthohepadnavirus* (Anabire *et al*., 2023).

The burden of hepatitis B virus infection in Ebonyi State is "6.9% which is far above 3% highest prevalence found in Sub Sahara Africa and 2% in America and Europe" (Chima *et al*., 2022). "Ebonyi North having the highest prevalence of 8.9% followed by Ebonyi Central, 7.1% and Ebonyi south, 5.2%" (Chima *et al*., 2022). "The present prevalence of accurately aligns with the global burden that affects hundreds of millions of people" (Okereke and Ufelle, 2025). In Izzi community in the state in particular, one in every family is living with vira l hepatitis B virus infection. "The major route for "transmission of HBV in Nigeria include sexual intercourse, local circumcision, local uvulectomy, scarification, tribal marks, surgical procedures, home birth, and blood transfusions" (Elom *et al*., 2024).

Hepatitis B virus is not responsible for the direct destruction of the liver, the disease related complications and prognosis are more related to immune system action, especially cytokines (Wong *et al*., 2017). For effective immune response to be initiated, immune cells must assume a mechanism to communicate with each other (Kim, 2025). Cytokines play a role in regulating immune and inflammatory responses, and these effector molecules are produced continuously at low levels but temporarily at exponential levels to locally control the rate and duration of the immune response (Kim, 2025). "The hepatocellular carcinoma development is closely related to the tumour microenvironment (TME), and cytokines produced by immune cells and cancer cells" (Oura *et al*., 2021; Chan *et al*., 2020). "Many cytokines play an important roles in the pathogenesis, progression, invasion, and metastasis of HCC that progresses from schronic

hepatitis B" (Chan *et al*., 2020; Jekarl *et al*., 2019; Ono *et al*., 2020). "The virus upon attachment and entry into the host nucleus, the deoxynucleic acid (DNA) molecule of the virion is changed into a covalently closed circular DNA (cccDNA), which serves as a template for initiation of replication" (Oyinloye and Bukbuk, 2021).

There is a lack of proofreading activity by the reverse transcriptase during replication, mutants, and different genotypes have emerged (Tsukuda and Watashi, 2020). At present, there are nine known HBV genotypes (A–I) with a greater than 8% nucleotide difference between each other (Fernandes da Silva *et al*., 2023). Severity of liver diseases and outcome of treatment with antiviral drugs depends on the HBV genotypes (Kramvis, 2016), each with a distinctive geographical distributions (Mahmood *et al*., 2016). Different HBV genotypes present different clinical outcome and response to treatment (Ahmad *et al*., 2019). An increase occurrence of transfer from infected mother to child has been linked to genotypes B, C, and I (Komatsu *et al*., 2015), while high transmission rates during sexual activity or drug injection has been linked to genotypes A, D and G (Anabire *et al*., 2023).
Chronic liver disease (CLD) causes wear and tear of the liver echotecture leading to fibrosis and cirrhosis, both being conferred end stages of the illness (Joshi *et al*., 2024).
Liver cirrhosis alters haematological indices and cause pancytopaenia (Fierro-Angulo *et al*., 2024), and the cause "is multifactorial, including splenic sequestration, bone marrow suppression, and disturbance in the balance of haematopoietic factors" (Peck-Radosavljevic, 2017).
The most common type of anaemia seen in liver cirrhosis is normocytic normochromic anaemia, due to the chronic inflammatory state (Kaur *et al*., 2021). Blood loss from oesophageal and rectal varices, portal hypertensive gastropathy (PHG) and gastric antral vascular ectasia (GAVE) can give rise to iron-deficiency anaemia (microcytic hypochromic anaemia) (Sharma *et al*., 2025). Another reason for anaemia and leukopaenia in patient with CLD is hypersplenism, a condition characterized by an over reactive spleen, which leads to the premature destruction of red and white blood cells and hepcidin deficiency (Kaur *et al*., 2023).

1.2 Statement of the problem

Liver dysfunction due to hepatitis B virus (HBV) is accompanied with bleeding and destruction of liver matrix and hepatocytes. These create a problem to the patients in that, blood cells may not perform its functions due to impairment. The functional immune cells may be affected leading to immune compromised status, which may lead to death. These complications may be silent, since HBV presents with high rate of latency in their respective hosts. It is necessary to understand the pattern of haematological and lymphocyte indices in association with the circulating HBV genotypes, immune response with some cytokines, with a view for appropriate therapeutic intervention.

1.3 Justification of the study

In Ebonyi State, the impact of hepatitis B virus (HBV) infection is enormous. This is due to high incidence of HBV spread, liver dysfunction and death within the individuals and communities. The resultant complications may affect blood volumes/parameters leading to anaemia and immune compromised status. Differences between genotypes of this virus affect disease severity and course, likelihood of complications and response to treatment.

Thus, it is necessary to ascertain the link between blood parameters, genotypes, and immune status of patients with HBV and liver dysfunction.

1.4 Significance of the study

The significance of this study lies in its potential to:

1. Improve understanding of HBV pathogenesis and pathophysiology,
2. Informed the development of personalized treatment strategies,
3. Enhance liver disease diagnosis and monitoring,
4. Contribute to the control and prevention of HBV transmission.

1.5 Research Questions

i. What are the alterations in haematological parameters in treated and untreated HBV infected individuals?

ii. What are the states of the liver enzymes in treated and untreated HBV infected individuals?

iii. What are the states of Interleukin-10 and tumour necrosis factor alpha on HBV infected individuals when compared to healthy controls?

iv. What are the genotypes of hepatitis B virus (HBV) circulating in Ebonyi State?

1.6 Aim of the Study

To assess haematological, cytokines, and hepatitis B virus genotypes among individuals infected by hepatitis B virus with liver dysfunction in Abakaliki, Ebonyi State, Nigeria.

1.7 Specific Objectives

1. To determine the haematological parameters among individuals with hepatitis B virus infection and liver dysfunction.
2. To estimate the liver enzymes among individuals with hepatitis B virus infection and liver dysfunction.
3. To estimate the levels of IL-10 and TNF-α among individuals with hepatitis B virus infection and liver dysfunction.
4. To determine HBV genotypes circulating in Abakaliki, Ebonyi State.

2. LITERATURE REVIEW

2.1 Conceptual Framework of the Study

The study used a conceptual framework adapted and modified to suit the present study from "the algorithm for management of hepatitis C virus (HCV) - positive patients with cancer" (Bozza *et al*., 2015). Hepatitis B virus infection is a fatal, life-threatening infection of the liver caused by the Hepatitis B Virus (HBV) (Liang *et al*., 2023). Transmission of HBV can either be through horizontal route from unprotected sexual contact, needle stick injuries, transfusions, and splashes or by vertical route from infected mother to child at birth (CDC, 2018). If HBV infection progresses to chronic hepatitis, it can possibly lead to liver failure, cirrhosis and hepatocellular carcinoma (HCC) (Madihi *et al*., 2020). The prevalence of HBV is evaluated based on the serological presence of hepatitis B surface antigen (HBsAg) in a given area (Karabey *et al*., 2025). The treatment of HBV aimed at preventing transmission, preventing liver cirrhosis and possibly death (Roma *et al*., 2024). This can be achieved by normalizing liver enzyme levels and stopping necroinflammatory activity through halting HBV replication (Lee *et al*., 2020). At present, about nine

HBV genotypes labelled A to I and more than 40 sub-genotypes based on genomic diversity have been identified (Rajbhandari *et al*., 2025). "These genotypes and sub-genotypes champion disease prognosis, response to antiviral therapy and route of viral infection" (Rajbhandari *et al*., 2025). There is variations in response to treatment among different genotypes with genotype C infection have a higher risk of cancer development than those with genotype B infection (Wai *et al*., 2022). The immune system plays a significant role in the pathogenesis and development of liver cancer (Chataa *et al*., 2024). It is known that HBV infection initiates a complex encompassing innate and adaptive immune response that plays a major role in the pathogenesis and clinical outcomes of the disease (Rajbhandari *et al*., 2025). Nevertheless, HBV has liberated several mechanisms to evade these early immune response which allow the virus to develop a chronic infection (Lannacone and Guidotti, 2022). "Interleukin-10 is a cytokine that modulates both innate and adaptive immune response by exerting anti-inflammatory effect" (Chataa *et al*., 2024). Tumour necrosis factor-alpha has been recorded to be one of the major pro-inflammatory cytokine that participates in the pathogenesis of HBV by inducing inflammation and apoptosis (Hassoon, 2024). Haematological parameters such as red blood cell (RBC), white blood cell (WBC), platelet count, and differential counts serve as valuable markers in close monitoring of the progression and severity of liver disease like hepatitis (Moore-Igwe *et al*., 2025). The alteration in these profiles can result in liver inflammation, fibrosis, and even hepatocellular carcinoma (Uche and Nwokediuko, 2023). The liver function tests are diagnostic procedures mainly to identify, diagnose, and evaluate hepatic diseases (Taiwo *et al*., 2024).

2.2 THEORETICAL FRAMEWORK

This study "Assessment of haematological, cytokine and hepatitis B virus genotypes among individuals infected by hepatitis B virus and liver dysfunction" adopted Immune Response "Pathogen interacts with the host and causes infection, leading to the development of disease in the host" (Rana *et al*., 2015). When the hepatitis B virus (HBV) (pathogen) enters into the human body (host), they will initiate interaction that will result to HBV infection. It is known that "when the pathogen enter the host cell, it has to face a strong inherent immune defense" of the host which when confined will eliminate the pathogen (Basset *et al*., 2003). The microbial pathogens express specific signalling molecules referred to as "Pathogen Associated Molecular Patterns (PAMPs), which is recognised by Pattern Recognition Receptors (PRRs), present on the surface of the host cells" (Carabeo, 2011), their interaction will result in activation and recruitment of immune cells (Carabeo, 2011). Similarly, the HBV when enters into human body will be recognised as foreign, the immune response will be initiated and consequently, the immune system will respond by producing some cytokines like interleukin-10 and tumour necrosis-alpha, all to get rid of the HBV. The serial activities will then activate the white blood cells and other immune cells to fight the HBV infection. In furtherance to that, the immune reaction will cause inflammation and organ damage, liver inclusive. A prolonged and severe immune response will definitely lead to inflammatory reaction which will then lead to multiple organ failure due to systemic inflammatory response syndrome that is marked by low or high body temperature, fast heart rate, and low or high white blood cell (Martin and Leibovich, 2005).

2.3 EMPIRICAL REVIEW

2.3.1 Virology of Hepatitis B Virus (HBV)

Hepatitis B Virus (HBV) is a member of the Hepadnavirus family (Aliu *et al*., 2022).

It is classified as "hepatotropic DNA virus where an envelope is formed by a lipid bilayer with a small, middle and large size surface antigens (S-HB_sAg, M- B_sAg and L-HB_5Ag) embedded as trans- membrane proteins" (Schieck *et al*., 2013; Patient *et al*., 2009). "The infectious virion, also known as the Dane particle, is 42–45 nm in diameter, made up of HBsAg embedded in a lipid envelope, encasing the viral nucleocapsid containing a reverse transcriptase attached to the nucleic-acid material" (Gerlich and Robinson, 1980; Liang, 2009). "The replication and transcription of HBV are controlled by its regulatory elements, such as enhancers, and promoters (S1, S2, pre-core, core and X)" (Karayiannis, 2017). "The key in the infection process" (Li *et al*., 2017) is the "deletions and non-synonymous mutations in overlapping regions which birthed the HBV genotypes evolution and the differentiation of the pre-s domain" (Li *et al*., 2017).

2.3.2 Hepatitis B Virus replicative cycle

The first step in HBV replicative process starts with binding large size surface antigen (L-HB_sAg) with the bile acid transporter-sodium taurocholate co-transporting polypeptides (NTCP) to mediate the entry of the viral particle into hepatocytes (Casillas *et al*., 2018). "The virus is then endocytosed and transported to the nucleus while the viral capsid is de-enveloped" (Huang *et al*., 2012; Mitra *et al*., 2018). "Upon entry into hepatocytes, HBV translocate the relaxed core DNA (rcDNA) to the nucleus, where it forms a minichromosome known as covalently closed circular DNA (cccDNA)" (Li *et al*., 2025). "The cccDNA subsequently undergoes transcription, facilitated by host RNA polymerase II, resulting in the production of five distinct HBV RNA species (0.7 kb, 2.1 kb, 2.4 kb, and two forms of 3.5 kb)" (Xia and Guo, 2020). "The 0.7 kb RNA is translated into HBV X protein (HBx), which functions as a transcriptional regulator" (Zheng *et al*., 2023). "The 2.1 kb RNA is translated into the small HBsAg and the middle HBsAg, while the 2.4 kb RNA is translated into the HBV large HBsAg" (Li *et al*., 2025). "The longer 3.5 kb RNA, referred to as preCore RNA, is translated into HBV e antigen (HBeAg) and the shorter 3.5 kb RNA referred as pre-genomic RNA serves two functions; it acts as a translation template for HBV polymerase (Pol) and core proteins (HBc), and as a replication template for intra-capsid reverse transcription by Pol, resulting in the formation of HBV rcDNA" (Xia and Guo, 2020). "These nucleocapsids can be transported back to the nucleus to enhance the cccDNA reservoir" (Zheng *et al*., 2023). "The mature capsids can either deliver the rcDNA into the nucleus, increasing the cccDNA content or binding to HBV surface proteins in the endoplasmic reticulum (ER) to be secreted as virions outside the hepatocytes" (Yuen *et al*., 2018).

2.3.3 Model system of Hepatitis B Virus Infection

2.3.3.1 Cell Culture Model

Diverse cell culture systems have been formulated for investigating HBV replication and its effect on the liver, as presented in table 2.1 and 2.2. "These models cannot be infected with hepatitis B virus naturally but they could express HBsAg, and DNA molecules after transferred with cloned HBV DNA in over length constructs" (Xiaoxiao *et al*., 2024). "These cell lines allow for the screening of assumed HBV replication inhibitors" (Huang *et al*., 2020), as well as traditional Chinese medicines" (Blanchet *et al*., 2019; Lander *et al*., 1997).

"MicroRNAs such as MiR-185, MiR-802 and MiR-192-3P, were up regulated in HepG2.2.15, promoting the expression of HBsAg and HBcAg" (Wang *et al*., 2019; Li *et al*., 2022).

"Primary Human Hepatocytes (PHHs) are considered as the most physiologically realistic model for investigating HBV infection" (Reuschel *et al.*, 2019; Sai *et al.*, 2016). "Primary Human Hepatocytes (PHHs) can be isolated from chimeric mice with humanized livers" (Michailidis *et al.*, 2020). "The molecular of HBV activity and related mechanisms were studied in this cell system" (Zeng *et al.*, 2020).
Animal models represent an essential tools for investigating the diverse of HBV and its host interactions (Xiaoxiao *et al.*, 2024). "Chimpanzees are one of the few nonhuman animals with complete immune systems that can support HBV infection" (Xiaoxiao *et al.*, 2024). "Recently, the rhesus macaque has been developed as a viable HBV animal model, bearing considerable compatibility to human hepatic physiology" (Burwitz *et al.*, 2017). Burwitz *et al.*, first "transfected rhesus macaque hepatocytes with Ad- and AAV-based viral vectors that expressed human NTCP; however, the transfection efficiency was low" (Burwitz *et al.*, 2017). "The Woodchuck hepatitis virus (WHV) is similar to HBV in terms of viral replication and course of infection" (Xiaoxiao *et al.*, 2024). "Young Tupaia (4–9-week-old Tupaia) exposed to HBV develop a chronic infection and exhibit similar hepatic pathological and immunohistochemical changes to humans" (Sanada etal., 2016; Yang *et al.*, 2015; Wang *et al.*, 2012). "Similarly, NTCP was identified as the main HBV receptor in Tupaia hepatocytes" (Zhing *et al.*, 2013; Konig *et al.*, 2014).
"The HBV transgenic mouse model has markedly contributed to the elucidation of the pathogenesis of HBV infection" (Xiaoxiao *et al.*, 2024). "It is highly useful in assessing antiviral compounds and pharmacological agents" (Julander *et al.*, 2003). "It cannot detect changes such as liver injury and liver fibrosis in this mouse, and only a few strains of transgenic mice can develop HCC" (Zong *et al.*, 2019; Gao etal., 2017).

2.3.4 Transmission of Hepatitis B Virus

It is confirmed that hepatitis B virus (HBV) can be detected in "serum, urine, saliva, nasopharyngeal secretions, tears, vaginal secretions, menstrual blood, and semen" (Latthaphasavang *et al.*, 2019). The transmission of "HBV occurs through vertical transmission from infected mother to child at birth and horizontal transmission via blood, body fluids, and sexual contact" (Khan *et al.*, 2021). "The most prevalent mode of HBV transmission is mother-to-child transmission especially in an endemic area" (WHO, 2021). When "newborn is born to an HBeAg-positive mother and does not receive timely hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine after birth" (Li *et al.*, 2024), about 70%–90% of new born will be infected with HBV (Li *et al.*, 2024). These transmission routes complicate the dynamics of HBV spread and require sophisticated modeling frameworks that consider latent infection periods and hidden carriers (Adama *et al.*, 2025).
In some areas, "sexual contact is also one of the main transmission routes of HBV" (Li *et al.*, 2024). Homosexual practice is also the risk of HBV transmission (Li *et al.*, 2024).
"In Low-prevalence areas, HBV spreads through sexual contact and percutaneous route primarily infecting drug users" (Burns and Thompson, 2014).

2.3.5 Pathogenesis of Hepatitis B Virus

Hepatitis B Virus does not involve direct killing of liver cells but the immune response to the virus is the main cause of hepatocyte injury and necrosis (Hong *et al.*, 2023). "The innate immune response plays an important role in the early stages of HBV infection and induces the subsequent adaptive immune response" (Kayesh *et al.*, 2021). Research has shown that "patients with chronic hepatitis B virus infection often

show a low frequency of myeloid dendritic cells (mDCs) and plasmacytoid dendritic cells (pDCs) in the peripheral blood, with impaired mDC maturation and reduced interferon α produced by pDC" (Hong *et al*., 2023). Low level of mDCS will reduced the capacity for body to directly clear the virus ultimately, negatively affecting virus clearance (Yonejima *et al*., 2019). However, "HBV-specific immune responses play an important role in HBV clearance" (Isogawa and Tanaka, 2015). "$CD8^+$ cytotoxic T lymphocytes can induce apoptosis of virus-infected hepatocytes and secrete interferon γ to suppress the expression and replication of HBV genes in hepatocytes" (Schuch *et al*., 2019).

2.3.5.1How does Hepatitis B virus infection induces cirrhosis and hepatocellular carcinoma?

The pathogenesis of chronic hepatitis B virus (CHBV) and its effects can be described by the interactions of pathogen and host mechanisms (Lannacone and Guidotti, 2022).

2.3.5.1.1 Pathogen-related Mechanisms

Hepatitis B virus (HBV) is a DNA virus which does not kill cell directly and has affinity to liver (Yao *et al*., 2025). It can be "transmitted through blood or mucosal exposure to infectious blood and bodily fluids" (Tu *et al*., 2025). The virion or Dane particle present in HBV is composed of "an envelope of lipids and hepatitis B surface antigens (HBsAg), a nucleocapsid composed of core proteins, and a partially double-stranded genomic DNA" (Tsukuda and Watashi, 2020). "As the virus particles enter hepatocytes through blood circulation, the HBV virion attaches to the host cell surface by binding to sodium taurocholate co-transporting peptide (NTCP) on the hepatocyte membrane" (Li *et al*., 2024). "The relaxed-circular DNA (rcDNA) form of HBV genome contained in the viral capsid is transported into the nucleus, where it is converted by the DNA repair pathway to covalently closed circular DNA (cccDNA) which serves as the template for HBV transcription" (Yeh *et al*., 2023). "The pregenomic RNA (pgRNA) and mRNAs that are formed are translated into viral proteins pgRNA which undergoes reverse transcription to form progeny viral rcDNA" (Shih *et al*., 2018).

2.3.5.1.2 Viral Protein-related Mechanisms

One of the viral proteins known as hepatitis B virus "X protein (HBx) is an oncogenic protein highly involved in hepatocellular carcinoma (HCC) formation" (Sivasudhan *et al*., 2022). "Hepatitis B virus X is a key driver of HBV infection by mediating cccDNA transcription, modulating cellular and viral promoters and enhancers, and influencing various signal transductions and cell cycle regulation" (Yao *et al*., 2025). The viral capsid "promote cell proliferation through the activation of multiple signalling pathways including Wnt/β-catenin, PI3K/AKT, and STAT3 signalling pathways" (Yan *et al*., 2024). It can also "inhibit cell apoptosis through various mechanisms, allowing damaged liver cells to survive and accumulate gene mutations, increasing the risk of cancer formation" (Agustiningsih *et al*., 2024).

Mutations in HBsAg have been attributed as one of the causes of HCC (Wang *et al*., 2021).

Also, the "gene deletions in HBV pre-S gene segments are valuable biomarkers for higher incidence rates of HCC" (Lin *et al*., 2022). The "pre-S deleted proteins of HBV are important oncoproteins that can induce DNA damage and promote growth and proliferation of hepatocytes through endoplasmic reticulum stress-dependent signalling activation pathways" (Choi *et al*., 2019). The "pre-S2 deleted proteins of HBV can by extension inhibit DNA repair, enhance hepatocyte survival and drug resistance" (Lin *et al*., 2021).

2.3.5.1.3 Hepatitis B virus DNA Integrated-related Mechanisms

"The assembling of HBV DNA into the hepatocyte is an important contributing factor to the development of Hepatocellular carcinoma" (HCC) (Yao *et al*., 2025). The integrations of HBV "can facilitate

chromosomal translocations, causing genomic instability" (Budzinska *et al*., 2018). "The genotypes and sub-genotypes of HBV are also related to the development of cirrhosis and HCC" (Chen *et al*., 2023).

2.3.5.2 Host-related Mechanisms

"The immune response of the host in chronic HBV infection can lead to a series of pathogenic reactions such as liver injury, necrosis, and inflammation" (Khanam *et al*., 2021). The interactions of "hepatocytes, lymphocytes, macrophages, hepatic stellate cells (HSCs), and stromal cells" as a dynamic process has resulted in chronic inflammation and fibrogenesis (CGARN, 2017). The "HBV-specific CD8+ T cells are the key effector of host adaptive immune response" (Schuch *et al*., 2019).

The chronic hepatitis B virus (CHB) infection is seen "as a disease in which HBV-specific CD8+ T cells that escape central tolerance and peripheral CD8+ T cells are not capable of eliminating the pathogen from the liver" (Lannacone and Guidotti, 2022). The Persistency of "CD8+ T cell dependent liver disease leads to continuous episodes of hepatocellular necrosis and regeneration resulting in fibrosis, cirrhosis, and HCC" (Yue *et al*., 2024). Outside "effector CD8+ T cells, HBV-related tumour-infiltrating regulatory T cells (Tregs) have also shown relationship with viral titre, carcinogenesis, and poor prognosis" (You *et al*., 2023).

Conclusively, "persistent hepatic inflammation induces an imbalance between liver fibrogenesis and fibrolysis, and promotes cell proliferation and genomic instability" (Yao *et al*., 2025).

2.3.6 Epidemiology of Hepatitis B Virus

2.3.6.1 Global Epidemiology of Hepatitis B Virus

The distribution of hepatitis B virus infection has changed over time and will continue to advance due to some factors like vaccination and migration patterns affecting the transmission of the virus (Patel Ankoor *et al*., 2024). "Hepatitis B virus affects approximately 296 million individuals worldwide and causes 820, 000 deaths per year" (Jeng *et al*., 2023). Burden of HBV infection is seen in the World Health Organization Western Pacific and African Regions (WHO, 2023). The prevalence of HBV is evaluated through the presence of hepatitis B surface antigen (HBsAg) in the general population of a given area (Karabey *et al*., 2025). The prevalence and endemicity of HBV has been categorized thus, "HBsAg of ≥8% is classified as high endemicity, 5-7% as high intermediate, 2-4% as low intermediate, and <2% as low endemicity" (WHO, 2023). About 45% - 60% of the global population live in areas of high endemicity (Chemin and Pujol, 2022). Regions where HBV is highly endemic include parts of Asia, Sub-Saharan Africa, the pacific, parts of the Amazon Basin, Parts of the Middle East, the Central Asian Republics, the Indian Subcontinent and some countries of Central and Eastern Europe (Liaw and Zoulim, 2015).

Ebonyi State is considered high intermediate endemic area for HBV, with HBsAg prevalence of 6.9%. It has been documented that 15% - 40% of people with chronic HBV (CHB) will develop liver cirrhosis, liver failure or hepatocellular carcinoma (HCC) with a 15% - 25% estimated rate of mortality (Suzuki *et al*., 2021).

2.3.6.2 Prevalence of Hepatitis B Virus (HBV) in Africa

Even though, data on hepatitis B virus (HBV) in Africa is scarce, its prevalence is among the highest in the world (Nalda *et al*., 2024). Approximately, 80 million people are infected with HBV in Africa (Gnyawali *et al*., 2022), representing a prevalence of 6.1% (Spearman *et al*., 2017). In furtherance to that, a high prevalence of hepatitis B surface antigen (HBsAg), exceeding 8% in the general population, is

observed in Sub-Saharan Africa (Polaris Observatory Collaborators, 2018). Hepatitis B virus is under-diagnosed on the continent, stemming from the latency nature of Chronic HBV infection and a lack of public information on its prevention, risks and outcomes (Nalda *et al*., 2024).

2.3.6.3 Prevalence of Hepatitis B Virus (HBV) in Nigeria

The Nigerian National Survey of prevalence of HBV infection showed prevalence rate of 12.2% classifying Nigeria as a highly endemic region (WHO, 2024). It has been reported a prevalence rate of 7.6% of hepatitis B surface antigen in South-East Nigeria (Odita *et al*., 2022).

2.3.7 Hepatitis B Virus Genotypes

The variation in genetic make-up of the hepatitis B virus (HBV) is largely attributed to a lack of proofreading nature of HBV reverse transcriptase which is responsible for replication of the HBV genomic DNA (Petal *et al*., 2024). Hepatitis B virus (HBV) has a high mutation rate, with an estimated nucleotide replacement ate of 1.4 – 3.2 x 10^{-5} per site per year (Liu *et al*., 2021). Errors are resulted in nucleotide substitutions during viral replication contributing to genotype, sub-genotype, and quasi-species diversity (Liu *et al*., 2021).

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The genotypes and sub-genotypes of HBV are identified by a divergence in their DNA base-pair sequences, and each genotypes differs by more than 8% in their nucleotide, while each sub-types differs by 4% - 8% (Lin and Kao, 2017). The specific locations of the nucleotide differences that define the genotypes and sub-genotypes varies and can be based on the nucleotide sequence variability of the S gene or mutations in the pre core/core region (Kumar, 2022).

It has been proven that the various genotypes influence the HBV epidemiology, natural history, outcomes of the infection, and treatment response (Fletcher *et al*., 2020). Suggestion has been made that viral genotypes can lead to diverse clinical manifestations, including acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (Liu *et al*., 2021). Chronic infection with genotypes C and D have been associated with a high risk of advanced liver disease and hepatocellular carcinoma (Khan *et al*., 2022).

The HBV genotypes and sub genotypes have distinct geographical distribution (Thijssen *et al*., 2020). Genotypes B (with sub genotypes B1–B10) and genotype C (with sub-genotypes C1–C17) are more frequent in Asia (Lin *et al*., 2019). Genotype D (with sub-genotypes D1–D12) is most prevalent in southern Europe Mediterranean, India and Russia (Ren, 2019). Genotype F (with 6 sub-genotypes F1–F6) has a worldwide distribution but mainly endemic in central and southern America (Mojsiejczuk *et al*., 2020). Genotype E is most common in west and central Africa and has no sub-genotype (Suzuk *et al*., 2003).

It has been established that HBV genotype C infection has the highest risk for liver fibrosis progression and cirrhosis (Carolina *et al*., 2023). Genotype C has higher HBV DNA levels, a lower rate of HBV surface antigen (HBsAg) loss, and lower blood T follicular helper cells, which have been reported to increase risk of severe liver damage (Xibing *et al*., 2013). Genotypes A and D have been associated with higher and genotype E with lower likelihood of HBV surface antigen (HBsAg) loss in nucleos(t)ide recipients (Sonneveld *et al*., 2022). Hepatitis B surface antigen (HBsAg) as well as HBV DNA levels decline more

rapidly with interferon-alpha (IFN-α) therapy than HBV/A and B compared to HBV/C and D infection (Zhang *et al*., 2013).

2.3.8 Biomarkers of Hepatitis B Virus

The mjor component of the hepatitis B virus (HBV) covalently closed circular DNA (cccDNA) and life cycle of the virus is produced initially from the virus and resides in non-dividing hepatocytes as a stable minichrosome (Alotaibi, 2023). For the detection of HBV and their phases in an individual, hepatitis B e antigen, Hepatitis B surface antigen, serum glutamic-pyruvic transaminase, and HBV-DNA are currently employed in practice (Tong and Revil, 2016; Testoni and Levrero, 2017).

For the management of chronic hepatitis B infections, various markers are used like HBc Ag, measures cccDNA intrahepatic, M2BPG; tracks the liver fibrosis and risk of HCC, serum HBV RNA and qHBsAg (Coffin *et al*., 2019).

2.3.8.1 Serum Hepatitis B Virus RNA

Hepatitis B virus (HBV) produced Pregenomic RNA, Core RNA, Pre-core, X-RNA, Pre S1 and 2 as viral transcripts (Gerlich *et al.*, 2020). Since precore, pregenomic RNA are overlength molecules of size roughly 3.5 kb, they can be translated from cccDNA (Stadelmayer, 2020). Hepatitis B virus RNA has also been found in complexes of capsids and antibodies as well as in naked capsids (Bai, 2018). Clients who are HBeAg positive can estimate their treatment response based on their HBV RNA kinetics (Van Bommel, 2015).

2.3.9 Laboratory Diagnosis

2.3.9.1 Hepatitis B Virus Serological Markers

Hepatitis B Virus (HBV) serological markers include HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, and anti-HBc immunoglobulin M (IgM) (You *et al*., 2023). Some markers like HBsAg and anti-HB core are useful in diagnosis while others like HBeAg, anti-HBe, anti-HBs, and HBV DNA are useful in defining the different phases of chronic hepatitis and monitoring patients (Vachon and Osiowy, 2021). The hallmark for diagnosis and screening of HBV infection and its detection is hepatitis B surface antigen (Agarwal *et al*., 2024). It is secreted by both cccDNA and integrated DNA, therefore representing intra-hepatic viral burden (Agarwal *et al*., 2024).

2.3.9.2 Hepatitis B Virus Virological Markers

2.3.9.2.1 Hepatitis B DNA Quantification: High HBV DNA is indicative of active replication and correlates with disease progression (Agarwal *et al*., 2024). Quantification of HBV DNA is essential for guiding treatment decisions, monitoring treatment response and may indicate the emergence of resistant variants (Agarwal *et al*., 2024). High Serum levels of HBV DNA is a prediction of risk of cirrhosis and hepatocellular carcinoma (Liu *et al*., 2021).

2.3.9.2.2 Hepatitis B Virus Genotyping: HBV genotyping assists in predicting the effectiveness of interferon therapy and prognosis (Zhang *et al*., 2021).

2.3.9.2.3 Detection of Drug-resistant Mutations: the HBV mutations has been related to decreased sensitivity of antiviral drugs (Rajoriya *et al*., 2017)

2.3.9.2.4 Hepatitis B virus Core-related Antigen

Studies have shown that hepatitis B core-related antigen in differentiating disease phases and predicting the antiviral efficacy of peg-IFN-α, recurrence after nucleoside analogs (NAs) withdrawal, disappearance of HBsAg, and risk of hepatocellular carcinoma (HCC) (Sonneveld *et al*., 2022; Wong *et al*., 2017).

2.3.9.2.5 Quantification of Anti-HBc Antibody: The level of anti-HB core antibody decreases with liver inflammation during antiviral therapy (Zhang *et al*., 2022) and positively correlated with the degree of liver fibrosis (Li *et al*., 2017).

2.3.9.2.6 Serum Biochemical Examination: The alpha-fetoprotein (AFP) and its heterogeneous L3 protein induced by vitamin k absence or antagonist (PIVKA-II) serve as important diagnostic markers for HCC (Tayob *et al*., 2023).

2.3.9.3 Imaging Diagnosis

The main purposes of imaging examinations are to monitor the clinical progression of chronic HBV infection, to determine whether liver cirrhosis and portal hypertension exist (De Francis *et al*., 2022), and to locate space-occupying lesions and identify their characteristics, and to monitor and diagnose HCC timely (Cunha *et al.*, 2021).

2.3.10 Treatment of Chronic Hepatitis B Virus Infection (CHBVI)

The major focus in treatment option is to improve the patient's quality of life and survival, preventing the disease from progressing to cirrhosis, terminal liver disease, HCC and death (Seto *et al*., 2014). Current treatments primarily focus on suppressing viral replication through nucleos(t)ide analogs (NAs) and modulating immune responses using interferon therapy (Haidari, 2025). The major outcome of the treatment is the clearance of the HBsAg (functional cure), which only occurs in a minimal number of patients (Likhitsup *et al*., 2019). It should be borne in mind that total eradication of the infection is impossible, given the persistence in the cells of cccDNA in the nucleus of the hepatocytes (Rodriguez *et al*., 2020).

The efficacy of both drugs in bringing about a response that is biochemical (normalisation of ALT) and virological (clearance of HBV-DNA) is over 95% (Marcellin *et al*., 2019). Treatment option should be personalized based on patient and virus characteristics (De Clercq *et al*., 2010). Currently, two types of treatment options are available against hepatitis B viral infection, interferon α derivatives (IFNs), and nucleos(t)ide analogues (NAs) (Prifti *et al*., 2021).

Nucleoside analogues (NAs) inhibit the HBV reverse transcriptase activity and therefore block HBV DNA replication (Prifti *et al*., 2021). About eight NAs have been approved against the HBV, of which the current recommended ones are entecavir and the two tenofovir prodrugs, disoproxil and alafenamide (Man, 2005). The first approved NA which was effective against HBV was lamivudine, and it was administered once daily with few side effects (Man, 2005). It is no longer widely used because it is less potent than newer drugs and most patients develop resistance within one to five years (Tu *et al*., 2018). Entecavir (ETV) was approved in 2005 (Man, 2005). It is a first-line treatment with exceptional resistance profile (Tu *et al*., 2018), and it has been proved that it reduces the incidence of HCC (Hou *et al*., 2020). The monitoring of serum alanine aminotransferase (ALT), an enzyme released by dead hepatocytes, is recommended at 6 and 12 months of treatment with ETV, since normal ALT levels are related to a reduced risk of developing

HCC (Prifti *et al*., 2021). Moreover, the follow-up monitoring of serum alpha-fetoprotein as a biomarker for HCC is suggested (Pierra *et al*., 2020). To some extent, tenovofir disoproxil fumarate (TDF) is not susceptible to resistance development, and hence, its use provides sufficient virological suppression (Liu *et al*., 2017).

2.3.11 Cytokines

Cytokines can be seen as small molecular weight proteins or peptides messengers between tissues and the immune system (Guven-Maiorov *et al*., 2014), and participate in many physiological processes (Ai *et al*., 2013). They are also an important chemical mediators synthesized and secreted by immune cells that act on their corresponding receptors and regulate the differentiation, proliferation, and function of immune cells, thus coordinating the responses and progression of inflammatory diseases (Shi *et al*., 2015). Better still, they are small secreted proteins (<40KDa), which are produced by a wide range of immune cells, as well as endothelial cells, fibroblasts, and various stromal cells (Meng-ju *et al*., 2023).

They can be classified as anti-inflammatory, suppress the activity and production of pro-inflammatory signals limiting inflammation and host damage or pro-inflammatory, inducing inflammation as a result of infection or injury (Sultani *et al*., 2012). The release of pro-inflammatory cytokines lead to activation of immune cells and release of further cytokines (Kany *et al*., 2019). When both anti and pro inflammatory cytokines combined, they give rise to distinct consequences, such as inflammation, proliferation, and angiogenesis (Allavena *et al*., 2011). Almost all cytokines have multiple cellular sources and targets, as well as many natural inducers and inhibitors (Matei and Matei, 2002). The anti-inflammatory ones are fundamental regulators of body metabolism (Pasha *et al*., 2013). They have a low molecular weight and act as messengers that alter the behaviour of its own or another (Turner *et al*., 2014).

The cytokine receptors are localized on the surface of human cells and few are found in plasma and referred as cells regulating cytokine function since they influence the nature of disease state (Nicholas and Lesinski, 2011). They bind to specific receptors, cytokines transmit intracellular signals by influencing cell activation, division, apoptosis or movement (Gabriela *et al*., 2023). Interleukins are products of leukocyte acting on other white blood cells, interferons have a defensive role; colony-stimulating factors serve for differentiation and proliferation of stem cells (Hewison, 2011). A variety of experiments have shown that either excessive or insufficient production of cytokines may contribute significantly to the pathophysiology of a range of diseases (Ai *et al*., 2013) including hepatic diseases (Seklyama *et al*., 1994).

2.3.11.1 General Properties of Cytokines

Certainly, cytokines can be produced by every nucleated cell type in response to injurious stimuli (Oppenheim, 2001). Little of them enter the systemic circulation in biologically relevant amounts and a few have an important physiological role there (Mannaa and Abdel-Wahhab, 2016). Whereas the purpose of endocrine hormones is to ensure the efficient function of normal tissues and the whole organism, cytokines with a physiological role in the circulation are concerned with restoring normal function to the tissue in which they are produced (Mannaa and Abdel-Wahhab, 2016).

When tissues are severely damaged, larger amounts of cytokines do enter the circulation and upset systemic homeostasis, inducing fever, sickness behaviour, cachexia and a variety of endocrine hormone imbalances (Slifka and Whitton, 2000). Individual cytokine either in tissues or in the circulation may exhibit a range of activities and many of these overlap with activities of other cytokines (Angkasekwinai and Dong, 2011).

Based on the phase of inflammation, cytokines can be divided into acute inflammation-inducing cytokines (IL-1, TNF-α, IL-6, IL-11, IL-8, chemokines, G-CSF) and chronic inflammation-inducing cytokines (humoral- IL-4, IL-5, IL-6, IL-7, IL-13, and cellular- IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12) (Medzhitov, 2008).

2.3.11.2 Classification of Cytokines based on Immune Cells involved in HBV Infection

2.3.11.2.1 Th1-associated Cytokines and HBV Infection

"Th1 cells are differentiated from naïve $CD4^+$ T cells (Th0 cells) in the presence of IL-2 and IL-8 and mediate cellular immune responses by producing anti-inflammatory cytokines such as IFN-γ, and IL-2" (Bauer *et al*., 2011; Rossol *et al*., 1997; Huang *et al*., 2012). The Th1-associated cytokines are known for playing a central role in HBV clearance of both acute and persistent infection and also contribute to immunopathology and pathogenesis of liver diseases (Bauer *et al*., 2011).

2.3.11.2.1.1 Interferon (IFN)

Interferon has a pivotal role in the resolution of an acute hepatitis B virus infection and may be the main mediator in noncytolytic control of HBV infection (Guidotti and Chisari, 2001). The antiviral mechanism of action of interferon is the expression of major histocompatibility complex (MHC) molecules on the cell surface leading to antigen presentation to $CD8^+$ T cells and consequently dissolution of target cells (Fisicaro *et al*., 2009).

It is documented that viral replication often results in rapid induction of IFN by the infected cells (Bertolino *et al*., 2002). A strong activation of IFN-γ production by innate and adaptive immune cells such as NK and NK T cells, HBcAg specific $CD4^+$ and $CD8^+$ T cells in self-resolving acute HBV infection is characterised with significant inhibition of HBV replication (Bauer *et al*., 2011).

Triggering of serum IFN-γ levels on treatment in chronic hepatitis B (CHB) patients can lead to virological control and HBeAg seroconversion (Chokshi *et al*., 2014).

2.3.11.2.1.2 Tumour Necrotic Factor-alpha (TNF-α)

Tumour necrosis factor –alpha (TNF-α) is known to be a key cytokine involved in the immune pathogenesis of HBV infection (He *et al*., 2006). It is already documented that TNF-α is one of the major pro-inflammatory cytokine that participates in proliferation, inflammation and programmed cell death (Hassoon, 2024). Infection by HBV leads to elevated levels of TNF-α, which is relevant to cirrhosis and hepatic encephalopathy (Odeh *et al*., 2004).

2.3.11.2.1.4 Interleukin-12 (IL-12)

IL-12 can cooperate with IL-2 to promote proliferation and differentiation of cells including CTL and NK, and help CTL cells, which eliminate virus-infected hepatocytes (Rossol *et al*., 1996). A decrease in IL-12 can prompt the balance of Th1/Th2 to tilt forward Th2 and cause non-cytotoxic T lymphocyte response against virus clearance, resulting in chronic HBV infection (le Song *et al*., 2003). Studies revealed that IL-12 plays important roles in HBV infection and patients with long-term forms of hepatitis are unable to produce a sufficient amount of this cytokine in chronic stage (Dadmanesh *et al*., 2014). Low serum level of IL-12 suggest body's non response to antiviral drug during treatment (He *et al*., 2012).

2.3.11.2.2 Th2-associated Cytokines and HBV infection

Type 2 helper T cells are subset of $CD4^+$ plays vital role in the host defense against pathogen like HBV and allergens by producing Th2 cytokines (Kokubo *et al*., 2022). Therefore, disorder of Th1/Th2 balance is the main factor that causes chronic HBV infection (Gao *et al*., 2009).

2.3.11.2.2.1 Interleukin-4 (IL-4)

Secretion of IL-4 by activated Th2 cells prompts the biological effects of stimulating proliferation and differentiation of B cells to produce antibodies, promoting $CD4^+$ T cells to differentiate into Th2 cells, and improving activity of macrophages, which is of great significance in the regulation of humoral and adaptive immunity (Nelms *et al*., 1999).

A sudden rise in serum level of IL-4 in the peripheral blood of HBV-infected patients can suppress the production of IFN-γ and Th1-type immune response, resulting in persistent HBV replication and consequently promotes the formation of immune tolerance (Jiang *et al*., 2010).

Furthermore, IL-4 might suppress the production of HBsAg, as well as the replication of HBV and thus mediate the host's antiviral response (Yao *et al*., 2011).

2.3.11.2.2.2 Interleukin-6 (IL-6)

Interleukin-6 exert its role by regulating growth and differentiation of B cells, promoting synthesis of acute phase protein from hepatocytes, and enhancing the killing effect of CTL and NK cells, leading to liver inflammation and immune damage (Barathan *et al*., 2013). It also has an important role in the induction of hepatitis, cirrhosis, and hepatocellular carcinoma (Barathan *et al*., 2013). Recent studies have demonstrated that serum IL-6 expression was significantly higher in CHB patients than that in naturally immune subjects, which plays key roles in the elimination of HBV with the induction of humoral and cellular immune responses (Gur *et al*., 2005).

2.3.11.2.3 Regulatory T cells (Treg)-associated Cytokines and HBV Infection

Regulatory T cells (Treg) causes persistence of HBV by producing immunosuppressive cytokines such as tumour growth factor- beta (TGF-β), IL-10, and IL-35 and inhibit the activation of Th1 or Th2 cells by down regulating the immune response (Pillai *et al*., 2011). Elevated viral load suppress $CD4^+$ and $CD8^+$ T cells in addition to the induction of Treg cells in patients with chronic Hepatitis B and is strongly linked to liver cirrhosis and primary hepatocellular carcinoma caused by chronic Hepatitis B (Liu *et al*., 2011)

2.3.11.2.3.1 Tumour Growth Factor-beta (TGF-β)

TGF-β is a 25- KDa cytokine with three homodimeric isoforms in humans, including TGF-β1, TGF-β2, and TGF-β3 (Zhang *et al*., 2002). TGF-β not only induces immune tolerance against HBV antigens via stimulating the differentiation of Treg cells, but also has an important role in the development of cirrhosis of the liver and HCC via increasing Th17 cells (Karimi-Googheri *et al*., 2014).

2.3.11.2.3.2 Interleukin-10 (IL-10)

IL-10 which is associated with regulatory T cells cytokines inhibit Th1 cell activation thereby negatively regulating immune response in virus persistence state (Pillai *et al*., 2011). It has been established that IL-10 is a cytokine with an anti-inflammatory effects, exercising a central role in several infections, limiting the immune response to pathogens and hence, preventing damage to the host (Saraiva and O'Gara, 2010). IL-10 owns the main biological effects of inhibiting the production and release of pro-inflammatory cytokines, thus limiting and terminating inflammation and promoting the proliferation and differentiation

of B cells to produce antibodies (Sabat *et al*., 2010). Increased expression of IL-10 is the primary factor that causes chronic HBV infection (Das *et al*., 2012).
An elevated level of IL-10 is relevant to HBV serum titres and degree of liver inflammation and also directs the processes of fibrogenesis and further disease progression (Wu *et al*., 2010).

2.3.11.2.3 Th17-associated Cytokines and HBV Infection
T helper 17 (Th17) cells are a newly identified subset of T helper cells and are closely related to the progression of HBV disease (Wang *et al*., 2013).

2.3.11.2.4.1 Interleukin-17 (IL-17)
It has been established that IL-17 binding to IL-17 receptor on target cells can induce the expression and secretion of proinflammatory factors, such as IL-6 and IL-8, which in turn promotes the inflammatory response (Zhang *et al*., 2012). Studies have identified that IL-17 is unregulated in chronic HBV-mediated inflammation and could be relevant to the development of liver cirrhosis and hepatocellular carcinoma (Du *et al*., 2013). IL-17 exerts its antiviral effects by inducing the expression of MxA and OAS both from messenger RNA (mRNA) levels and protein levels, which directly inhibits HBV replication (Wang *et al*., 2013). This cytokine can stimulate monocytes and dendritic cells (DCs) to express IL-17R and produce pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-6 which are important for liver damage during HBV infection progression (Zhang *et al*., 2010).

2.3.11.3 Cytokines and Liver Disease
2.3.11.3.1 Cytokines and Hepatic Hepatitis B Virus Infection
Hepatic injury and regeneration from chronic inflammation are the main driving factors of liver fibrosis and cirrhosis in chronic hepatitis B virus (HBV) infection (Jia *et al*., 2015). Most of these effects are mediated directly or indirectly by IFN-α, β, and γ (Wieland *et al*., 2000). IL-12 can inhibit the replication of HBV through the induction of IFN-γ (Cavanaugh *et al*., 1997).

2.3.11.3.2 Cytokines and Alcoholic Liver Disease
Chronic alcohol consumption leads to hepatocellular injury, fat accumulation, and liver inflammation, and sometimes leads to liver cirrhosis or hepatocellular carcinoma (Ceni *et al*., 2014).

In the liver, TNF-α is mainly produced by kupffer cells (McClain *et al*., 2004). The role of TNF-α as a critical inflammatory cytokine in the progression of ALD is well known (Kitazawa *et al*., 2003). Kupffer cells secrete inflammatory cytokines (Tsujimoto *et al*., 2001) and reactive oxygen species (ROS) (Bilzer *et al*., 2006) which activate cells such as hepatocytes, hepatic stellate cells (HSCs), and endothelial cells (Diehl, 2005). After chronic alcohol consumption, KC exhibit enhanced sensitivity to lipopolysaccharide (LPS)-stimulated TNF-α production (Aldred and Nagy, 1999). Treatment with pentoxifylline (an inhibitor of TNF-α synthesis) improved the survival of patients with severe alcoholic hepatitis (Akriviadis *et al*., 2000). Anti-TNF-α antibody, infliximab, is also effective in severe alcoholic hepatitis patients (Tilg *et al*., 2003).
Interleukin-6 (IL-6) protect against hepatocyte apoptosis and participates in mitochondrial DNA repair after alcoholic liver injury (Hong *et al*., 2002). It is already documented that IL-6 and IL-10 are two cytokines that play roles in reducing alcoholic liver injury and inflammation (Gao, 2005).

2.3.11.3.3 Cytokines and Fatty Liver Disease

The most frequent chronic liver disease in recent time is non-alcoholic fatty liver disease (NAFLD) that occurs across all ages and its prevalence ranges from 14 - 30% among general population, debiting a serious and growing clinical problem as a result of obesity and overweight (Abd El-kader *et al*., 2015). The first manifestation of hepatic injury is the accumulation of fat within hepatocytes (steatosis), this is followed by the development of necroinflammatory (steatohepatitis) activity that leads cirrhosis (Lalor *et al*., 2007). TNF-α is involved in the progression from steatohepatitis to cirrhosis since it promotes activation of stellate cells, matrix-gene expression, and matrix remodelling (Brenner *et al*., 1989). Obesity, especially visceral adiposity, is a major risk factor for non-alcoholic steatohepatitis (NASH) in humans (Ikejima *et al*., 2007).

Adipose tissue is a source of free fatty acids (FFA) that are delivered to the liver and a depot for triglycerides that are synthesized by hepatocytes and released into the blood as a producer of TNF-α and IL-6, adipocytes are considered a component of the immune system (Rajah and Scherer, 2003). Another adipocyte secretions known as leptin appears to play a critical role in the inflammatory response by stimulating leukocyte proliferation and the resulting increased plasma levels of the proinflammatory cytokines such as IL-6 and TNF-α (Hukshorn *et al*., 2004). These cytokines influence nitric oxide that induces free radicals production and lipid peroxidation (You *et al*., 2004). Elevation of the inflammatory markers above normal levels is an independent predictor of several chronic diseases, including coronary heart disease, stroke, diabetes, atherosclerosis and insulin resistance (Pravo *et al*., 2006).

2.3.11.3.4 Cytokines and Hepatic Cholestasis

Cholestasis is defined as a decrease in canalicular bile flow that results in accumulation of bile in hepatocytes and canaliculi (Popper, 1981). Bile acids retention causes liver cell damage and pruritis (Keith *et al*., 1999). TNF-α plays a critical role in epithelial injury as well as in immune-mediated cholangiocyte injury (Spirli *et al*., 2003). Systemic levels of TNF-α is increased following biliary obstruction in experimental cholestasis produced by ethinylestradiol in rats (Ahmed and Mannaa, 2004). Nitric oxide causes ductular cholestasis by a reactive nitrogen oxide species mediated inhibition of adenyl cyclase and cAMP-dependent HCO_3^- and Cl^- secretory mechanisms. This pathogenetic sequence may contribute to ductual cholestasis in inflammatory cholangiopathy (Spirli *et al*., 2003).

2.3.11.3.5 Cytokines and Hepatic Hepatitis C Virus (HCV) Infection

Cytokine gene polymorphisms located within the coding/regulatory regions have been shown to affect the overall expression and secretion of cytokines (Pasha *et al*., 2013). The pathogenesis of liver cell damage in HCV infection may be related to several immunologic mechanisms and the subsequent T-cell responses (Ferrari *et al*., 1994). Patients with chronic HCV infection, viral persistence which is a characteristic feature of chronic hepatitis C may be due to selective immune responses deficiencies and the production of inappropriate cytokine patterns (Abd-Elghaffar *et al*., 1999).

The involvement of macrophages derived cytokines such as TNF-α and IL-1β in the production of inflammation has been described (Lee *et al*., 2015). Moreover, TNF-α is positively related with the extent of liver necrosis (Zalata *et al*., 2007).

2.3.12 Abnormalities in Complete Blood Count in Chronic Liver Disease

The abnormalities observed in blood count are influenced by factors associated with the aetiology of chronic liver damage (such as viral hepatitis and alcohol), the impact of liver failure (resulting in poor protein synthesis) and portal hypertension (Qamar and Grace, 2009).

2.3.12.1 Erythrocytes

The main cause of anaemia in liver cirrhosis is acute or chronic bleeding in the context of portal hypertension (Gonzalez-Casas *et al*., 2009). The prevalence of iron deficiency in these patients approach 50% compared to 24.3% in the general population (Paternostro *et al*., 2020). Causes of iron deficiency include bleeding (Paternostro *et al*., 2020), as well as deficiencies in vitamin B12 deficiency (Muro *et al*., 2010) in patients with cirrhosis.

This abnormality is typically accompanied by a high red cell distribution width (Maruyama *et al*., 2001). The main alteration in mean corpuscular haemoglobin is hypochromia (<80pg), typically linked to iron deficiency (Paternostro *et al*., 2020).

Proposed mechanisms for these abnormalities include hypersplenism and alterations in lipoprotein metabolism (Roy *et al*., 2023), with the reported prevalence of spur cells ranging from 5% to 19% in some series (Nassiliadis *et al*., 2010).

2.3.12.2 Leukocytes

Leukopaenia is a frequent finding with advanced liver disease (Tan *et al*., 2015). It has been estimated that up to 26% of patients with cirrhosis exhibit dysplastic changes in leukocytes during bone marrow examination without fulfilling the definition of myelodysplastic neoplasm (Koschade *et al*., 2023).

2.3.12.3 Platelets

Thrombocytopaenia, defined as a platelet count less than 150,000/uL (Raadsen *et al*., 2021). It is a common clinical problem that is always discovered in laboratory test and always leads to further investigation (Lewis, 2006). The pathophysiology of thrombocytopaenia in patients with chronic liver disease due to HBV infection is multifactorial (Weksler, 2007). These includes sequestration of platelets in the enlarged spleen secondary to portal hypertension simply known as hypersplenism (Adinolfi *et al*., 2000). For a patient to have thrombocytopaenia, it is considered as an indicator of advanced liver diseases (Peck-Radosavljevic, 2000). Interestingly, individuals who develop thrombocytopaenia and leukopaenia have an increased risk of hepatic decompensation, hepatocellular carcinoma, mortaliry, or requiring transplant, even when controlling for other factors such as Child-pugh score, hepatic vein pressure gradient, or alcohol use (Qamar *et al*., 2009).

2.3.12.4 Prothrombin Time/International Normalized Ratio

Prothrombin time (PT) evaluates the extrinsic and common pathways of coagulation, including the activity of tissue factor, factors II, V, VII, X, and even fibrinogen (Daneshi *et al*., 2023). International normalized ratio (INR) is calculated as a ratio between patient's PT to the mean normal PT adjusted with a correction factor called the international sensitivity index (Dorgalaleh *et al*., 2021). The increase in PT and INR reflects worse liver function in cirrhosis, nevertheless, common tests such PT/INR oversimplify the adaptations in the balance between procoagulant and anticoagulant factors in liver cirrhosis (Turo *et al*., 2019).

It has been considered to function as a compensatory mechanism (Lisman *et al*., 2006). Due to synthetic liver dysfunction, both anticoagulant and procoagulant factors decrease as it progresses, leading to a rebalancing of blood coagulation mechanisms between prohaemeostatic factors (McMurry *et al*., 2021).

2.3.13 The Liver

In the normal liver, three major components are found: (i) Hepatocytes, which represent over 70% of total liver cells, (ii) the biliary system, that communicates within and outside with the adjacent organs and, (iii) a highly complex vascular system (Sanz-Garcia *et al*., 2021). The hepatic sinusoids are formed by liver sinusoidal endothelial cells, highly fenestrated cells that allow flow of plasma from the sinusoids throughout the space of Disse, a thin perisinusoidal area of contact with the hepatocytes (Sanz-Garcia *et al*., 2021).

Blood flowing through the sinusoidal lumen carries its contents of oxygen, nutrients, hormones, and among other substances like inflammatory factors, toxins and neoplasms; this makes the space of Disse a unique area for the bidirectional interchange between cells (Arraez-Aybar *et al*., 2018). The space of Disse is an area where several cell types are located: pit cells, a liver-associated natural killer cells, mesenchymal stem cells and multifunctional cell-type known as hepatic stellate cells (Brunt *et al*., 2014). Natural killer cells patrol from the sinusoids to the hepatocytes, looking for virus-infected cells and tumour cells, which they kill by using cell-to-cell cytotoxic activity (Wisse *et al*., 1997)

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Quiescent hepatic stellate cells (qHSCs) store vitamin A in lipid droplets, but after activation, hepatic stellate cells proliferate, and progressively lose vitamin A storage and start the deposition of extracellular matrix (ECM) in the injured liver (Sanz-Garcia *et al*., 2021). The liver is an organ with an extensive regenerative power, an impairement of regeneration is associated with the progression of a fibrotic liver (Aydin and Akcali, 2018).

3. MATERIALS AND METHODS

3.1 Study Area

This study was conducted at two healthcare facilities in Abakaliki, Ebonyi State, Nigeria; Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AE-FUTHA) and Ebonyi State University Teaching Hospital (EBSUTH), both located at the hub of Abakaliki town. AE-FUTHA is a 1500 beds capacity federal government owned hospital that function as one of the major health care centre in the state. EBSUTH is a 1200 beds capacity state government owned hospital. Abakaliki is the state capital of Ebonyi State and it is situated within the boundary of three Local Government Areas namely; Abakaliki, Izzi and Ebonyi. It covers an area of 533km^2 with an estimated population of 134,102. It is located at 6.32^{0}N Latitude, 8.12^{0}E Longitude and 117 metres elevation above sea level (Oroke *et al*., 2020). The people in the area are predominantly Igbo people and are civil servants, business men and women, artisans, and farmers.

3.2 Study Design

The study adopted cross-sectional research design. Study subjects (Cases) were recruited from clinics/hospitals; controls are recruited from the hospital visitors and confirmed to be HBV-negative.

3.3 Study Population

A total of 112 subjects consisting of 78 males and 34 females (≥15 years) with confirmed HBV infection (HBsAg and HBcAg positive) and clinical/biochemical evidence of liver dysfunction and 15 apparently healthy individuals from hospital visitors as controls were used for the study.

3.4 Inclusion Criteria

1. HBV positive individual with liver dysfunction.
2. Individual who has duly signed informed written consent form.

3.5 Exclusion Criteria

1. Individual with co-morbidities like HCV, HIV, diabetes mellitus, stroke, hypertension, leukaemia, and sickle cell anaemia.
2. Pregnant women.
3. Individual who did not sign informed written consent form.

3.6 Enrollment of the Subjects

The subjects included in this study were individuals that have occult HBV infection, current HBV infection, and those diagnosed with liver disease due to HBV. These individuals were recruited from tertiary/specialized hospitals. They were administered with the structured questionnaire that captured their demographic status such as age, sex, marital status/occupation, and the other component of social life behaviours and clinical manifestations as observed by the individuals. The subjects were also issued with informed written consent form detailing the reason to participate in the study.

3.7 Ethical Approval

Ethical approval was obtained from the Directorate of Health Research and Ethical Committee of the Ebonyi State Ministry of Health, (EBSHREC/2025/010/0010), Abakaliki, Ebonyi State.

3.8 Sample Collection

3.8.1 Sample Size

The sample size will by calculated using Cochran formula.

$$n = \frac{Z^2 PQ}{d^2}$$

Where

n = sample size

z = standard variation which is commonly set at 1.96 at a 95% confidence interval.

p = prevalence (proportion in the population having the particular trait). (8

Q = 1 – p

d = precision (in proportion of one; if 5%, d = 0.05).

To determine the required minimum sample size, the calculation employed a 95% confidence interval, a P value of 0.125, indicating a prevalence rate of 6.5% for HBV infection based on the previous study conducted by Chima *et al*.,(8) (Chima *et al*., 2022). Additionally, a margin of error (d) will be utilized. In order to enhance the study's robustness and account for potential non-compliance, an attrition of 20% of the sample size will be incorporated to minimize errors.

$n = \frac{Z^2PQ}{d^2} = \frac{(1.96)^2(0.065)(1-0.065)}{(0.05)^2} = \frac{3.8416\ x\ 0.065\ x\ 0.935}{0.0025}$ =93.38 ~ 93

Then, 20% of 93 = 18.6 ~ 19

Sample size, n will be therefore: 93 + 19 = 112

3.8.2 Collection of Blood Samples

Ten (10.0) ml of blood sample was collected from each participant by venepuncture of the antecubital vein and dispensed 4.0 ml into ethylene diamine tetra-acetic acid (EDTA) well labelled container for the assessment of haematological parameters, and 6.0 ml into an appropriately labelled plain tube. This was allowed to clot at room temperature and spun for 5 min at 3000 revolution per minute (rpm). The resultant sera were harvested into well-labelled cryovials and stored at −20 °C and transported to Marylmas diagnostic centre, Abakaliki for liver function test (LFT), and to Molecular Pathology Labs, Warri for quantification of tumour necrotic factor-alpha (TNF-α) and interleukin-ten (IL-10), and to DNA LABS Kaduna where Hepatitis B Viral genotyping was conducted.

3.9 Laboratory Analysis

3.9.1 Detection of Hepatitis B Surface Antigen

This was done using Hepatitis B surface antigen (HBsAg) rapid diagnostic test kit (VAL-CARE, with LOT number, 20240706 and Expiration date, 20290705) following manufacturer's instructions. Positive results were considered HBV active and contagious infection.

3.9.2 Detection of Hepatitis B Core Antigen

This was done using Hepatitis B Core Antigen (HBcAg) rapid diagnostic test kit (VAL-CARE, LOT number, 20250209 and Expiration date, 20300208) following manufacturer's instructions, reactive results are considered ongoing HBV infection.

3.9.3 Estimation of Blood Parameters

A fully automated machine, Zybio Z3 (China), was used for the analysis of the complete blood count (CBC) of the studied subjects. The EDTA anticoagulated blood samples were mixed and introduced to the probe of the machine, after the subjects ID, age, sex and the appropriate test were keyed into the machine. The machine then analysed and generated a printed report for the samples. The machine was well calibrated and an external quality control sample consisting of high values, normal values and low values was run before batches of subjects` samples were analysed.

3.9.4 Estimation of Liver Enzyme Markers (LFT)

3.9.4.1 Alanine Aminotransferase (ALT)

Principle:

ALT is incubated at 37^0C for exactly 30 min in a P^H 7.4 buffered substrate containing L-alanine and α-oxogluterate. ALT catalyses the transfer of the aminopyruvate reacts with 2, 4-dinitrophenylhydrazine to form pyruvate hydrazine which in alkaline medium gives a brown colour. The absorbance of the colour produced is measured in semi-auto Analyzer/spectrophotometer at a wavelength of 546 nm.

Reagent: RANDOX

Procedure:

Reagent	Test	Blank
R1 (buffered substrate)	250 µL	250 µL
Sample (serum)	50 µL	------
Mix thoroughly and incubate for 30 minutes at 37^0C		
R2 (2, 4-dinitrophenylhydrazine	250 µL	250 µL
Sample (serum)	------	50 µL
Mix thoroughly and allow to stand at room temperature for 20 minutes		
Add 0.4M NaOH	2.5 ml	2.5 ml
Mix thoroughly and allow to stand at room temperature for 5 minutes		

Measure the absorbance of test against blank.

3.9.4.2 Aspartate Aminotransferase (AST)

Principle:

AST is incubated at 37^0C for exactly 30 minutes in a PH 7.4 buffered substrate containing L-aspartate and α-oxoglutarate. AST catalyses the transfer of the amino group from L-aspartate to α-oxoglutarate forming L-glutamate and oxaloacetate. The oxaloacetate reacts with 2, 4-dinitrophenylhydrazine to form oxaloacetate hydrazine which in an alkaline medium gives brown colour. The absorbance of the colour produced is measured using semi chemistry auto Analyzer/spectrophotometer at a wavelength of 546 nm.

Procedure:

Reagent	Test	Blank
R1 (buffered substrate)	250 µL	250 µL
Sample (serum)	50 µL	------
Mix thoroughly and incubate for 30 minutes at 37^0C		
R2 (2, 4-dinitrophenylhydrazine	250 µL	250 µL
Sample (serum)	------	50 µL
Mix thoroughly and allow to stand at room temperature for 20 minutes		
Add 0.4M NaOH	2.5 ml	2.5 ml
Mix thoroughly and allow to stand at room temperature for 5 minutes		

Measure the absorbance of test against blank.

3.9.4.3 Alkaline Phosphatase (ALP)

Principle:

Serum alkaline phosphatase hydrolyses a colourless substance of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at PH values turns to pink colour. The absorbance is measured at 520 nm wavelength.

Procedure:

Reagent	Test	Standard
Distilled water	500 µL	500 µL
Solution A (substrate)	25 µL	25 µL
Mix thoroughly and incubate for 5 minutes at 37^0C		
Solution C (standard)	----	50 µL
Sample (serum)	50 µL	-----
Mix well and incubate for 20 minutes at 37^0C		

Solution B (colour developer)	2.5 µL	2.5 µL

Mix well and measure the absorbance of test against a standard. Water is use as blank.

3.9.4.4 Total Bilirubin

Principle:

Sulphanilic acid is diazotised by nitrous acid produced from the reaction between sodium nitrite and hydrochloric acid. Bilirubin reacts with diazotised sulphanilic acid to form azobilirubin. Caffeine serves as an accelerator and gives a rapid and complete conversion to azobilirubin. The pink acid azobilirubin is converted to blue azobilirubin by an alkaline tartrate reagent and the absorbance of the blue-green solution read using semi-auto analyser/spectrophometer at wavelength 578 nm.

Reagent: RANDOX

Procedure:

Reagent	Test	Blank
R1	100 µL	100 µL
R 2	25 µL	------
R 3	100 µL	100 µL

Mix well and incubate for 10 minutes at room temperature

R 4	500 µL	500 µL

Mix well and incubate for 5 minutes at room temperature

Measure the absorbance of test against blank at 578 nm wavelength.

3.9.4.5 Conjugated/Direct Bilirubin

Principle:

Conjugated bilirubin reacts with diazotised sulphanilic acid in an alkaline medium to form a blue coloured complex. The absorbance of the colour is measured using spectrophometer at 546 nm wavelength.

Reagent: RANDOX

Procedure:

Reagent	Test	Blank
R 1	100 µL	100 µL
R 2	25 µL	-----
0.9% Nacl	1000 µL	1000 µL
Sample (serum)	100 µL	------

Mix well and incubate for 10 minutes at room temperature

Measure the absorbance of test against blank.

3.9.5. Estimation of Interleukin-10 (IL-10) (ELISA Method)

Principle: The target antigens are the specific interleukins tumour necrotic factor-alpha (TNF-α) and interleukin-10 (IL-10). The assay involves immobilization of a capture antibody specific o the interleukin on a solid micro-plate surface. Upon incubation with the sample, the interleukins bind to the capture antibody. A detection antibody, also specific to the interleukin but recognizing a different epitope, is then added. Upon addition of a suitable TMB substrate, the enzyme catalyses a colorimetric reaction, producing a measurable signal. The intensity of the colour is directly proportional to the concentration of the interleukin in the sample and is quantified using a micro-plate reader at 450 nm.

Procedure:

- Set the number of micro-plate wells according to the number of samples,
- Labelled the plate very well,
- Add 100 µL of the standard and 100µL of sample to the standard well and sample wells respectively,
- Shake and incubate for 1 hour @ room temperature sealed,
- Aspirate and wash the wells for 4 times,
- Add 100 µL of IL-10 antibody to all the plates wells and shake,
- Incubate for 1 hour @ room temperature ,
- Aspirate and wash for 4 times,
- Add 100 µL of IL-10 conjugate and shake,
- Incubate for 30 minutes @ room temperature,
- Aspirate and wash for 4 times,
- Add 100 µL of TMB substrate and shake to all the wells,
- Incubate for 15 minutes @ room temperature,
- Add 100 µL of stop solution (1NHCL) to all the wells,
- Read the optical density @ 450 nm.

3.9.6 Estimation of Tumour Necrotic Factor-alpha (TNF-α) (ELISA METHOD)

Procedure:

- Set the micro-plate wells according to the number of samples,
- Labelled them properly,
- Add 100 µL of standard into the standard well and 100 µL of sample into the corresponding labelled micro-plate well already coated with TNF-α antibody,
- Mix them in mixer for 30 seconds and sealed,
- Incubate for 2 hours @ 37^0C,
- Aspirate and wash out the unbound antibodies for 4 times with wash buffer,
- Blot out very well,
- Add 100 µL of TNF-α antibody into the well,
- Incubate for 1 hour @ 37^0C,
- Wash out the unbound Ag-Ab for 4 times,
- Add 100 µL of human TNF-α conjugate to each of the micro-plate well,
- Incubate for 30 minutes @ 37^0C,
- Wash for 4 times,
- Add 100 µL of TMB substrate to all the wells,
- Incubate @ room temperature for 30 minutes,
- Add 100 µL of stop solution (1 NHCL),
- Read the optical density @ 450 nm.

3.9.7 Molecular Genotyping of HBV

Multiplex-nested polymerase chain reaction (PCR) using type-specific primers as shown in table 3.1 was used to designate genotypes A through F based on pre-S1 across S genes of the HBV genome. The design of the HBV genotype-specific primers is based on the conserved regions of the sequences in a particular

genotype and discordance in homology with the sequences of other HBV genotypes (Doumbia *et al*., 2013).(17) Five different sets of primers were used: P_1 and S_{1-2} being the universal outer primers and B_2 was used as the inner sense (forward) primer with a combination of BA1R, BB1R and BC1R as anti-sense (reverse) inner primers for genotypes A, B and C, respectively, in a multiplex system named "MIX A". For genotypes D, E and F, an anti-sense primer B2R was used in combination with BD1, BE1 and BF1 as sense (forward) primers, also in a multiplex system named "MIX B".

3.9.7.1 Hepatitis B Virus DNA Extraction

HBV DNA was extracted from sera of HBsAg-positive individuals using the Accu Prep Genomic DNA extraction kit from Bioneer according to the manufacturer's instructions ((Bioneer Corporation, Daejeon, South Korea).

3.9.7.2 First-round Polymerase Chain Reaction (PCR) Procedure: Hepatitis B Virus DNA Detection

3.9.7.3 Second-round Polymerase Chain Reaction (PCR) Procedure: Hepatitis The final reaction volume for the first round of the nested PCR was 20 µL. The premix tubes were labelled with the sample numbers. Two microliters (2µL) of extracted DNA was added to a Master Mix (cocktail of 16 µL of deionised water [D.H2O] and premix of 250 mM of each dNTP, 1X PCR buffer, 15 mM of $MgCl_2$ and 1U of thermostable Taq polymerase) and 1 µL each of P1 (forward) and S1-2 (reverse) outer primers. The PCR was performed using thermal cycler (thermal cycler PTC 100, MJ Research) and reaction conditions were set as: initial activation at 95°C for 5 min; denaturation at 94^0C for 20 sec; annealing at 55°C for 20 sec and extension at 72°C for 1 min. Complete sets of 30 cycles from denaturation to extension were obviously observed. Then, the final extension was set at 72°C for 5 min.

B Virus DNA Detection

Second-round PCR was performed in two different tubes for each sample, one with the common universal sense primer (hepB B2) and type-specific primers for genotypes A, B, C in "MIX A" and the other with the common universal anti-sense primer (hepB B2R) and type-specific primers for genotypes D, E, F in "MIXB." Seventeen microliters (17 µL) of DH2O was added into each tube of premix A and B. Two microliters (2 µL) of second-round primers were added into the mixtures. One microliter (1 µL) of the first-round PCR product into each tube of the premix. The mixture was mixed gently and centrifuged. The PCR condition was set as: initial activation at 94°C for 5 min, followed by denaturation at 94°C for 20 sec, annealing at 59°C for 20 sec and extension at 72ºC for 30 sec for b "MIX A." Complete sets of 15 cycles from denaturation to extension, and denaturation at 94°C for 20 sec, followed by annealing at 61ºC for 20 sec, and extension at 72ºC for 30 sec with the final extension at 72°C for 5 min. Complete sets of 15 cycles were observed. Primers used were adopted from previous studies by Lindh *et al*., (Lindh *et al*., 1998). Five microliters (5 µL) of each of negative and positive control, samples and the ladder were run on 2% agarose gel (2% w/v in 1 × TAE buffer) and electrophoresed in 1 × TAE buffer for 45 min at 100V. The bands were depicted under gel documentation system (BioRad Gel Doc-XR, USA) and screenshots taken. The size of the separated bands (DNA fragments) was compared with the 100 base pair (bp) DNA ladder (MBI Fermentas, Life Sciences, Canada).

Figure 3.1

3.10 Statistical Analysis

The data obtained was analysed along with the questionnaire. Descriptive statistics was used to calculate the frequency, means and standard deviations, while t-test and Fisher exact test were employed to compare the variables, between the haematological indices, liver function test, cytokines, and hepatitis B virus genotypes. The strength of association was estimated by logistics regression analysis. Any value less than or equal to 0.05 was regarded as significant. All the analysis were done using statistical package for social science (SPSS) version 23.0.

4. RESULTS

4.1 Characteristics of the Study of HBV Infected Subjects

A total of 112 individuals suffering from HBV infection were recruited for this study. It showed the frequency and percentage of general characteristics of the subjects. They comprised of 69.6% males and 30.4% females with mean age of 33.96 ± 7.57 years old. The majority of the subjects were between the age groups of 25 – 44 collectively accounting for 84% of the subject. Majority of the participants (75.9%) were married while 22.3% were single. The educational level of the participants showed that 22.3%, 50.9%, and 26.8% had primary, secondary and tertiary education respectively; while 32.1% were involved in trading, 22.3% were civil servants, 21.4% were farmers and 18.8% were artisans. In regards to residential area, 68.8% and 31.2% were of urban and rural dwellers respectively (Table 4.1).

4.2 Frequency and the Percentage of Clinical and Social Life habits of the HBV infected Subjects.

It was observed that 53.6% were not on medication but harbours HBV infection whereas 46.4% were on different antiviral therapy. Majority of those on therapy, 67.3% were on tenofovir alafenamide, 19.3% were on livolin forte, 7.6% were on lamivudine and 3.8% were on tenofovir fumarate. In relation to duration of therapy, 53.8% have been on therapy for 1 – 5 years and 42.3% in less than 1 year while 3.8% were above five years. It was observed that majority of the subjects, 98.2% have not received HBV vaccine, and only 1.8% have received. There were no history of HBV co-morbidities like high blood pressure, hepatitis C virus (HCV), diabetes mellitus, and sickle cell anaemia. It was observed that 3.6% have had surgical operation while 96.4% had no history of surgical operation. None have had the history of blood transfusion and only 0.9% had history of tooth extraction. It was also observed that 76.8% had no family history of HBV infection and liver disease whereas 23.2% had the history. In regards to cigarette smoking, it was 4.5% that smokes while 95.5% do not smoke. Considering residency, 75% dwelled on blocks of flat while 23.2% lived on slum. The alcohol intake showed that 51.8% drink alcohol whereas 48.2% were non-alcohol drinkers. Most of the subjects, 58.9% were self-employed and 23.2% were earning above sixty thousand naira (Table 4.2).

Table 4.1: Characteristics of the Study of HBV-infected Subjects

n = 112

Variables	Frequency	Percentage (%)
Age:		
15 – 24 years	11	9.8
25 – 34years	47	42.0
35 – 44 years	47	42.0
45 – above	7	6.3
Sex:		
Male	78	69.6
Female	34	30.4
Marital Status:		
Single	25	22.3
Married	85	75.9
Divorced	2	1.8
Highest Level of Education:		
Primary	25	22.3
Secondary	57	50.9
Tertiary	30	26.8
Occupation:		
Farming	24	21.4
Civil Servant	25	22.3
Artisan	21	18.8
Business	36	32.1
Student	6	5.4
Residency:		
Urban	77	68.8
Rural	35	31.

Table 4.2: Frequency and Percentage of Clinical and Social Life habits of the HBV-infected Subjects

Variables	Frequency	Percentage (%)
HBV Therapy:		
Yes	52	46.4
No	60	53.6
Antiviral Therapy (n=52):		
Tenofovir Fumarate	2	3.8
Lamivudine	4	7.6
Livolin Forte	10	19.3
Tenofovir Alafenamide	35	67.3
Duration of Therapy (years):		
≤ 1	22	42.3
1 – 5	28	53.8
6 – 10	2	3.8
Receipt of HBV Vaccine:		
Yes	2	1.8
No	110	98.2
Presence of High Blood Pressure:		
Yes	0	-
No	112	100
Presence of Diabetes Mellitus:		
Yes	0	-
No	112	100
History of Surgical Operation:		
Yes	4	3.6
No	108	96.4
History of Blood Transfusion:		
Yes	0	-
No	112	100
History of Tooth Extraction:		
Yes	1	0.9
No	111	99.1
Family History of HBV/Liver Disease:		
Yes	26	23.2
No	86	76.8
Use of Barbing Instrument:		
Personal	94	83.9
Commercial	18	16.1
Sharing of Sharp Objects:		
Yes	5	4.5
No	107	95.5
Cigarette Smoking:		

Yes	5	4.5
No	107	95.5
Type of Residence (Housing):		
Slum	26	23.2
Blocks of Flat	84	75
Self-Contain(Bungalow/Duplex)	1	1.8
Alcohol Intake:		
Yes	58	51.8
No	54	48.2
No. of family members per household:		
≤ 5	51	45.5
5 – 11	60	53.6
11 – 17	1	. 9
Employment Status:		
Employed	22	19.6
Unemployed	22	19.6
Self-Employed	66	58.9
Student	2	1.8
Monthly Income:		
≤ 20,000	21	18.8
20,000 – 40,000	53	47.3
40,000 – 60,000	12	10.7

4.3 Estimation of Blood Parameters among the HBV Infected Subjects

Blood Parameters were measured and indicates variations among the subjects. The mean value of WBC obtained were 9.11 ± 17.1x10^9/L. The distribution of WBC indicated that 1.8% (2/112), 56.3% (63/112) and 42.0% (47/112) had low, normal and elevated total white blood cell (TWBC) respectively. The mean value of the low WBC was 3.04 ± 0.13x10^9/l while the mean value of the elevated WBC was 12.8 ± 2.29x10^9/l. The differential leucocytes showed that 8.0% (9/112) had low neutrophil count with mean value 34.6 ± 3.7%, majority, 86.6% (97/112) of the subjects had normal neutrophil values with the mean value of 59.29 ± 9.03% while 5.4% (6/112) had elevated neutrophil values with mean value of 77.41 ± 1.7%. Similarly, 8.9% (10/112) and 10.7% (12/112) of HBV infected subjects had low and elevated lymphocyte values, with mean values of 16.2 ± 2.6% and 56.5 ± 3.7% respectively. The estimated red blood cell (RBC) values indicated that 6.3% (7/112) and 52.7% (59/112) had reduced and RBC values, with the mean values of 3.49 ± 0.31 x10^{12}/l and 6.78 ± 1.04 x10^{12}/l. Greater number of subjects, 53.6% (60/112) had reduced haemoglobin (HB) with the mean value of 9.88 ± 0.99%. The absolute values, mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were inadvertently altered as majority of the HBV infected subjects had low values. It was also observed that majority of the subjects 62.5% (70/112) had normal platelet count with mean value of 166.32 ± 35.69x10^9/l and 37.5% (42/112) had low values with mean value 107.02 ± 10.65x10^9/l as no elevated values were recorded (Table 4.3).

Table 4.3: Estimation of Blood Parameters among the HBV-infected subjects

n = 112

Blood Parameters	Frequency	Percent	Range	Mean ± SD
WBC ($x10^9$/L)			2.95-17.30	9.11±3.71
- Low	2	1.8		3.04±0.13
- Normal	63	56.3		6.26±1.62
- High	47	42.0		12.80±2.29
Neut (%)			28.6-80.0	58.36±12.00
- Low	9	8.0		34.61±3.75
- Normal	97	86.6		59.29±9.03
- High	6	5.4		77.38±1.67
Lymph (%)			12.0-62.0	34.05±11.95
- Low	10	8.9		16.20±2.62
- Normal	90	80.4		33.03±8.17
- High	12	10.7		56.47±3.69
Mid (%)			1.0-28.0	7.58±5.54
- Low	12	10.7		2.03±0.41
- Normal	90	80.4		7.06±3.28
- High	10	8.9		21.30±3.47
RBC ($x10^{12}$/L)			2.66-9.50	5.59±1.50
- Low	7	6.3		3.49±0.31
- Normal	46	41.1		4.38±0.38
- High	59	52.7		6.78±1.04
HBG (g/dl)			7.5-16.6	11.51±2.07
- Low	60	53.6		9.88±0.99
- Normal	46	41.1		13.01±0.83
- High	6	5.4		15.83±0.44
HCT (%)			22.0-51.0	34.66±6.05
- Low	58	51.8		29.79±3.02
- Normal	51	45.5		39.37±2.75
- High	3	2.7		48.20±2.66
MCV (fl)			68.4-117.5	86.66±5.60
- Low	16	14.3		77.80±3.45
- Normal	95	84.8		87.80±3.20

- High	1	0.9		117.50±0.00
MCH (pg)			21.5-45.9	28.63±2.85
- Low	17	15.2		25.17±1.68
- Normal	93	83.0		29.13±1.77
- High	2	1.8		42.45±4.88
MCHC (g/dl)			28.0-39.1	32.61±1.90
- Low	33	29.5		30.55±0.79
- Normal	68	60.7		33.08±1.07
- High	11	9.8		36.28±0.93
RDW-CV (%)			9.6-16.8	13.04±1.53
- Normal	1	0.9		16.20±0.00
- High	111	99.1		13.10±1.48
RDW-SD (fl)			13.9-51.2	40.38±3.45
- Low	1	0.9		13.90±0.00
- Normal	111	99.1		40.88±2.71
PLT (x10^9/L)			70-315	141.58±39.98
- Low	42	37.5		107.02±10.65
- Normal	70	62.5		166.32±35.69
MPV (fl)			8.9-16.2	11.42±1.73
- Normal	72	64.3		9.98±1.22
- High	40	35.7		13.40±1.01
PDW (fl)			8.7-18.4	12.65±2.37
- Low	7	6.3		8.81±0.09
- Normal	100	89.3		12.61±2.01
- High	5	4.5		17.82±0.50
PCT (%)			0.101-.423	0.15±0.06
- Low	1	0.9		0.10±0.00
- Normal	107	95.5		0.15±0.03
- High	4	3.6		0.38±0.05

4.4 Comparison of Blood Parameters between HBV Infected Subjects and Apparently Healthy Individuals

Blood Parameters were measured and compared with the HBV infected subjects and the apparently healthy individuals. The results revealed a significantly increased in white blood cell (WBC), red blood cell (RBC), and mean platelet volume (MPV) with the mean values of 9.11 ± 3.71x10^9/l, 5.59 ± 1.50x10^{12}/l and 11.42 ± 1.73fl respectively and significantly decreased in haemoglobin (HB), haematocrit (HCT), mean cell volume (MCV), red cell distribution width-standard deviation (RDW-SD), and platelet with the mean values of 11.51 ± 2.07g/dl and 34.66 ± 66.05%, 86.66 ± 5.60fl, 40.38 ± 3.45fl, and 141.58 ± 39.98x10^9/l respectively when compared to control ($P < 0.01$). A slight decrease in neutrophil count with mean value of 58.36 ± 12.00% as compared to control, 58.65 ± 6.18% even though, it was not significant was observed. Similarly, a slight increase in lymphocyte with mean value of 34.05 ± 11.95% when compared to control, 32.97 ± 6.66% was also observed (Table 4.4).

Table 4.4: Comparison of Blood Parameters between HBV Infected Subjects and apparently healthy Individuals

Comparing Test Group and Control Group	N	Mean ± SD	t-test	p-value
WBC (x10^9/l)			9.565	<.001
- Test group	112	9.11±3.71		
- Control	15	5.06±0.92		
Neut (%)			-.150	.881
- Test group	112	58.36±12.00		
- Control	15	58.65±6.18		
Lym (%)			.525	.604
- Test group	112	34.05±11.95		
- Control	15	32.97±6.66		
Mid (%)			-.817	.420
- Test group	112	7.58±5.54		
- Control	15	8.31±2.84		
RBC (x10^{12}/L)			-2.897	.009
- Test group	112	5.59±1.50		
- Control	15	4.18±0.52		
HB (g/dl)			7.196	<.001
- Test group	112	11.51±2.07		
- Control	15	12.83±0.78		
HCT (%)			-4.718	<.001
- Test group	112	34.66±6.05		
- Control	15	39.27±1.67		
MCV (fl)			-6.436	<.001
- Test group	112	86.66±5.60		
- Control	15	86.93±4.11		

MCH (pg)			-1.951	.053
- Test group	112	28.63±2.85		
- Control	15	30.13±2.39		
MCHC (g/dl)			-1.424	.157
- Test group	112	32.61±1.90		
- Control	15	33.33±1.18		
RDW-CV (%)			-1.706	.091
- Test group	112	13.04±1.53		
- Control	15	13.74±1.07		
RDW-SD (fl)			-2.487	.014
- Test group	112	40.38±3.45		
- Control	15	42.80±4.14		
PLT ($x10^9$/l)			-4.099	<.001
- Test group	112	141.58±39.98		
- Control	15	185.00±24.05		
MPV (fl)			6.622	<.001
- Test group	112	11.42±1.73		
- Control	15	8.32±1.51		
PDW (fl)			.580	.563
- Test group	112	12.65±2.37		
- Control	15	12.27±2.34		
PCT (%)			1.038	.301
- Test group	112	0.15±0.06		
- Control	15	0.14±0.03		

4.5 Comparison of Liver Enzyme Markers between HBV Infected Subjects and Apparently Healthy Individuals

The liver enzyme markers between the HBV infected subjects and apparently healthy control indicated that alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and direct bilirubin were statistically significant. The mean ALT value of the study subjects was 23.25 ± 13.54U/L as against 6.16 ± 2.72U/L of the controls, which indicated that the study subjects had higher ALT values than the control; $P < 0.001$. It was observed that the AST, total bilirubin and direct bilirubin values with $P < 0.001$, $P = 0.011$ and $P < 0.001$ were significantly elevated among the study subjects than the controls (Table 4.5).

Table 4.5: Comparison of Liver Enzyme Markers between HBV Infected Subjects and Apparently Healthy Individuals

Comparing Test Group and Control Group	N	Mean ± SD	t-test	p-value
ALT (U/L)			4.859	<.001
- Test group	112	23.25±13.54		
- Control	15	6.16±2.72		
AST (U/L)			6.033	<.001
- Test group	112	14.82±7.91		
- Control	15	8.09±3.20		
ALP (U/L)			-.790	.431
- Test group	112	99.88±27.74		
- Control	15	105.80±22.71		
TBIL (umol/L)			2.658	.011
- Test group	112	11.34±6.45		
- Control	15	8.92±2.61		
DBIL (umol/L)			14.496	<.001
- Test group	112	8.05±3.61		
- Control	15	1.57±1.12		

4.6 Estimation of IL-10 and TNF-α

Interleukin-10 (IL-10) and tumour necrosis factor-alpha (TNF-α) were measured and compared to study subjects and apparently healthy individuals. The results revealed a significantly increased in IL-10 and TNF-α with the mean values of 198.68 ± 217.96pg/mL and 85.93 ± 89.04pg/mL as against the controls 46.93 ± 13.86pg/mL and 23.73 ± 7.27pg/Ml respectively; $P < 0.001$ (Table 4.6).

Table 4.6: Estimation of IL-10 and TNF-α

Comparing Test Group and Control Group	n	Mean ± SD	t-test	p-value
IL-10 (pg/ML)			7.259	<.001
- Test group	112	198.68±217.96		
- Control	15	46.93±13.86		

TNF-α (pg/ML)			7.215	<.001
- Test group	112	85.93±89.04		
- Control	15	23.73±7.27		

4.7 Differences in Blood Parameters of HBV Infected Individuals on Therapy and Non-therapy

Blood Parameters were measured and compared with those on treatment and untreated among HBV-infected subjects. The results revealed a significantly increased in WBC of those untreated with the mean value of 10.13 ± 3.48x10^9/l and a significantly decreased in WBC of those on treatment with the mean value of 794 ± 3.67x10^9/l; P = 0.02. In differential leucocytes count, reduced value of neutrophil counts with the mean value of 56.88 ± 13.34% and increased value in lymphocyte counts with the mean value of 34.63 ± 13.15% were observed. Although, they are not statistically significant. Significantly increased in RBC among the untreated with the mean value of 5.86 ± 1.4x10^{12}/l was also observed; P = 0.04. In haematocrit (Hct), there was a significantly increased among those on treatment with the mean value of 36.02 ± 5.73%; P = 0.03. There was also a significantly increased in platelet among those on treatment with the mean value of 150.44 ± 40.56x10^9/l; P = 0.03. A significant increase in platelet distribution width among those on treatment with the mean value of 13.16 ± 2.38 fl was observed; P = 0.032 (Table 4.7).

5. DISCUSSION

Hepatitis B Virus (HBV) infection despite the enormous intervention still poses a serious threat to life worldwide especially in developing countries like Nigeria, and particularly in Ebonyi state. Inflammation and tissue infection can lead to abnormal results in haematological parameters (Ginan *et al*., 2024). In the study, we observed an elevated white blood cells (WBC) among the subjects with hepatitis B virus (HBV) infection. The findings were consistent with that of Al-mammory *et al*., (2024) and Rasheed *et al*., (2022), who reported increase in WBC in infected patients when compared with the healthy persons. However, the report contrasts with Ahsan *et al*., (2024) and Nyarko *et al*., (2023), who reported a decreased in WBC among the HBV infected individuals. The observed leucocytosis in this study may reflect an active immune response to viral replication or hepatic inflammation. White blood cells play an important role in the immune system, during infection, higher levels in the WBC among the infected patients are not sudden, as white blood cells are likely released to combat the viral pathogen (Onwuasoanya *et al*., 2017). The difference between this finding and previous reports suggest that alterations in the white blood cell counts during HBV infection might depend on the stage of infection and the subject's immunological status.

In this study, we observed a slight decrease in neutrophil count when compared to control. Although, the difference was not statistically significant. The results were in contrast with Urip *et al*., (2025) and a research conducted by Mentari and Zahrah, (2022), who showed that there was an increase in neutrophils in HBV infected subjects. Neutrophils are the most abundant type of phagocyte, usually 50 – 60% of all leucocytes in circulation, bone marrow produces more neutrophil during acute inflammation (Naully and Nursidika, 2019). Evidently, it is likely that the subjects in this study were those on chronic stage of HBV infection as neutrophil decreases at the latter stage due to bone marrow suppression as a result of persistent inflammation. Similarly, the result showed an increase in lymphocytes count of the HBV infected subjects when compared to control. Though, not significant. Increase in the percentage of lymphocyte can occur if there is damage to cells in the tissue or organs of the body (Saputri, 2020).

In this study, individuals infected by HBV showed significantly elevated red blood cells (RBC) counts. This observation contrasts with most previous studies, which have reported reduced RBC levels among HBV infected individuals (Shuaib *et al*., 2023; Al-mammory *et al*., 2024). The elevated RBC count observed in this study may indicate a compensatory response to mild hepatic hypoxia, which could stimulate erythropoietin secretion by the liver or kidney (Odusanya *et al*., 2023). In the study, we recorded decrease in haemoglobin, haematocrit, and mean cell volume. The findings were consistent with Shuaib *et al*., (2023). In contrast to the result, Gong *et al*., (2018) reported that the mean cell volume was unaffected by HBV infection in his study. The reduced level could be attributed to transient bone marrow suppression and autoimmune haemolytic anaemia which may accompany viral hepatitis (Shuaib *et al*., 2023).

Our study revealed that the platelet counts were decreased in study subjects compared to control groups. This was in support with "other studies that have reported reduced platelet counts among HBV infected persons" (Ahsan *et al*., 2024). This was further supported by Sulejmani *et al*., (2023). The reduction implies that individuals with HBV infection are more prone to develop thrombocytopaenia, a condition marked by a low platelet count (Raddsen *et al*., 2021). Thrombocytopaenia can be caused by a reduction in platelet production due to bone marrow suppression by the virus, increased in platelet sequestration or platelet destruction due to immune-mediated processes (Sulejmani *et al*., 2023). "It has been reported that in HBV infected persons, hypersplenism as a result of portal hypertension may lead to increased sequestration of blood cells including platelets" (Nyarko *et al*., 2023). Mean platelet volume (MPV), a biomarker of platelet activation was found to be increased in the study. This was consistent with research conducted by Wu *et al*., (2020) and Elsha *et al*., (2024), who also reported an elevated MPV in patients with HBV infection. In contrast, Xu *et al*., (2022), reported a reduced MPV in patients with decompensated liver cirrhosis. The possible explanation for his discrepancy could be that the subjects used by Xu *et al* were all receiving antiviral treatment thus, improving their symptoms and physical condition. In hepatic disease, platelet production is heightened in the bone marrow due to increased platelet utilization and destruction (Elsha *et al*., 2024); consequently, it will result in variations in platelet size, where younger platelets are larger and older platelets are smaller, thereby increasing the MPV value (Elsha *et al*., 2024). We compared the levels of WBC, RBC, HCT and platelet for both those on treatment and those untreated, we observed lower values in subjects on treatment than the untreated. The findings were in consonance with Obu *et al*., (2022), who reported pancytopaenia among HBV infected subjects in six months post treatment. The finding is likely due to side effects of some of the drugs they are taken as most of the drugs can cause bone marrow suppression.

Alanine aminotransferase (ALT) is an enzyme produced in response to cell damage or death in the liver (Shuaib *et al*., 2024). As biomarker, its serum levels can help in diagnosing and predicting the course of diseases and injuries from viral hepatitis (Shuaib *et al*., 2024). Our study observed elevated ALT among the HBV infected subjects. This is in line with what Kesete *et al*., (2022) observed in patients suspected of liver diseases. Elevated ALT levels are generally considered an indicator of liver inflammation and damage, often attributed to the immune response against the virus (Shuaib *et al*., 2024). Similarly, increased level of aspartate aminotransferase (AST) was also observed in the study. This correlate with the study conducted by Tahrim *et al*., (2025). Increased AST levels among the HBV infected subjects were strongly linked to the liver inflammation and fibrosis, which aligns with previous research highlighting its diagnostic and prognostic value in chronic hepatitis B virus management (Garcia-Cortes

et al., 2020). Hepatocytes are primarily responsible for metabolism and excretion of bilirubin (Ma *et al*., 2023). The total bilirubin and direct bilirubin were significantly increased in the study subjects compared to control, indicating the hepatocytes injury caused by HBV infection. This is in agreement with Cheng *et al*., (2018), who reported increase in both total and direct bilirubin in patients with chronic HBV infection. Both total bilirubin and direct bilirubin are valuable for determining disease severity and prognosis in patients with liver diseases (Lee *et al*., 2021).

In comparison of study subjects under treatment and the untreated, the liver enzymes were found to be reduced among the HBV treated subjects. The finding was in contrast to Vaillant, 2020, who reported an increase in ALT and AST due to effective therapy targeting infected cells in the bile duct epithelium and endothelial cells of hepatic blood vessels. The reduced viral load in the liver and blood by antiviral therapy would undoubtedly lower inflammation of the liver tissue which would result in less liver damage and inherently lower the liver enzymes (EASL, 2017).

Interleukin-10 (IL-10) is a cytokine with anti-inflammatory properties that plays a significant role in the immune response to HBV infection (Wang *et al*., 2024). In the study, we observed a significant increase in IL-10 among the HBV infected subjects. In agreement with the result, Gao *et al*., (2020) reported that several cytokines are released in response to chronic HBV infection. Interleukin-10 is known by its immunosuppressive property (Fabri *et al*., 2020) that has been reported to be secreted in response to HBV infection (Wang *et al*., 2024). In the study, we observed a significant increase in tumour necrosis factor-alpha (TNF-α) among the HBV infected subjects when compared to control. The finding is consistent with Hassoon *et al*., (2024), who also reported an increase in the level of TNF-α among the HBV patients. It is believed that TNF-α plays a dual function depending on the state of liver cells; it can promote inflammation by activating transcription factors or induce apoptosis when the hepatocytes are metabolically weak (Valaydon *et al*., 2016).

When cytokines were compared among those on treatment and those untreated, it was observed that both IL-10 and TNF-α for those on treatment were elevated. This could be attributed to the persistence of the virus indicating that functional cure has not been achieved (El Behery *et al*., 2025). In the study, there was no relationship between blood parameters and IL-10, but when the levels of TNF-α were compared to blood parameters, it was observed that TNF-α exhibited a negative relationship with the count of haemoglobin levels. This was consistent with the results of Ogbuabor, 2024, who reported a negative connection between TNF-α levels and haemoglobin levels in cases of anaemia in chronic kidney disease patients and also in malaria (Sarangi *et al*., 2012). The elevated levels of TNF-α was attributed to the cause of the impairment in red blood cell formation and the increased destruction of red blood cells by programmed cell death as a result of inflammation (Jelkmann, 2009).

Our study observed the distribution of HBV genotypes in Abakaliki, Ebonyi State as genotype E was predominant (36.7%). This finding was consistent with the study of Ezea *et al*., (2022), who reported the predominance of genotype E of 75% in Enugu State. Although, some studies had reported the predominance of genotypes A, B, and E in different parts of Nigeria (Kuhns *et al*., 2004; Odemuyiwa *et al*., 2001). We also reported genotype B, mixed infection of B and E while some showed no amplification. Genotype B was at 13.3%, B/E was also at 13.3% and 36.7% had no amplification. A study in Central part of Nigeria scored 34.5% of genotype B, genotype E (44.8%) and a mixed B/E (6.9%) and documented that it was the first time genotype B was detected in Nigeria (Odemuyiwa *et al*., 2001). The mixed infection reported in this study could be as a result of recombination between genotypes (Maylin *et al*., 2015), or it could be as a result of migrations from different parts of the world either for businesses or studies (Ezea

et al., 2022). Those subjects observed no amplifications could be attributed to the stage of infection like patients who are chronic carriers with inactive infection (Ezea *et al*., 2022). It might also be linked by intermittent viraemia or very low and undetectable levels of HBV-DNA either due to preceding treatment or natural clearance (Allain *et al*., 1999). The high rate of mortality of HBV infection seen in Abakaliki can be attributed to super infection with genotype B/E which is associated with several clinical outcomes, such as the development of liver fibrosis and hepatocellular carcinoma (Ghoshi *et al*., 2013). In our study, there was no relationship between blood parameters and the genotype.

5.1 CONCLUSION

The study established the association between haematological, cytokines, liver enzyme markers, and HBV genotypes in HBV infection since the level of each parameters varied in both those with HBV infection and healthy individuals. It also established the variations of these variables for those on treatment and those untreated. It revealed the HBV genotypes circulating in Abakaliki, Ebonyi State. Thus, this study have proven that unified assessment of haematological variables, cytokine profiles, liver enzyme markers and hepatitis B virus genotypes contribute thorough knowledge on the pathophysiology, variations in clinical manifestations and treatment outcomes of HBV infection.

5.2 RECOMMENDATION

From the findings and conclusions of this study, the following recommendations are made:

1. Further studies should include larger sample size, wider geographical coverage, and additional biomarkers such as liver fibrosis markers, and genetic polymorphisms.
2. Advocacy for Policy Development and Implementation by Government and Health agencies on HBV genotype surveillance, subsidized testing, and treatment programs.

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Conflict of Interest

The author declared that there is no conflict of interest.