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Host cytokine genetic polymorphisms in a selected population of persons living with hepatitis B virus infection in the central region of Ghana

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Abstract

Background Hepatitis B virus (HBV) infection is a public health concern in resource limited settings like Ghana. Over the past decades, it is noted that the natural course of HBV in persons infected are taking a worse turn leading to liver cirrhosis and cancer. The outcome of HBV infection is influenced by viral and host factors including genetics. Cytokine variations affect virus survival and progression and may even influence associated complications. Cytokines such as tumor necrosis factor alpha (TNF-α), interleukins (IL-4, IL-6, IL-8, IL-10, IL-18), interferon gamma (IFN)-γ, and tumor growth factor-beta (TGF-β) have key roles in HBV infection and modulation. In this study, polymorphisms occurring in five cytokines were analysed to understand how they can influence prognosis of HBV infection.

Methods The study is a single centre cross-sectional study involving 227 participants made up of HBV infected participants and HBV-negative controls. Recruitment was from March 2021 to April 2022. Blood samples were taken for full blood count, HBV antigen profile, liver function tests, HBV DNA quantification and cytokine genotyping. FIB score was calculated using available tools. Statistical analysis was undertaken with $p < 0.05$ set as statistically significant.

Results The 20–39-year-old group formed majority of the HBV infected participants with 60% of all participants being classified as healthy HBsAg carriers. *IL2 rs1479920 GG carriers* ((1258.93; 0.00–5011.87) IU/mL had reduced HBV DNA in comparison to *IL2 rs1479920 AA* ((5011.87; 2113.49–5956.62) */AG* (3548.13; 0.00–6309.57) IU/mL carriers. *TNF-α rs1800629 AA* carriers (1258.93; 0.00–3981.07) IU/mL had a reduction in HBV DNA levels in comparison to *TNF-α rs1800629 GG* carriers (1584.89; 0.00–5011.87) IU/mL. The results of univariate (OR = 0.08, 0.00–0.93; $p = 0.043$) and multivariate (OR = 0.02, 0.00–0.67; $p = 0.029$) analysis, showed that carrying TNF-α rs1800629 *AA* genotype reduce susceptibility to high FIB score compared with *GG* genotypes. In univariate analysis, subjects aged 20–39 years (OR = 5.00, 1.13–6.10; $p = 0.034$) and 40–59 years (OR = 41.99, 3.74–47.21; $p = 0.0002$) were more susceptible to high FIB score compared to subjects aged 1–19 years. Being female (OR = 2.42, 1.03–5.71; $p = 0.043$) in the univariate models showed

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higher odds of having high levels of HBV DNA in the multivariate model. There was a reduced likelihood of herbal medicine usage influencing HBV DNA levels significantly (OR = 0.29, 0.10–0.86; $p = 0.025$).

Conclusion In conclusion, variations in *IL2 rs1479920 GG* and *IL2 rs1479921 AA* could offer protective effects by reducing HBV DNA. *TNF-α rs179924CT* may also cause elevation in HBV DNA levels whiles *TNF-α -308A/G,* showed a potential protective effect on liver scarring in HBV infected participants. It is therefore important to take a further look at such variations for understanding of HBV modulation in the Ghanaian population.

Keywords HBV, Cytokines, Hepatitis, Genetics, TNF-α, IL-2, IL-10, IL-18, IFN-γ

Introduction

The global prevalence of hepatitis B virus (HBV) is soaring with a significant associated mortality. It is estimated that the global prevalence of HBV infections is 3.2% which translates into approximately 257 million cases of infections [1]. Approximately 70% of global HBV infections occurs in the African region with 125, 000 associated deaths [2]. Despite being a vaccine-preventable infection, HBV infection is a public health concern in sub-Sahara Africa (SSA). It is estimated that most HBV infections occur in Eastern and other Sub-Saharan African countries, where persistent liver diseases and liver cancer contributes significantly to mortality [3, 4]. In Ghana, HBV prevalence is relatively high with a reported prevalence of 5–27% [5–8]. This high prevalence is resulting in reported life-threatening illnesses such as liver cirrhosis, cancer and other liver diseases [9]. The virus that causes HBV infection has long being considered as non-cytopathic [10, 11] which means, the associated clinical effects of the virus results from host immune mechanisms in dealing with the virus [11, 12]. Host immune modulation mechanisms may lead to diverse clinical outcomes ranging from asymptomatic self-limited infection, inactive carrier status, and chronic hepatitis to other complicated phases of liver diseases including fatal liver failure [13–15].

Although viral pathogenesis plays a significant role in the outcome of HBV infections, individual differences in the immune responses to HBV infection are more crucial for these outcomes [16–19]. The functionality and activity of an active immune system which can lead to the successful elimination of the hepatitis B virus, only occurs in just about 10–30% of infected persons [20].

Several risk factors have been explored to understand and identify persons infected with HBV who may be predisposed to less favourable outcomes. One such risk factor includes cytokine modulation and potential genetic influence [21, 22]. Cytokines influence the natural course of HBV infection and play an important role in the initiation and regulation of immune responses [23–25]. It is noted that persons infected with HBV may have a significant decrease in CD4 + T-helper and CD8 + cytotoxic T-lymphocyte response cascade, which is postulated to be key to HBV clearance [26, 27]. Immuno-modulatory mechanisms involving cytokines such as tumor necrosis factors alpha (TNF-α), interleukins (IL-4, IL-6, IL-8, IL-10, IL-18), interferon gamma (IFN)-γ, and tumor growth factor-beta (TGF-β) have a key role at the beginning of infection and modulatory dynamics of immunological reactions, and hence can influence vulnerability to HBV and the normal course of infection [21, 28]. As part of immunomodulatory activity towards HBV infection, cytokines may influence liver fibrosis through hepatic stellate cell activation [29, 30]. This therefore means that immune cells and cytokines within the immune system potentially participate in the progression of liver scarring collectively exerting significant immunomodulatory effects.

The influence of host genetics in HBV progression cannot be over emphasized, as variations in cytokines that contribute to the modulation of immune responses in HBV infection are affected by polymorphisms. Globally, polymorphisms have been linked to host vulnerability to HBV infection and viral clearance [31, 32]. Several studies have shown that host genetic polymorphisms influence the transition from acute to chronic status in HBV [33] and increase or reduce the chance of severity of illness [22, 34]. The body of evidence available has shown that host immunity is associated with relevant gene variants, particularly single nucleotide polymorphisms (SNPs) occurring in promoter regions of genes [21, 28].

With the knowledge that immune response is important to HBV progression and prognosis, it is important to understand and appreciate the genetic profile of cytokines in persons infected with HBV. There is very limited data on genetic polymorphisms in cytokines involved in HBV modulation and their potential relationship to HBV progression in Ghanaians. The aim of this study therefore was to profile for polymorphisms in cytokines including *IL-2, IL-10, IL-18, INF-γ*, and *TNF-α* implicated in immunomodulation of HBV infection and understand how these polymorphisms influence prognoses using HBV DNA and the non-invasive fibrosis-4 (FIB-4) score as indicators.

Methods

Study design and subjects

The study is a single-centre cross-sectional study carried out at the hepatitis clinic at the Cape Coast Teaching Hospital, Cape Coast, Ghana. We recruited participants from March 2021 to April 2022. The targeted population was made up of patients from both genders above 18 years old who have been diagnosed with HBV infection and had available in their medical records viral loads results not less than 6 months old. Both acute and chronic HBV patients were recruited whiles HBV negative participants served as healthy controls. Acute hepatitis B was defined as acquired hepatitis B virus infection that lasts for less than 6 months whiles chronic hepatitis refers to infection persists for more than 6 months.

Sample size

The prevalence of HBV in Ghana is estimated to be 5–27% with the central region having a prevalence of 11.1–12.0%. Based on the data at the hepatitis clinic at the Cape Coast Teaching Hospital, with an average patient population of 1000, a confidence interval of 95%, 5% margin of error and a 5% provision for contingency, a response distribution of 84.8%, we calculated a sample size for this exploratory study as 174. The Raosoft software was used to calculate the sample size. However, to make up for contingencies, the total samples size was 227 made up of HBV infected patients (207) and HBV-negative controls [20].

Ethical considerations

The Cape Coast Teaching Hospital Ethical Review Board Committee granted ethical approval for the research, with reference number CCTHERC/EC/2021/005. Participants who met the inclusion criteria were approached at the clinic during their visit and informed of the study and those who were willing were enrolled. In addition, both verbal and/or written consent was sought for. Unique codes were used for participants to ensure confidentiality and anonymity.

Laboratory analysis

A volume of 5 ml blood using BD vacutainer needles (BD Life Sciences, Plymouth, UK), were drawn from patients into two tubes. A volume of 2.5 ml of the whole blood was collected into ethylenediamine tetraacetic acid (EDTA) vacutainer tubes for full blood count (FBC) and separated at 3000G using the Universal 320R (Hettich Centrifuge, Germany) for the plasma for HBV profile. A 2.5 ml blood from a plain tube was then centrifuged at 3000G for serum for LFT and viral loads. Samples were further processed downstream for DNA extraction and genotyping.

HBV antigen profile test

The Wondfo One Step HBV Whole Blood/ Serum/ Plasma kits (Wondfo, Guangzhou, China) were used for obtaining the HBV antigen profile from the patients. Three drops of plasma were placed in a labelled cassette and reaction allowed to settle for 15 min before reading the results. If a band appears in any of the HBeAg, HBsAg and HBsAb designated zone, then it is considered as reactive while if no band appears in the then it is considered non-reactive.

Liver function test

Liver function test was performed using the PRO XL chemical analyzer (ElitechGroup, Puteaux, France). Serum samples were used to evaluate liver function markers such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Viral load

The HBV DNA quantification was undertaken using a fully automated DNA extraction and RT-PCR amplification process on the COBAS® AmpliPrep Instrument (Roche Diagnostics, USA) and Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) HBV test kits, v2.0 following manufacturer's protocol [35–37].

FIB 4 index

Fibrosis-4 Index (FIB-4) is a simple non-invasive tool developed to determine the presence of advanced hepatic fibrosis, with scores categorized into low (< 1·30), indeterminate (1·30–2·67), or high (> 2·67) risk of fibrosis. It is calculated using age, AST, ALT, and platelet concentrations [38].

FIB-4 calculation formula is shown below:

$$\text{FIB 4} = \text{Age (years)} \times \text{AST (U/L)} / (\text{platelet count } (10^9/\text{L}) \times \text{ALT}^{1/2} \text{ (U/L)})$$

DNA Extraction and cytokine genotyping

The samples collected into the EDTA vacutainer tube were stored at 20 °C and subsequently used for the DNA extraction. The omega BIO-TEK E.Z.N.A R Blood DNA Mini Kit (Omega Bio-tek, Inc. Norcross, USA) was used for the extraction process according to the manufacturer's instruction. Genotyping was undertaken using the Iplex GOLD SNP genotyping protocol on the Agena MassARRAY ®system (Agena Bioscience™, San Diego, CA, USA) [39] and PCR–RFLP. PCR was performed in a total reaction volume of 25µL made up of 0.5µL of 5X GoTaq poly reaction buffer containing $MgCl_2$, 0.625µL of 5 mM dNTPs, 1µL of forward and reverse primer, 0.2µL of 5U/µL GoTaq Flexi DNA polymerase, 17.175µL of ddH_2O and 3µL DNA. The selected SNPS included

IL2 (*rs1479920 A > G, rs1479921 G > A, rs1479922 T > C), TNF-α (rs1799724 C > T, rs1800629 G > A), IL10 (rs1800782 T > G), IFN-γ (rs2069722 G > A, rs2069723 T > C, rs121913163 T > C), IL18 (rs360722 A > G, rs549908 T > G* and *IFN-γR1 (rs11914 A > C, rs1887415 A > G).* The primer for *TNF-α rs1800629* was F: 5´-AGGCAATAG GTTTTGAGGGCCAT-3´; R: 5´ACACTCCCCATCCTC CCTGCT-3´ and the enzyme used for restriction digestion was NcoI. The expected band patterns were AA: 116 PCR fragment, AG: 116, 96, 20 PCR fragments and GG: 96, 20 PCR fragments.

Statistical analysis

Kobo toolbox was used to gather data for this investigation [40]. Data on age, gender, employment, ethnicity, education, smoking status and clinical parameters such as viral loads, full blood count and liver function were collected and entered using a structured questionnaire in the Kobo Toolbox. Baseline features from participants with acute and chronic infections, and HBV-negative controls were compared. Statistical analysis was undertaken using Graph Pad Prism 8 (Prisma, San Diego, California) and STATA, version 18, (StataCorp,

Table 1 Demographic data of study participants

Parameter	Total population n (%)	Acute HBV n (%)	Chronic HBV n (%)	Controls n (%)	*p*-value
Gender					**< 0.001**
Male	102 (44.93)	22 (33.33)	77 (54.61)	3 (15)	
Female	125 (55.07)	44 (66.67)	64 (45.39)	17 (85)	
Ethnic group					0.19
Akan	196 (86.34)	61 (92.42)	120 (85.11))	15 (75.00)	
Ewe	13 (5.73)	4 (6.06)	8 (5.67)	1 (5.00)	
Dagbani	3 (1.32)	0(0.00)	2 (1.42)	1 (5.00)	
Non-Ghanaian	2 (0.88)	0(0.00)	2 (1.42)	0(0.00)	
Ga	2 (0.88)	0(0.00)	2 (1.42)	-	
Ga-Adangbe	3 (1.32)	0(0.00)	1 (0.71)	2 (10.00)	
Others	8 (3.52)	1 (1.52)	6(4.26)	1 (5.00)	
Religion					0.84
Christian	209 (92.07)	60 (90.9)	130 (92.2)	19 (95)	
Muslim	18 (7.93)	6 (9.1)	11 (7.8)	1 (5)	
Age					0.18
1–19	8 (3.54)	3 (4.55)	4 (2.84)	1 (5.26)	
20–39	138 (61.06)	43 (65.15)	85 (60.28)	10 (52.63)	
40–59	64 (28.32)	17 (25.76)	43 (30.5)	4 (21.05)	
60–79	15 (6.64)	2 (3.03)	9 (6.38)	4 (21.05)	
80 +	1(0.44)	1 (1.52)	0(0.00)	0(0.00)	
Missing	1			1	
Marital status					0.53
Cohabiting	3 (1.33)	0(0.00)	2 (1.42)	1 (5.26)	
Divorced	7 (3.11)	1 (1.54)	6 (4.26)	0(0.00)	
Married	118 (52.44)	32 (49.23)	75 (53.19)	11 (57.89)	
Single	91 (40.44)	29 (44.62)	55 (39.01)	7 (36.84)	
Widowed	6 (2.67)	3 (4.62)	3 (2.13)	0(0.00)	
missing	2	1		1	
Educational level					**< 0.001**
JHS	42 (19.27)	8 (12.31)	29 (21.64)	5 (26.32)	
No formal education	15 (6.88)	8 (12.31)	4 (2.99)	3 (15.79)	
Primary	12 (5.5)	-	6 (4.48)	6 (31.58)	
SHS	51 (23.39)	19 (29.23)	29 (21..64)	3 (15.79)	
Tertiary	96 (44.04)	30 (46.15)	64 (47.76)	2 (10.53)	
Other	2 (0.92)	-	2 (1.49)	-	
Missing	9	1	7	1	

Others: Moose, Bimbo, Hausa and Dagare

College Station, Texas, USA). Median and Interquartile ranges were computed for the liver enzymes and viral loads between acute, chronic and controls. The Wilcoxon signed and Kruskal Wallis tests were used to test for non-parametric data. Genotype and allele frequencies were calculated using ShesisPlus. SNPs were tested for departure from Hardy–Weinberg Equilibrium (HWE) using a chi-square goodness of fit test. The web based tool LDlink [41] and Shesis [42] Univariate and Multivariate logistic regression analysis were done using the SNPs of the study and age, duration of diagnosis, treatment, age and gender as covariates.

Table 2 Clinical and Anthropometric characteristics of participants

Parameter	Total population n (%)	Acute HBV n (%)	Chronic HBV n (%)	Controls n (%)	*p*-value
Healthy HbsAg carrier	137 (60.35)	43(65.15)	94(66.67)	0(0.00)	**< 0.001***
Late incubation period	47 (20.7)	17(25.76)	30(21.28)	0(0.00)	
Persistent carrier state	21 (9.25)	6 (9.09)	15 (10.64)	0(0.00)	
Resolved	1 (0.44)	0(0.00)	1 (0.71)	0(0.00)	
Possible resolution of CHBV	1 (0.44)	0(0.00)	1 (0.71)	0(0.00)	
HBV-negative control	20 (8.81)	0(0.00)	00(0.00)	20 (100.00)	
Cigarette usage					0.44
Never used	221 (97.36)	66 (100)	135 (95.74)	20 (100)	
Irregularly used	4 (1.76)	_0(0.00)	4 (2.84)	0(0.00)	
Regularly used	2 (0.88)	_0(0.00)	2 (1.42)	0(0.00)	
Alcohol usage					0.14
Never used	172 (76.79)	46 (71.88)	106 (75.71)	20 (100)	
Irregularly used	43 (19.20)	14 (21.88)	29 (20.71)	0(0.00)	
Regularly used	9 (4.02)	4 (6.25)	5 (3.57)	0(0.00)	
BMI					**< 0.001***
Underweight	15 (11.11)	6 (9.23)	15 (11.11)	1 (5.26)	
Normal	57 (42.22)	32 (49.23)	57 (42.22)	7 (36.84)	
Overweight	34 (25.19)	13 (20.0)	34 (25.19)	3 (15.79)	
Obese	29 (21.48)	14 (21.54)	29 (21.48)	8 (42.11)	
FIB-4 Score					
0–1.30		6 (12.50)	11 (11.22)	6 (42.85)	**0.008**
1.31–2.67		15 (31.25)	25 (25.51)	2 (14.28)	
> 2.67		27 (56.25)	62 (63.27)	6 (42.86)	
Medication					**0.01***
TDF	23(11.17)	3 (4.55)	20 (14.29)	0(0.00)	
Levolin	2 (0.97)	_0(0.00)	2 (1.43)	0(0.00)	
Lamivudine	1 (0.49)	_0(0.00)	1 (0.71)	0(0.00)	
No treatment	180 (87.38)	63 (95.45)	117 (83.57)	0(0.00)	
HBV Herbal groups					0.72
Lev 52	2 (0.99)	_0(0.00)	2 (1.46)	0(0.00)	
COA mixture	6 (2.69)	1 (1.52)	5 (3.65)	0(0.00)	
Power herbal	2 (0.99)	_0(0.00)	2 (1.46)	0(0.00)	
Others	8 (3.92)	2 (3.04)	5 (3.65)	0(0.00)	
None	185 (91.13)	63 (95.45)	122 (89.05)	0(0.00)	
HBV status of partner					0.67
No	47 (22.6)	13 (19.70)	34 (24.11)	0(0.00)	
Yes	161 (77.4)	53 (80.30)	107 (75.89)	0(0.00)	
Liver disease					0.06
No	163 (78.74)	54 (81.82)	109 (77.3)	0(0.00)	
Unknown	28 (13.53)	11 (16.67)	17 (12.06)	20 (100)-	
Yes	16 (7.73)	1 (1.52)	15 (10.64)	0(0.00)	

TDF Tenofovir disoproxil fumarate, *BMI* Body mass index

*$p < 0.05$ statistically significant

Results

This study included a total of 227 participants made up of individuals diagnosed with acute and chronic HBV infection and HBV-negative controls. Of the 227, 125 (55%) were females whiles 102 (45%) were males. Participants from the Akan tribe comprised 88% of the overall numbers. Approximately 62% of overall participants had been diagnosed with chronic HBV infection with 29% being diagnosed with acute HBV infections whiles 8% were HBV-negative participants. The demographics of the study participants are displayed in Table 1.

Table 2 shows the clinical and anthropometric characteristics of study participants. Of those with HBV infection, 60% were classified as Healthy HbsAg carriers. The participants were HBV positive but had no symptoms or history of chronic liver disease, normal AST levels and no detectable levels of HbeAg or HBV DNA in serum. Only 12% were on treatment with tenofovir disoproxil fumarate (TDF), lamivudine or levolin. Among HBV-positive participants, 9% were on some form of herbal treatment.

To ascertain the biochemical characteristics of patients to determine extent of liver damage by the HBV infection, AST, ALT and VL levels were measured. The median AST levels for acute HBV infected individuals were higher than both chronic HBV infected individuals and HBV-negative controls ($p < 0.001$) (Table 3) although they were still within the acceptable reference ranges. However, the median viral load for chronic HBV infected individuals was 6620 (3466 -30111) IU/mL in comparison to acute HBV infected individuals 4386 (2674.50–6482) IU/mL, although it was not statistically significant.

Using a selection criteria of HBV DNA levels, gender, age and availability of clinicodemographic data, a total of 175 samples were selected for further genotyping. Genotype frequency distribution of the cytokines is presented in Table 4. All polymorphisms were in HWE equilibrium except *rs1479922 T>C* and *rs11914 A>C* that exhibited departure ($p < 0.05$).

Variant allele frequencies (VAF) were compared between this study, other Africans, East Asians, South Asians, Americans and Europeans as reported in dbSNP (https://www.ncbi.nlm.nih.gov/snp/) (supplementary Table 1). This implies that the studied variations were adequately represented in our population.

The effect of cytokine polymorphisms on HBV DNA levels is shown in Fig. 1. Variations in *IL2 rs1479920 A>G* saw a reduction in HBV DNA levels. The median HBV DNA for GG genotype (1258.93; 0.00–5011.87) IU/mL was lower than AG (3548.13; 0.00–6309.57) IU/mL and AA (5011.87; 2113.49–5956.62) IU/mL genotypes. The was a significant difference in HBV DNA among *IL2 rs1479921 A>G* variant carriers ($p = 0.0424$). Carriers of the AA genotype (1258.93;0.00–4731.51) IU/mL, had lower HBV DNA than AG (3548.13; (0.00–6309.57) IU/mL and GG (5011.87;2818.38–39810.72) IU/mL carriers. However, *IL2 rs1479922 C>T* carriers of the homozygous variant allele TT (5011.87; 2113.49–5956.62) IU/mL had higher HBV DNA than CT (3548.13;0.00–5308.84) IU/mL and CC (1258.93; 0.00- 5011.87) IU/mL carriers. *TNF-α rs1800629 AA* carriers (1258.93; 0.00–3981.07) IU/mL had a reduction in HBV DNA levels in comparison to *TNF-α rs1800629 GG* carriers (1584.89; 0.00–5011.87) IU/mL. Significance was again observed in HBV DNA levels among *TNF-α rs179924C>T variant carriers* ($p = 0.0156$) with lower HBV DNA levels for CC carriers (1258.9; 0.00–5011.99) and elevated for CT carriers (5011.99;22.39–35481.00). Similar patterns are observed in the other cytokine variations where carriers of heterozygous and homozygous variations have alternating patterns of HBV DNA levels.

Univariate and multivariate analysis of factors including variations in cytokines on HBV DNA levels and higher FIB-4 score is shown in Table 5. In the univariate model there was an association for a carrier of *TNF-α rs1800629 AA* genotype to FIB score (*OR = 0.08, 0.01–0.93; p = 0.043*) with reduced odds. The multivariate model

Table 3 Biochemical characteristics of participants

Parameter	n (%)	Acute Median (IQR)	Chronic Median (IQR)	Controls Median (IQR)	*p*-value
AST U/L					
male		33.4 (25.6—38.9)	27.3 (22.5—36)	13.5 (12.2—21.6)	0.06
female		27.3 (16.3—35.8)	26.4 (20.7—38)	21.8 (17.6—23.9)	0.33
ALT U/L					
male		8.65 (6.5—13.9)	9.3 (5.85—15)	10.2 (7.1—12.9)	**< 0.001***
female		5.9 (2.8—10.3)	7.45 (4.2—11.6)	11.2 (9—14.6)	**< 0.001***
VL IU/mL					
≤ 2000	186 (89.86)	29.3 (0–708)	0 (0—480)	n/a	0.44
> 2000	21 (10.14)	4386 (2674.50–6482)	6620 (3466 -30111)	n/a	0.24

AST Aspartate aminotransferase, *ALT* Alanine transaminase, *VL* Viral load n/a = not applicable

*$p < 0.05$ statistically significant

Table 4 Genotypic distribution of cytokine polymorphisms

	***Acute HBV* (*n* = 52)**	***Chronic HBV* (*n* = 104)**	***HBV-negative Control* (*n* = 20)**	***p*-value**
SNP ID	*Genotype, n (freq)*			
IL2				
rs1479920 A > G				
GG	36 (0.69)	89 (0.86)	17 (0.85)	0.089
AG	13 (0.25)	14 (0.13)	3 (0.15)	
AA	3 (0.06)	1 (0.01)	0 (0.00)	
HWE p^{∇}	0.127			
rs1479921A > G				
AA	36 (0.69)	89 (0.86)	17 (0.85)	0.089
AG	13 (0.25)	14 (0.13)	3 (0.15)	
GG	3 (0.06)	1 (0.01)	0 (0.00)	
HWE p^{∇}	0.127			
rs1479922 T > C				
CC	37 (0.71)	93 (0.89)	17 (0.85)	**0.044***
TC	12 (0.23)	10 (0.10)	3 (0.15)	
TT	3 (0.06)	1 (0.01)	0 (0.00)	
HWE p^{∇}	**0.030***			
TNF-α				
rs1799724 C > T				
CC	50 (0.96)	100 (0.96)	20 (1.00)	1.000
TC	2 (0.04)	4 (0.04)	0 (0.00)	
TT	0(0.00)	0(0.00)	0(0.00)	
HWE p^{∇}	0.818			
rs1800629 G > A				
GG	34 (0.65)	74 (0.71)	13 (0.65)	0.862
AG	17 (0.33)	28 (0.27)	7 (0.35)	
AA	1 (0.02)	2 (0.02)	0 (0.00)	
HWE p^{∇}	0.330			
IL10				
rs1800872 T > G				
GG	23 (0.45)	28 (0.27)	5 (0.25)	0.133
GT	22 (0.43)	49 (0.47)	11 (0.55)	
TT	6 (0.12)	27 (0.26)	4 (0.20)	
HWE p^{∇}	0.544			
IFN-γ				
rs2069722 G > A				
GG	37 (0.71)	91 (0.88)	17 (0.85)	**0.048***
AG	15 (0.29)	13 (0.12)	3 (0.15)	
AA	0(0.00)	0(0.00)	0(0.00)	
HWE p^{∇}	0.200			
rs2069723 T > C				
TT	46 (0.88)	97 (0.93)	19 (0.95)	0.596
TC	6 (0.12)	7 (0.07)	1 (0.05)	
CC	0(0.00)	0(0.00)	0(0.00)	
HWE p^{∇}	0.583			
rs121913163 T > C				
TT	52 (1.00)	104 (1.00)	20 (1.00)	N/A
TC	0(0.00)	0(0.00)	0(0.00)	
CC	0(0.00)	0(0.00)	0(0.00)	

Table 4 (continued)

	Acute HBV (*n* = 52)	*Chronic HBV* (*n* = 104)	*HBV-negative Control* (*n* = 20)	*p*-value
HWE p^{∇}				
IFN-γR1				
rs11914 A > C				
AA	44 (0.85)	97 (0.93)	19 (0.95)	0.388
CA	7 (0.14)	6 (0.06)_	1 (0.05)	
CC	1 (0.02)	1(0.01)		
HWE p^{∇}	**0.017***			
rs1887415 A > G				
AA	49 (0.94)	102 (0.98)	19 (0.95)	0.355
AG	3 (0.06)	2 (0.02)	1 (0.05)	
GG	0(0.00)	0(0.00)	0(0.00)	
HWE p^{∇}	0.818			
IL18				
rs360722 A > G				
GG	17 (0.33)	47 (0.46)	6 (0.30)	0.201
GA	30 (0.58)	41 (0.39)	11 (0.55)	
AA	5 (0.01)	16 (0.15)	3 (0.15)	
HWE p^{∇}	0.998			
rs549908 T > G				
TT	42 (0.81)	76 (0.73)	14 (0.70)	0.663
GT	10 (0.19)	26 (0.25)	6 (0.30)	
GG	0 (0.00)	2 (0.02)	0 (0.00)	
HWE p^{∇}	0.505			

HWE $p^{\nabla} < 0.05$ = Deviation from Hardy-Weinberg

*$p < 0.05$ statistically significant

further confirmed the association with reduced odds (*OR = 0.02, 0.00–0.67; p = 0.029*). There was an association and higher odds of age ranges of 20–39 (*OR = 5.00, 1.13–6.10; p = 0.034*) and 40–59 years (*OR = 41.99, 3.74–47.21; p = 0.0002*) of having higher FIB-4 scores in the univariate model. Being female (*OR =. 2.42, 1.03–5.71; p = 0.043*) in the univariate models showed higher odds of having high levels of HBV DNA in the multivariate model. There was a reduced likelihood of herbal medicine usage influencing HBV DNA levels significantly (*OR = 0.29, 0.10–0.86; p = 0.025*).

Analysis of LD on the *IFN-γR1 rs11914, IFN-γ rs121913163, IFN-γR1 rs1887415, IFN-γ rs2069722* and *IFN-γ rs2069723* was undertaken as show in Fig. 2. There was strong LD between *rs11914, rs2069723, rs1887415* and *rs2069723* (D' = 0.99-

The LD for *IL2 rs1479920, rs1479921* and *rs1479922* is shown in Fig. 3. Strong LD was again observed for all three variants with D' values at 0.99 and R' values from 0.88–0.99. These means the variations are linked and could be inherited together and have some associated functional effects.

Discussion

The objective of this work was to study the association of polymorphisms of genes coding for cytokines IL-2, IL-10, IL-18, INF-γ, and TNF-α with plasma HBV DNA level and FIB score in patients with HBV infection in Ghana. Irrespective of drug treatment, HBV infection can lead to a range of clinical outcomes, from viral clearance to infection persistence resulting in chronicity and complex liver disorders. Factors that influence hepatitis outcomes include viral, environmental, co-infections, lifestyle and host genetics. Due to the important role of cytokines in immunomodulation, some studies have looked at the role of SNPs in influencing the activities of interleukins (*IL2, IL4, IL10, 1L18*), *TNF-α* and *IFNγ* [43–46].

This study focused on thirteen [13] SNPs in cytokines including *IL2, IL10, IL18, TNF-α, IFN-γ* and *IFN-γR1*. The effect of the cytokines polymorphisms was compared in terms of HBV DNA where it was found out that some carriers of the variant alleles in for example *IL2 rs1479920 GG* had lower median HBV DNA than their homozygous

wildtype *AA* and *AG* allele carriers respectively. *IL2* contributes to HBV infection by boosting host immunological activity [47, 48] so this reduction in HBV DNA due to the *IL2 rs1479920 GG* variation may augur well for carriers of this genotype. The variation is an intronic variant which may affect function and therefore carriers of this variant may be offered a certain protective effect. This is also observed in reduced median HBV DNA of *AG* carriers compared to *AA*. There was variable HBV DNA levels in relation to being a carrier of *IL10, IL18, TNF-α, IFN-γ* and *IFN-γR1* variation. Although, there was an observed significance in HBV DNA levels ($p=0.0424$) among carriers *IL2 rs1479921A > G* variants, those with AA genotypes still had lower HBV DNA levels in comparison with their *GA* and *GG* genotypes. Although no studies have reported this level of significance, it has been previously reported that IL2 may have a heterogenous effect in immunomodulation and genetic variation may further influence its effect and function [24]. On another hand, there was a significant difference in the HBV DNA levels ($p=0.0156$) of *TNF-α rs1799724 C > T (-857C/T)* carriers. The promoter polymorphisms at *-857C/T* regulate TNF-α production thus affecting immune regulation. There is also a relationship between TNF-α and HLA II expression which facilitates viral antigen presentation [49]. This could account for the differences in the HBV DNA levels as this promoter polymorphism could influence the levels TNF-α subsequently HLA. Previous studies and meta-analysis have shown that *TNF-α rs1799724 C > T* variations were markedly related and associated with HBV infection and persistence [50–52]. Variations that exists in cytokines may offer protective or deleterious effects and for this study, the differences in HBV DNA observed when comparing the various studied cytokines supports the body of evidence that variations in cytokines can influence viral DNA levels and persistence and possibly cirrhosis [53–58].

TNF-α is produced by macrophages, monocytes, neutrophils, T-lymphocytes and NK cells. It is an important stimulatory factor that promotes the secretion of other cytokines and expression of adhesion molecules on endothelial cells [59, 60]. The study observed an

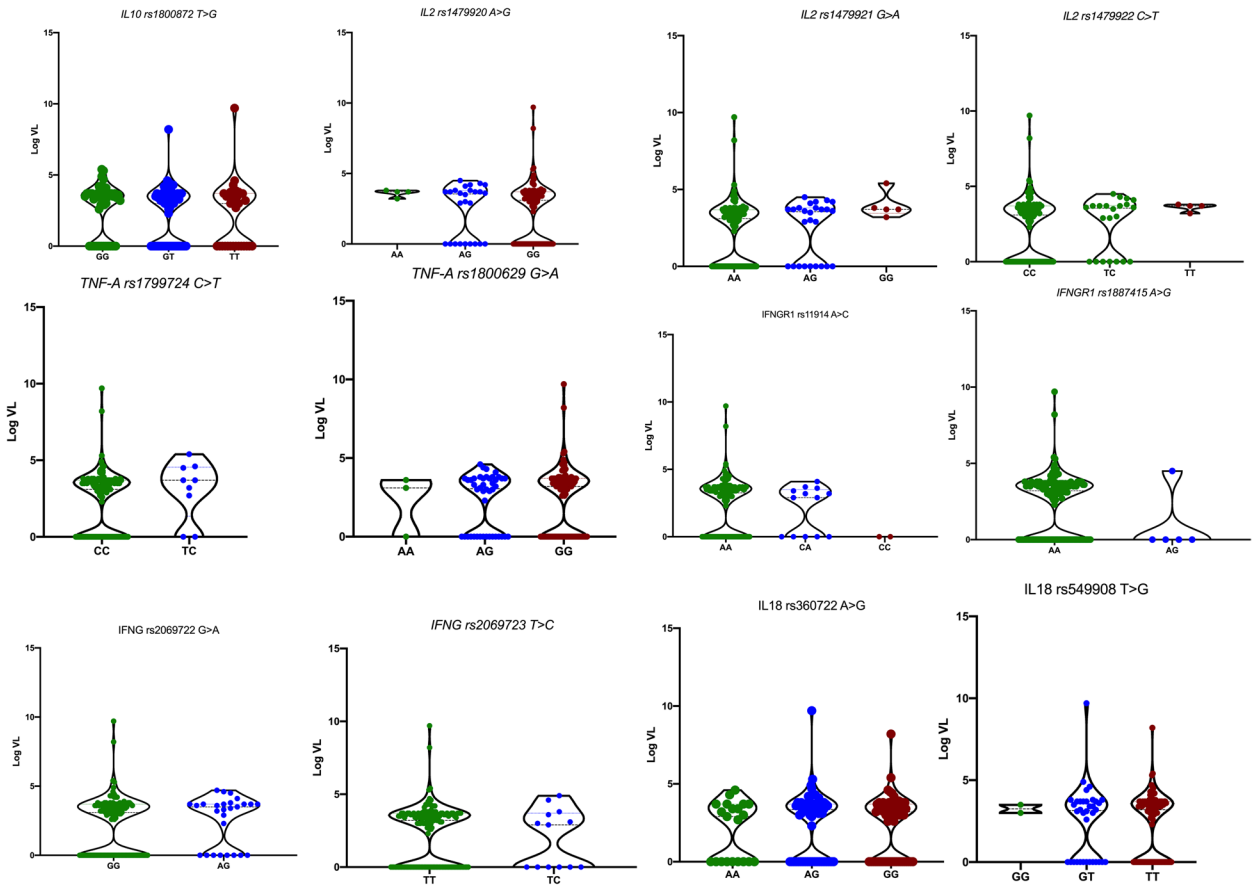

Fig. 1 Effect of cytokine variations on HBV DNA levels in HBV-positive participants. There was a significant difference in HBV DNA levels among *IL2 rs1479921 G > A* ($p=0.0424$) and *TNF-α rs179924C > T* ($p=0.0156$) variant carriers

Table 5 Association of Genetic Polymorphisms and Clinical Factors with HBV DNA Levels and FIB Score in Patients

	HBV DNA				FIB Score			
Factor	**COR (95% CI)**	***p*-value**	**AOR (95% CI)**	***p*-value**	**COR (95% CI)**	***p*-value**	**AOR (95% CI)**	***p*-value**
IFNGR1 rs11914 A > C								
AA (ref)							1	
CA	0.92 (0.28–2.95)	0.884	2.32 (0.46–11.76)	0.309	0.76 (0.15–3.81)	0.742	1.54 (0.07–33.87)	0.787
IL2 rs1479920 A > G								
GG (ref)					1			
AG	1.23 (0.51–2.99)	0.641	1.05 (0.13–8.64)	0.967	2.07 (0.45–9.61)	0.349	0.14 (0.01–2.69)	0.191
IL2 rs1479921 A > G								
AA	1				1		-	-
AG	1.23 (0.51–2.99)	0.641			2.07 (0.45–9.61)	0.349	-	-
IL2 rs1479922 T > C								
TT	1		1		1		1	
TC	1.40 (0.53–3.67)	0.493	1.26 (0.17–13.56)	0.851	3.8 (0.49–30.34)	0.202	1.3 (0.39–4.58)	0.152
TNF-α rs1799724 C > T								
CC (ref)	1				1	-	-	-
TC	1.24 (0.22–7.02)	0.804	0.45 (0.02–7.71)	0.585	1	-	-	-
TNF-α rs1800629 G > A								
GG (ref)	1				1			
AG	1.07 (0.52–2.22)	0.853	0.74 (0.28–1.95)	0.548	0.97 (0.34–2.74)	0.948	1.36 (0.23–8.03)	0.734
AA	1.22 (0.10–3.93)	0.871	0.93 (0.5–1.72)	0.960	0.08 (0.01–0.93)	**0.043***	0.02 (0.00–0.67)	**0.029***
IL10 rs1800872 T > G								
GG (ref)	1				1			
GT	0.70 (0.33–1.50)	0.361	1.04 (0.41–2.66)	0.939	1.48 (0.48–4.53)	0.497	3.05 (0.46–20.03)	0.245
TT	0.59 (0.24–1.47)	0.255	0.66 (0.19–2.36)	0.535	0.53 (0.16–1.70)	0.282	0.27 (0.03–1.84)	0.180
IFN-γR1 rs1887415								
AA	1				1		1	
AG	0.14 (0.02–1.29)	0.083	-		0.32 (0.056–1.90)	0.212	0.12 (0.00–3.79)	0.231
IFN-γ rs2069722 G > A								
GG	1				1		1	1
AG	1.49 (0.60–3.67)	0.388	1.75 (0.54–5.66)	0.348	1.49 (0.60–3.67)	0.388	3.24 (0.27–3.94)	0.356
IFN-γ rs2069723 T > C								
TT (ref)	1				1		1	
TC	0.66 (0.21–2.07)	0.478	0.36 (0.09–1.42)	0.146	1.80 (0.22–14.86)	0.585	0.81 (0.04–14.81)	0.890
IL18 rs360722 A > G								
GG (ref)	1				1			
GA	0.95 (0.47–1.92)	0.885	0.77 (0.30–1.96)	0.589	0.62 (0.23–1.74)	0.366	2.70 (0.53 -13.87)	0.233
AA	0.75 (0.27–2.04)	0.571	0.43 (0.11–1.65)	0.219	0.70 (0.22–1.74)	0.639	1.21 (0.13–11.55)	0.866
IL18 rs549908 T > G								
TT (ref)	1				1			
GT	1.12 (0.51–2.40)	0.799	1.64 (0.55–4.90)	0.373	1.25 (0.39–4.02)	0.711	2.21 (0.28–2.76)	0.453
Treatment								
TDF (ref.)	1		1		1			
No treatment	1.07 (0.45–2.55)	0.877	0.48 (0.10–2.16)	0.335	1.07 (0.45–2.55)	0.877	-	-
Age (years)								
1–19 (ref.)	1				1			
20–39	0.83 (0.24–2.87)	0.773	0.83 (0.12–5.65)	0.847	5.00 (1.13–6.10)	**0.034***	19.83 (0.89–23.54)	0.060
40–59	1.17 (0.32–4.25)	0.815	1.26 (0.16–9.73)	0.826	41.99 (3.74–47.21)	**0.002***	2.47 (0.50–4.77)	**0.11***
Gender								
Male (ref.)	1		1		1			
Female	1.55 (0.89–2.68)	0.121	2.42 (1.03–5.71)	**0.043***	0.80 (0.32–2.00)	0.633	0.56 (0.14–2.28)	0.45
Herbal Medicine Usage								
No	1		1		1		-	-
Yes	0.29 (0.10–0.86)	**0.025***	0.22 (0.01–3.92)	0.303	0.26 (0.02–3.01)	0.280	-	-

Abbreviations: COR Crude odds ratio, *AOR* Adjusted odds ratio, *95% CI* 95% confidence interval, Crude and adjusted odds ratios (OR) and 95% confidence intervals (variables found to be associated through a univariate analysis were entered into the multivariate model)

* Significant association ($P < 0.05$)

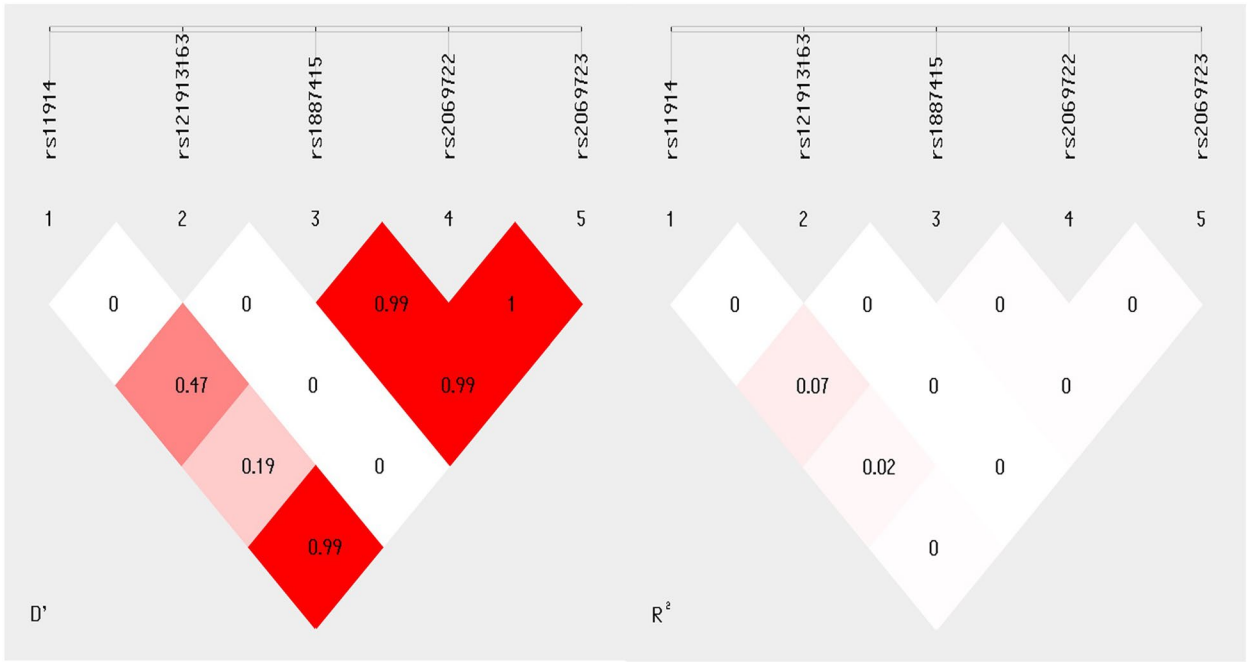

Fig. 2 Linkage disequilibrium (L.D) plot of *IFN-γR1 rs11914, IFN-γ rs121913163, IFN-γR1 rs1887415, IFN-γ rs2069722* and *IFN-γ rs2069723.* Legend: *For the pair-wise LD association between five SNPs and the corresponding D' and R' values, the colour gradient from red to white reveals higher to lower LD (D' 1–0; R' 1–0)*

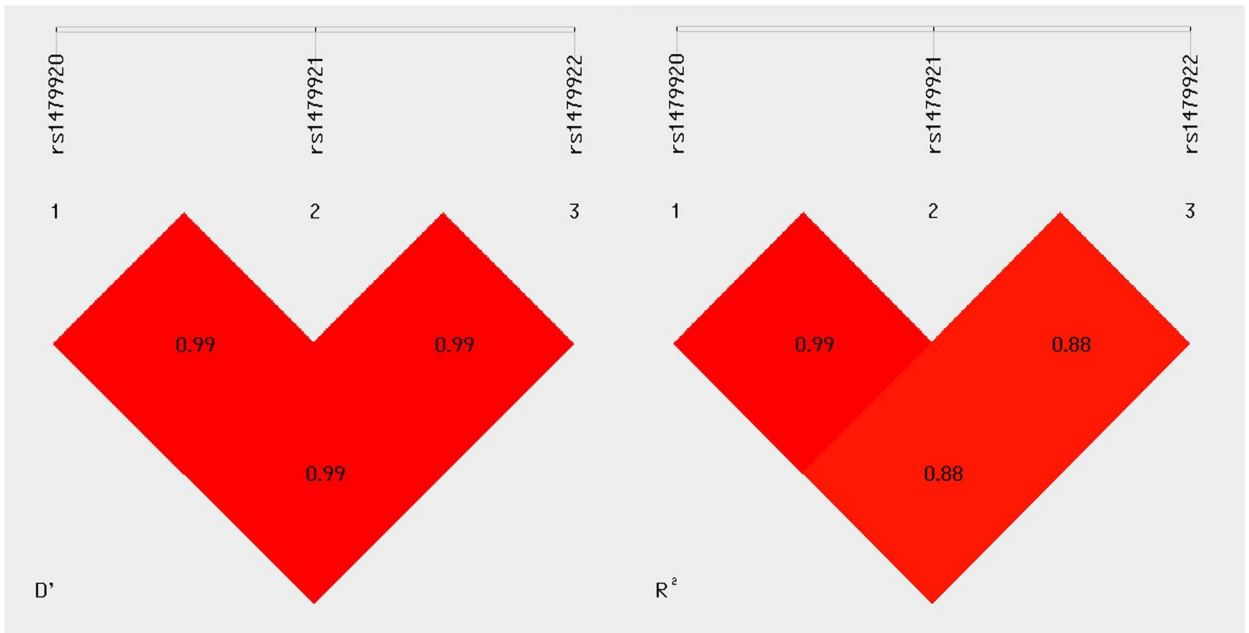

Fig. 3 Linkage disequilibrium (LD) plot of *IL2 rs1479920, rs1479921* and *rs1479922* and observed D' and R' values (Fig. 4.6). The pair-wise LD association between three SNPs and the corresponding D' and R' values

association between *TNF-α -308A/G* (*rs1800629)* and FIB-4 score which is a non-invasive indicator of liver scarring. Carriers of *AA* genotype had a reduced likelihood of getting a higher FIB-4 score in both univariate (OR 0.08; 0.01–0.93; p = 0.043) and multivariate (OR 0.02; 0.03–1.84; $p = 0.029$) models. This could mean that the presence of the *-308A* allele is associated with a better prognosis of liver scaring and potentially other complications. This finding though showing an association between *TNF-α -308A/G* (*rs1800629)* and the non-invasive FIB-4 score is similar to a study that showed that *TNF-α -308A/G* is an independent risk factor for hepatocellular carcinoma [61]. *TNF-α* plays a role in the inflammatory process in the liver and in HBV infection, excessive *TNF-α* production triggers inflammation and subsequent death of liver cells which increases the development of liver connective tissue. If this process continues, even healthy liver cells may be damaged and replaced by connective tissue. What this means for this study therefore is that for carriers of *A* allele, the rate of liver inflammation in such HBV positive persons may be reduced reducing the risk of liver scarring. Though other findings have showed an increased likelihood of liver related complication such as HCC, the findings from this study showed a reduced likelihood. This further underscores the need to consider ethnic background of populations under study in such analysis. It will, however, be important to undertake further studies on this relationship in future studies among Ghanaians.

Other variations in the univariate and multivariate analysis such as *IL2 rs1479920 AG, IL2 rs1479921 AG, IL2 rs1479922 TC, TNF-α -308A/G* and *TNF-α -857C/T* showed increased likelihood of HBV-positive persons having high HBV DNA although the association was not significant. Such patterns of increased likelihood of having high FIB-4 scores were found in a non-significant association with *IL2 rs1479920 AG, IL2rs1479921 AG, IL2 rs1479922 TC, IL10 rs1800872GT,* and *IFN-γ rs2069722AG.* Previous studies had shown that some of these variations may offer protective effects in HBV infection [43, 62, 63]. These cytokines may also contribute to liver scarring and HBV DNA progression through immune dysfunction where liver inflammation can cause HBV associated complications.

The lack of comparable numbers for acute, chronic, and HBV-negative controls is one of the limitations of this study. This was because we were undertaking random recruitment without targeting specific patients. This led to different numbers although all analyses undertaken arestill valid. Another limitation is that not all recruited persons were genotyped due to availability of resources. However, selection of genotyped samples was done to represent all aspect of available data.

Conclusion

Variations in some cytokines such as *IL2 rs1479920 GG* and *IL2 rs1479921 AA* could offer protective effects by reducing HBV DNA. *TNF-α rs179924CT* may also cause an elevated HBV DNA level in HBV infected persons leading to persistence. *TNF-α -308A/G* showed a protective effect on liver scarring in HBV infected patients. The observation from the study showing that *TNF-α -308A/G* could offer a potential protective effect against liver damage in HBV-positive patients advances the overarching sustainable development goals (SDG) objective of hepatitis prevention. Through the identification of genetic markers that impact the course of the disease and the effectiveness of therapy, focused medical treatments and public health measures may be developed, ultimately lowering the prevalence of HBV and enhancing health outcomes as stated in SDG3.3. What this means for this study therefore is that for carriers of A allele, the rate of liver inflammation in such HBV positive persons may be reduced reducing the risk of liver scarring. Therefore, it will be important to undertake further studies on this relationship in future studies within the Ghanaian population.

Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

NET conceived the idea, supervised and secured funding for the project. NET, FA,ASO and SBN were involved in recruitment, data retrieval, laboratory analysis. NET, EAB, CK, AA and ROA performed analysis. All authors contributed to the writing of the manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Raw data has been deposited in GEO submission with accession number GSE274827.

Declarations

Ethics approval and consent to participate

The Cape Coast Teaching Hospital Ethical Review Board Committee granted ethical approval for the research, with reference number CCTHERC/EC/2021/005. Participants who met the inclusion criteria were approached at

the clinic during their visit and informed of the study and those that willing were enrolled. Informed consent was obtained from all participants. In addition, both verbal and/or written consent was sought for. Unique codes were used for participants to ensure confidentiality and anonymity. The study was undertaken in accordance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interest
The authors declare no competing interests.

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