**Evaluation of** **inflammatory and liver injury biomarkers among drug-naive viral hepatitis B patients: A study in a referral laboratory, Ghana**

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**Running Title: Inflammatory and liver injury biomarkers among HBV patients**

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**Abstract**

**Background and Aim:** Globally, hepatitis B virus (HBV) infection is among the commonest chronic infections and the leading cause of liver cancer. This study evaluated inflammatory and liver injury biomarkers among newly diagnosed HBV-infected patients to reveal inflammation and liver injury levels.

**Patients and Methods:** This case-control study was conducted among 146 newly diagnosed drug-naive patients and 64 blood donors. Questionnaires were administered to obtain demographic data. Blood samples were collected to assess viral serological markers, inflammatory markers, liver function, and hematological indices. Also, non-invasive markers of liver fibrosis (APRI: aspartate transaminase - platelet ratio index, FIB-4: fibrosis 4 index, and AAR: aspartate - alanine transaminase ratio) were mathematically derived. The patients were categorized into acute and chronic infections based on their viral serological markers.

**Results:** Overall, 81.5% of the patients had an acute HBV infection, whereas 18.5% had a chronic HBV infection. There was a significant increase in the biomarkers of inflammation, C-reactive protein (CRP) and interleukin 6, and liver injury (liver transaminases, FIB-4 index, and APRI) among the drug-naive chronic HBV-infected patients. The study also revealed significant anemia and leucocytosis in patients with chronic HBV infection. Further, the study showed a strong correlation between CRP and alanine transaminase among patients with chronic HBV infection.

**Conclusion:** There was increased anemia, inflammation, and liver fibrosis among the drug-naive chronic HBV-infected patients; hence, public education is required so patients with viral hepatitis B in Ghana would visit the clinic earlier enough for proper clinical management.

***Keywords:*** inflammation; liver fibrosis; liver injury; biomarkers; hepatitis B virus infection, liver transaminases; C-reactive protein; interleukin 6; anemia; leucocytosis.

**Background**

Globally, hepatitis B virus (HBV) infection is among the most common infections, with an estimated 300 million persons chronically infected and the leading cause of hepatocellular carcinoma (HCC) [1, 2]. According to the Global Cancer Statistics 2020 Report, HCC is the sixth most common cancer worldwide, with 905,677 new cases in 2020 [3]. Furthermore, the national prevalence of chronic HBV in Ghana was estimated to be between 8.36% (2020) and 12.30% (2016) [4], suggesting a likely increase in the future burden of HCC in Ghana [5]. However, most adults with HBV infection recover, with about 5 – 10% becoming asymptomatic carriers or developing chronic viral hepatitis B [6]. Moreover, patients with chronic HBV infection have a 15 – 40% risk of developing liver fibrosis, cirrhosis, and, eventually, HCC [7]; hence, newly diagnosed HBV-infected patients need to be assessed for better disease management.

The hepatitis B virus mainly affects the functions of the liver by multiplying in the liver cells leading to inflammation of the hepatocytes [8]. These inflammatory processes comprise tissue breakdown and repair mechanisms; therefore, the high tissue turnover in chronic active viral hepatitis B often leads to scarring and hepatocyte damage [9]. Subsequently, serum levels of aspartate and alanine transaminases are raised. These enzymes are associated with the liver parenchymal cells and are useful biomarkers of liver injury [10]. In addition, the hepatocytes also produce C-reactive protein (CRP), an acute phase protein [11], which causes the release of pro-inflammatory cytokines, specifically interleukin-6 (IL-6), from macrophages and T cells; hence, C-reactive proteins and IL-6 are used to assess the degree of inflammation [12, 13].

Assessing liver fibrosis using biopsies can be used to estimate prognosis, treatment urgency, and the necessity of HCC screening [14, 15]. The use of liver biopsies requires expertise from specimen collection to examining stained tissues. However, surrogate methods can estimate liver fibrosis, including serum fibrosis markers. These fibrosis markers are used, separately or in algorithm models, to assess liver fibrosis [16]. Some of these non-invasive biomarkers of liver fibrosis are the aspartate transaminase-platelet ratio index (APRI), fibrosis 4 index (FIB-4), and the aspartate-alanine transaminase ratio (AAR) [17, 18]. A study carried out among persons with

non-alcoholic fatty liver disease (NAFLD) found that an AAR of 0.8 was better for predicting advanced fibrosis [19]. Also, a study reported that AAR ≥1 is specific for liver fibrosis in persons with hepatitis [20]. Further, another study has shown that in persons with chronic hepatitis B virus infection, APRI ≥ 1.5 has predictive power for predicting significant fibrosis [21]. Moreover, studies have reported that FIB-4 > 1.45 has better diagnostic accuracy for assessing liver fibrosis among persons with non-alcoholic fatty liver disease [22, 23].

Currently, available literature in Ghana indicates a paucity of information on the serum levels of inflammatory markers and biomarkers of liver injury in drug-naive HBV-infected patients. Here we report the presence of inflammation, impaired liver function, and liver fibrosis among drug-naive HBV-infected patients.

**Materials and Methods**

**Ethics statement**

Ethical approval was sought from the Committee on Human Research, Publications and Ethics (CHPRE) of the School of Medicine and Dentistry, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (approval letter number: CHPRE/AP/336/14). Also, informed consent was obtained from the study participants after explaining the purpose of the study in a language each participant understood.

**Study design, study site, and sample size**

This case-control study was conducted at Medilab Diagnostics Services Limited in Kumasi in the Ashanti Region of Ghana. Medilab Diagnostics Services Limited is among Ghana’s leading private medical laboratories, with branches in the regional capitals, and serves as the referral center for most of the clinical laboratory investigations in the country. The sample size was 146 patients and 64 age and sex-matched controls. Blood donors were recruited as controls from the Regional Blood Transfusion Centre, Kumasi.

**Blood sample collection**

After obtaining informed consent, structured questionnaires were administered to obtain patients’ demographic data. Next, 5 ml of venous blood samples were collected from the patients: 2 ml into a K2EDTA tube and 3 ml into a gel separator tube. Hematological analysis was performed on the samples in the K2EDTA tube within two (2) hours. First, however, the gel separator tube samples were allowed to clot and centrifuged at 3000 rpm for five (5) minutes to obtain the sera to assess viral serological markers, inflammatory markers, and liver function indices.

**Determination of viral serological and inflammatory markers**

The viral serological markers assessed were the sero-presence of IgM antibodies to the viral core antigen (anti-HBc IgM) and HBV “e” antigen (HBeAg). The iCARE One Step anti-HBc IgM Rapid Test Strip (JAL Innovation, Pte, Ltd., Singapore) was qualitatively used to detect the presence of anti-HBc IgM in the serum of the participants. Also, the Wondfo Five Step Rapid Test Kit (Wondfo Biotech Co. Ltd., China) was qualitatively used to detect the total anti-HBc antibodies and HBeAg in the serum of newly diagnosed HBV-infected patients. The patients who tested positive for anti-HBC IgM were classified as having an acute infection. In contrast, those who tested negative for anti-HBC IgM but positive for the total anti-HBC antibodies were classified as having a chronic infection. The patients who tested positive for HBeAg were classified as having an active infection [24]. The inflammatory markers, CRP and IL-6, were quantified using the CRP ELISA Assay Kit (Eagle BioSciences, Inc., Nashua, USA) and the IL-6 ELISA Assay Kit (Eagle BioSciences, Inc., Nashua, USA). All the kits were used by following the manufacturers’ instructions.

**Determination of liver function and hematological indices**

Liver function indices such as serum levels of aspartate and alanine transaminases (AST and ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and albumin were analyzed using a semi-automatic biochemistry analyzer (BC-3000M, Sinnowa Medical Science and Technology Co. Ltd., China). Also, the hematological indices such as total white blood cell (WBC) count, lymphocyte count, neutrophil count, platelet counts, and hemoglobin concentration were assessed using the Mindray Auto Haematology Analyser (BC-3200, Shenzhen Mindray BioMedical Electronics Co. Ltd., China).

**Estimation of non-invasive markers of liver fibrosis**

The non-invasive markers of liver fibrosis, such as AAR, APRI, and FIB-4, were estimated from the liver function and hematological indices, as well as the patient’s age in years, where appropriate, as described previously [25-27]. The respective equations are ***AAR*** *= AST / ALT,* ***APRI*** *= [(AST / Upper Limit of Normal) / Platelet Count (×109/L)] × 100* and ***FIB-4*** *= [age (years) × AST (U/L)] / [(Platelet Count (×109/L) × ALT (U/L))1/2]*.

**Study inclusion and exclusion criteria**

Newly diagnosed HBV-infected patients between 18 and 60 years were included in the study. However, patients with other forms of inflammatory conditions and liver diseases, diabetes mellitus, hypertension, HIV/AIDS, rheumatoid arthritis, malignant diseases, and other forms of inflammatory diseases such as malaria and HBV-infected patients on therapy were excluded from this study. Also, co-infection of HBV with HCV was excluded.

**Statistical analysis**

Data were analyzed using the Statistical Package for Social Sciences Statistical Software (version 20.0, IBM Corporation USA). The categorical variables were expressed in proportions, whereas the continuous variables were expressed as mean ± standard error of the mean (SEM). The confidence intervals of the proportions were calculated using the binomial test. A comparison of proportions was made using Fisher’s exact test. In contrast, a comparison of means was made using a *t*-test or ANOVA (analysis of variance) and Bonferroni’s post hoc test where appropriate. Moreover, correlations were evaluated using Pearson’s correlation analysis. The statistical significance was accepted in all comparisons at a *p*-valueless than 0.05.

**Results**

**Demographic characteristics of study participants**

The study participants comprised 146 HBV seropositive patients (males: 74.7% and females: 25.3%) and 64 healthy controls (males: 79.7% and females: 20.3%). There was no statistical difference between the average ages of the HBV seropositive patients and the healthy controls, as shown in **Table 1**. However, the females with HBV seropositive were older than their healthy control counterparts (*p* = 0.024).

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| *Table 1. Demographic characteristics of the study participants.* |
| **Parameter** | **Healthy Controls****n = 64** | **HBsAg Sero-positive****n = 146** | **p-value** |
| **Age (years)** |  |  |  |
|  Male | 36.98±0.78 | 37.79±0.91 | 0.580 |
|  Female | 32.77±1.94 | 39.40±1.57 | 0.024\* |
|  Total | 36.13±0.76 | 38.17±0.78 | 0.064 |
| **Gender** Male Female | 51 (79.7)13 (20.3) | 109 (74.7)37 (25.3) | 0.4300.430 |
| *Age is expressed as mean ± SEM, and gender is defined as frequency (percentage). \*Difference calculated by t-test (p-value <0.05).* |

**The proportion of acute and chronic HBV infection among the patients**

The HBV-infected patients were grouped into acute or chronic infections based on the presence or absence of the anti-HBc IgM in their serum. Also, the patients were considered to have active or inactive infections based on the presence or absence of the HBeAg in their serum. Out of the 146 HBV-infected patients, 81.5% (CI: 74.2- 87.4) had an acute infection, whereas 18.5% (CI: 12.6 - 25.8) had a chronic infection. Among the patients with chronic HBV infection, 70.4% had an inactive infection, whereas 29.6% had an active infection (**Table 2**).

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| *Table 2. Acute and chronic HBV infections among the newly diagnosed patients* |
| **Parameter** | **Gender** | **Total** |
| **Male** | **Female** |
| **AHB** | 92 (63.0; 54.6 – 70.8) | 27 (18.5; 12.6 – 25.8) | 119 (81.5; 74.2 – 87.4) |
| **CHB** | 17 (11.6; 6.9 – 18.0) | 10 (6.9; 3.3 – 12.2) | 27 (18.5; 12.6 – 25.8) |
|  CHB1 | 11(40.8; 22.4 – 61.2)  | 8 (29.6; 3.8 – 50.2) | 19 (70.4; 49.8 – 86.2) |
|  CHB2 | 6 (22.2; 8.6 – 42.3) | 2 (7.4; 0.9 – 24.3) | 8 (29.6; :13.8 – 50.2) |
| *The data are expressed as frequencies (percentages; 95% confidence interval), AHB: Acute HBV infection, CHB: Chronic HBV infection, CHB1: inactive chronic HBV infection, CHB2: active chronic HBV infection.* |

**Inflammatory and liver injury markers, as well as hematological indices, among the study participants**

There was significant anemia, leucocytosis, and thrombocytopenia among the patients compared to the controls (**Table 3**), which was more prominent in the patients with chronic HBV infection (**Table 4**). Also, there was an increase in the markers of liver injury (AST and ALT), with a reduced hepatic synthetic ability (reduced serum albumin levels) among the patients (**Table 3**), which was more prominent in the patients with chronic HBV infection (**Table 4**). Similarly, the inflammatory markers were significantly increased among the patients compared to the controls (**Table 3**), which was more prominent in the patients with chronic HBV infection (**Table 4**). Within the patients, all the non-invasive markers of liver fibrosis were above their cut-off among the patients with chronic HBV infections, which was explicitly due to the active infection (**Table 4**).

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| *Table 3. Biomarkers of inflammation, liver injury, and hematological indices among controls and patients* |
| **Parameter** | **Total****n = 210** | **Controls****n = 64** | **Patients****n = 146** | **p-value** |
| ***Hematological indices*** |
|  HB (g/dL) | 13.46±0.76 | 14.76±0.20 | 12.89±0.20 | 0.000\* |
|  WBC (x109/L) | 10.64±1.37 | 6.78±0.18 | 12.33±0.34 | 0.000\* |
|  LYM(x109/L) | 5.05±0.88 | 2.67±0.14 | 6.09±0.22 | 0.000\* |
|  NEU (x109/L) | 4.98±0.82 | 3.53±0.12 | 5.62±0.23 | 0.000\* |
|  PLT (x109/L) | 238.56±23.47 | 268.95±5.90 | 225.24±6.61 | 0.000\* |
| ***Liver function indices*** |
|  AST (U/L) | 45.85±15.40 | 11.78±0.46 | 60.79±4.28 | 0.000\* |
|  ALT (U/L) | 31.18±7.44 | 15.36±0.59 | 38.11±2.08 | 0.000\* |
|  ALP (U/L) | 69.02±12.76 | 44.81±3.47 | 79.64±3.35 | 0.000\* |
|  GGT (U/L) | 43.65±8.98 | 32.65±2.32 | 48.48±2.53 | 0.000\* |
|  ALB (g/dL) | 3.82±0.37 | 4.32±0.18 | 3.61±0.08 | 0.000\* |
| ***Inflammatory markers*** |
|  CRP (mg/L) | 18.52±5.89 | 5.14±0.26 | 24.38±1.62 | 0.000\* |
|  IL - 6 (pg/mL) | 21.44±5.84 | 6.60±0.23 | 27.94±1.55 | 0.000\* |
| ***Non-invasive markers of liver fibrosis*** |
|  AAR | 1.36±0.25 | 0.83±0.06 | 1.60±0.07 | 0.000\* |
|  APRI | 0.59±0.25 | 0.11±0.01 | 0.80±0.07 | 0.000\* |
|  FIB-4 | 1.42±0.44 | 0.42±0.02 | 1.86±0.12 | 0.000\* |
| *The data are expressed as mean ± SEM, HB: Haemoglobin, WBC: White Blood Cell, LYM: Lymphocyte, NEU: Neutrophil, PLT: Platelet, AST: Aspartate Transaminase, ALT: Alanine Transaminase, ALB: Albumin, GGT: Gamma Glutamyl Transferase, ALP: Alkaline Phosphatase. \*Difference calculated by t-test (p-value <0.05).* |

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| **Table 4. Comparison of biomarkers of inflammation, liver injury, and hematological indices within the patients.** |
| **Parameter** | **AHB****n = 119** | **CHB****n = 27** | **p-value** | **CHB1****n = 19** | **CHB2****n = 8** | **p-value** |
| Hematological indices |
|  HB (g/dL) | 13.68±0.16 | 9.41±0.40 | 0.000\* | 9.65±0.49 | 8.85±0.65 | 0.345 |
|  WBC (x109/L) | 11.09±0.25 | 17.80±0.88 | 0.000\* | 16.74±1.00 | 20.32±1.52 | 0.070 |
|  LYM(x109/L) | 5.83±0.21 | 7.27±0.68 | 0.052 | 6.76±0.83 | 8.49±1.16 | 0.245 |
|  NEU (x109/L) | 4.67±0.16 | 9.80±0.55 | 0.000\* | 9.24±0.67 | 11.14±0.84 | 0.097 |
|  PLT (x109/L) | 236.96±7.23 | 173.59±12.12  | 0.000\* | 175.63±15.58 | 168.75±18.81 | 0.782 |
| Liver function indices |
|  AST (U/L) | 51.70±3.75 | 100.85±14.00 | 0.002\* | 56.48±4.52 | 206.23±8.86 | 0.000\* |
|  ALT (U/L) | 33.70±2.04 | 57.57±5.40 | 0.000\* | 42.50±4.06  | 93.35±2.21 | 0.000\* |
|  ALP (U/L) | 69.45±2.93 | 124.53±8.49 | 0.000\* | 121.72±9.99 | 131.20±16.91  | 0.638 |
|  GGT (U/L) | 43.11±2.14 | 72.14±8.69 | 0.003\* | 66.09±11.67 | 86.49±8.56 | 0.171 |
|  ALB (g/dL) | 3.69±0.09 | 3.23±0.12 | 0.003\* | 3.38±0.15 | 2.88±0.15 | 0.027\* |
| Inflammatory markers |
|  CRP (mg/L) | 18.48±0.59 | 50.40±6.37 | 0.000\* | 32.45±4.28 | 93.03±5.24 | 0.000\* |
|  IL - 6 (pg/mL) | 22.37±0.57 | 52.49±6.13 | 0.000\* | 36.27±4.94 | 91.03±4.45 | 0.000\* |
| Non-invasive markers of liver fibrosis |
|  AAR | 1.57±0.07 | 1.74±0.16 | 0.323 | 1.54±0.21 | 2.21±0.09 | 0.007\* |
|  APRI | 0.62±0.05 | 1.62±0.25 | 0.001\* | 0.90±0.11 | 3.31±0.35 | 0.000\* |
|  FIB-4 | 1.60±0.11 | 2.97±0.36 | 0.001\* | 2.18±0.25 | 4.83±0.71 | 0.007\* |
| The data are expressed as mean ± SEM, **AHB:** Acute HBV infection, **CHB:** Chronic HBV infection, **CHB1: inactive c**hronic HBV infection, **CHB2:** active chronic HBV infection., **HB**: Haemoglobin, **WBC**: White Blood Cell, **LYM**: Lymphocyte, **NEU:** Neutrophil, **PLT:** Platelet, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB:** Albumin, **GGT:** Gamma Glutamyl Transferase, **ALP:** Alkaline Phosphatase. \*Difference calculated by t-test (p-value <0.05). |

**Correlation between the biomarkers of liver injury and inflammation**

From Pearson’s correlation analysis, there was no significant association between the markers of liver injury (AST and ALT levels) and inflammation (CRP and IL-6 levels) among the patients with acute HBV infection, as presented in **Table 5**. However, for the patients with chronic HBV infection, there were significant associations between IL-6 and ALT (r = 0.855, *p* < 0.001), IL-6 and AST (r = 0.799, *p* < 0.001), CRP and ALT (r = 0.909, *p* < 0.001), and CRP and AST (r = 0.856, *p* < 0.001), as demonstrated in **Table 5**.

From Pearson’s correlation analysis, there were significant positive associations between ALT and CRP levels (r = 0.682, *p* = 0.001), CRP and IL-6 levels (r = 0.887, *p* < 0.001), ALT and IL-6 levels (r = 0.550, *p* = 0.015), and AST and CRP levels (r = 0.202, *p* = 0.406) among the patients with inactive chronic HBV infection (**Table 6**). Also, there were significant positive associations between CRP and ALT levels (r = 0.854, *p* = 0.007), ALT and IL-6 levels (r = 0.716, *p* = 0.046), IL-6 and CRP levels (r = 0.898, *p* = 0.002), and AST and IL-6 levels (r = 0.713, *p* = 0.047) among the patients with active chronic HBV infection (**Table 6**).

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| *Table 5. Pearson’s correlation between some parameters within the patients with acute (upper-right hand side) and chronic (lower-left hand side) infections* |
| **Parameters** | **HB****(g/dL)** | **AST****(U/L)** | **ALT****(U/L)** | **ALB****(g/dL)** | **CRP****(mg/L)** | **IL-6****(pg/mL)** |
| **HB (g/dL)** |  | 0.020 | -0.024 | 0.097 | 0.000 | -0.025 |
|  |  | (0.828) | (0.793) | (0.294) | (1.000) | (0.789) |
| **AST (U/L)** | -0.204 |  | 0.744\* | 0.058 | -0.057 | -0.067 |
|  | (0.309) |  | (0.000) | (0.528) | (0.535) | (0.469) |
| **ALT (U/L)** | -0.115 | 0.863\* |  | 0.066 | 0.005 | -0.041 |
|  | (0.568) | (0.000) |  | (0.479) | (0.958) | (0.658) |
| **ALB (g/dL)** | 0.268 | -0.366 | -0.404\* |  | -0.072 | 0.110 |
|  | (0.177) | (0.061) | (0.037) |  | (0.435) | (0.235) |
| **CRP (mg/L)** | -0.128 | 0.856\* | 0.909\* | -0.240 |  | 0.820\* |
|  | (0.524) | (0.000) | (0.000) | (0.227) |  | (0.000) |
| **IL-6 (pg/mL)** | -0.168 | 0.799\* | 0.855\* | -0.275 | 0.959\* |  |
|  | (0.401) | (0.000) | (0.000) | (0.166) | (0.000) |  |
| *The data are expressed as correlation coefficients (p-values), HB: Haemoglobin, AST: Aspartate Transaminase, ALT: Alanine Transaminase, ALB: Albumin, IL-6: Interleukin 6, CRP: C-Reactive Protein. \*Correlation coefficient significant (p<0.05).* |

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| **Table 6: Pearson’s correlation between some of the parameters within the chronic HBV-infected patients with inactive (upper-right hand side) and active (lower-left hand side) infections** |
| **Parameters** | **HB****(g/dL)** | **AST****(U/L)** | **ALT****(U/L)** | **ALB****(g/dL)** | **CRP****(mg/L)** | **IL-6****(pg/mL)** |
| **HB (g/dL)** |  | 0.071 | 0.108 | 0.254 | 0.176 | 0.052 |
|  |  | (0.774) | (0.659) | (0.294) | (0.472) | (0.833) |
| **AST (U/L)** | -0.528 |  | 0.420 | -0.003 | 0.202 | 0.047 |
|  | (0.179) |  | (0.073) | (0.991) | (0.406) | (0.849) |
| **ALT (U/L)** | -0.218 | 0.300 |  | -0.231 | 0.682\* | 0.550\* |
|  | (0.604) | (0.471) |  | (0.34) | (0.001) | (0.015) |
| **ALB (g/dL)** | 0.071 | -0.246 | 0.250 |  | 0.123 | 0.044 |
|  | (0.867) | (0.557) | (0.551) |  | (0.617) | (0.859) |
| **CRP (mg/L)** | -0.417 | 0.458 | 0.854\* | 0.271 |  | 0.887\* |
|  | (0.304) | (0.253) | (0.007) | (0.516) |  | (0.000) |
| **IL-6 (pg/mL)** | -0.510 | 0.713\* | 0.716\* | -0.046 | 0.898\* |  |
|  | (0.196) | (0.047) | (0.046) | (0.914) | (0.002) |  |
| The data are expressed as correlation coefficients (p-values), **HB**: Haemoglobin, **AST:** Aspartate Transaminase, **ALT:** Alanine Transaminase, **ALB**: Albumin, IL**-6**: Interleukin 6, **CRP:** C-Reactive Protein. **\***Correlation coefficient significant (p<0.05). |

**Discussion**

This study assessed biomarkers of inflammation and liver injury to reveal the existence of liver inflammation and the extent of liver injury among drug-naive chronic HBV-infected patients in Ghana to help manage newly diagnosed HBV-infected patients. Most HBV-infected patients in this current study were males. The finding was consistent with previous research in three densely populated suburbs in Kumasi, Ghana, that reported a higher proportion of HBV infection among males than females [28]. Females produce higher estrogen levels than males; hence females have antibodies at a higher frequency against the HBsAg and HBeAg than males [29]. The gender disparity in HBV infection supports the findings of this study. Most newly diagnosed patients have an acute infection, and in Sokoto, Nigeria, a high proportion of acute HBV infections has also been reported [30]. The higher proportion of acute infection is because most adults infected with HBV recover entirely from the infection, with about 5 – 10% progressing to a chronic HBV infection [6]. Male dominance was recorded in all the clinical categories of HBV infection, and a similar outcome with male dominance has been found with chronic HBV-associated hepatocellular carcinomas [31, 32].

The hemoglobin concentration was lower among the HBV-infected patients compared to the healthy controls, and among the patients, there was significant anemia among those with chronic infection. This finding is similar to studies where lower hemoglobin concentrations have been reported among HBV-infected patients, especially those with chronic infection [33-35]. The chronic nature of HBV infection contributes to the observed anemia [36]. Also, it was observed that most chronic HBV-infected patients have leucocytosis and thrombocytopenia. The leucocytosis may be due to viral infection, inflammation, and tissue necrosis [37]. The low platelet count indicates the varying grade of liver fibrosis, buttressed by a study that showed that a low platelet count is independently associated with significant liver fibrosis [38].

The serum levels of liver injury markers (ALT and AST) were higher in the HBV-infected patients compared to the healthy controls, especially among those with chronic infection. Also, serum levels of ALP and GGT were significantly higher among the chronic HBV-infected patients. This finding aligns with a previous study that reported higher levels of liver transaminases in chronic hepatitis and attributed this to more profound liver damage because of the spread of the viral infection and replication [10, 39]. In addition, the chronic HBV-infected patients have lower levels of serum albumin, which could be associated with impaired hepatic synthetic capacity among these patients since hepatocytes produce albumin [40, 41].

The serum levels of the inflammatory markers, CRP, and IL-6 were higher among the HBV-infected patients than the healthy controls, especially those with chronic infection. These findings are consistent with studies that reported increased CRP and IL-6 levels in chronic HBV-infected patients than those with acute infection and healthy controls [42, 43]. The study also showed significant liver fibrosis, as assessed by the non-invasive markers (FIB-4 and APRI), among the chronic HBV-infected patients. There were significant associations between the markers of inflammation and liver injury among the chronic HBV-infected patients, which may be a result of viral activity in the hepatocytes, which causes the release of CRP and IL-6 [17] and liver transaminases. However, the rise in the CRP levels in the presence of impaired hepatocyte function may result from the increase in IL-6 and other cytokines that initiate the production of CRP by the liver [12, 44].

**Limitations of the study**

The sample size was small, which could affect the study's outcome; hence, a more extensive study is required to bolster the current findings. Also, the assessment of liver fibrosis was based only on the non-invasive markers, dependent on the patient’s age, liver function and hematological indices. The evaluation of the liver fibrosis could have been supplemented with an ultrasound scan and fibroscan, but with limited resources, these scans were not done during the study.

**Conclusion**

There was increased anemia, inflammation, and liver fibrosis among the drug-naive chronic HBV-infected patients compared to those with acute infection. Hence, public education is required so patients with viral hepatitis B in Ghana would visit the clinic early enough for proper clinical management to prevent future complications.

**Footnotes.**

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**Data availability**

All the data obtained and analyzed are included in this manuscript.

**Conflicts of interest**

The authors declare that they have no competing interests.

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**Abbreviations**

AAR: aspartate - alanine transaminase ratio, ALP: alkaline phosphatase, ALT: alanine transaminase, APRI: aspartate transaminase - platelet ratio index, AST: Aspartate transaminase, CRP: C-reactive protein, ELISA: enzyme-linked immunosorbent assay, FIB-4: fibrosis 4 index, GGT: gamma-glutamyl transferase, HBV: hepatitis B virus, HCC: hepatocellular carcinoma, IgM: Immunoglobulin M, IL-6: interleukin 6,

**Authors’ contributions**

MTF conceived and supervised the work. SAD planned and carried out the experiments. EOA analyzed the data. SAD and SAS wrote the manuscript. PWN and MM reviewed and edited the manuscript. All authors read and approved the final manuscript.

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