Original article

GUT MICROBIOTA DYSBIOSIS, ENDOTOXIGENIC INDEX, ATHEROGENIC INDEX OF PLASMA, DYSLIPIDEMIA, LIVER ENZYMES IN CHRONIC HEPATITIS B VIRUS: A CASE CONTROL STUDY

Authors: Raymond E. Eworo[1]*, Delia D. Johnson[2], Unwana C. Usoro,[3] Nathaniel E. Usoro[4], Augusta C. Nsonwu-Anyanwu [5],

[1,2,5]*Department of Clinical Chemistry & Immunology, University of Calabar, Nigeria.*

[3,4] *Faculty of Clinical Sciences, University of Calabar, Nigeria*

***Corresponding Author:** Raymond Ekong Eworo**,** Department of Clinical Chemistry & Immunology, University of Calabar, Cross River State, Nigeria. **E-mail:** raymondeworo@yahoo.com; raymondeworo@unical.edu.ng. Orcid ID: 0000-0003-2139-665X. **Telephone:** +234 8069363545

ABSTRACT

Context: Gut microbiota dysbiosis has been linked with chronic inflammation, dyslipidemia and progression of liver damage in chronic hepatitis B virus (CHBV). **Aim:** This study aimed to evaluate serum endotoxin (LPS), lipid profile, liver enzymes and platelets count (PLT) in subjects with CHBV. **Settings and design:** This case-control study was conducted to assess the impact of gut microbiota dysbiosis on dyslipidemia in subjects with CHBV attending a Tertiary Health Care Facility. **Materials and Methods:** The study enrolled 40 subjects with CHBV, liver fibrosis (LF) 20, and 40 controls. The LPS was determined by ELISA. Liver enzymes, lipids and PLT by spectrophotometry and Automated Haematology Analyzer respectively. Low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, endotoxigenic index (EI) and atherogenic index of plasma (AIP) were computed. **Data analysis:** Data were analyzed using ANOVA, Turkey-HSD post hoc analyses, receiver operator characteristic curve and Pearson's correlation, at $p<0.05$. **Results:** Liver enzymes, lipids, LPS, EI and AIP significantly increased while PLT significantly reduced in LF, CHBV than in controls ($p<0.05$). Liver enzymes and LPS significantly increased in dyslipidemia than in CHBV with normal lipid levels ($p<0.05$). The EI correlated positively with AIP ($r=0.559$, $p=0.000$). The ROC of EI and ALT showed AUC (1.000

and 0.916), maximum Kolmogorov-Smirnov metrics (1.000 and 0.717), sensitivity (100% and 92%) and specificity (100% and 80%) respectively. **Conclusion:** This study suggests that gut dysbiosis, dyslipidemia, EI, and AIP may be useful adjunctive markers in evaluating CHBV severity and progression, but further longitudinal and mechanistic studies are needed.

Key word: Gut microbiota dysbiosis, chronic hepatitis B virus, dyslipidemia, endotoxigenic index, liver fibrosis

Introduction

The gut microbiota is a complex and dynamic consortium of microbial conformation in the gut. Chronic HBV infection can interrupt the balance of the gut microbiota, resulting in dysbiosis and the release of lipopolysacharride (LPS) endotoxins. Various lipid fractions and LPS have significant immunomodulatory effects. 'Endotoxigenic index' (EI) (a measure of the balance between endotoxin production and HDL-C that binds and neutralize endotoxin) and Atherogenic Index of Plasma (AIP) may be useful in assessing the impact of microbiota dysbiosis and dyslipidemia on the severity of CHBV and its progression. The configurational and functional shift in gut microbial ecology allowing LPS (endotoxins) through the compromised gut barrier into blood stream (endotoxaemia) has been linked recently to dyslipidemia and chronic hepatitis B related liver complications.[1] .

Chronic hepatitis B virus (HBV) infection is one of the most severe public health concern affecting about 296 million people worldwide and is the leading aetiology of cirrhosis and liver cancer globally and the principal cause of liver cirrhosis and hepatocellular carcinoma (HCC) the second leading cause of cancer-associated deaths globally.[2,3] The need for clarification of the mechanisms that regulate HBV replication, persistence and the development of HBV-associated liver complications is timely.[4,5] Hepatitis B virus (HBV) is considered a "metabolovirus" that affects many hepatic metabolic pathways. The connection between chronic hepatitis B virus infection, liver fibrosis, dyslipidemia and gut microbiota dysbiosis is not fully understood. However, studies have established that the composition of gut microbiota changes at different phases of HBV-related liver disease progression. [6,7]. Thus, gut microbiota transformation and the associated dyslipidemia may constitute a natural aspect in the progression of liver damage. [8,9] Mechanisms of HBV-induced LF and HCC have been proposed involving changes in gut microbiome and an increased gut lipid absorption.[10] Lately, many diseases have been reported to be regulated by processes in the gut microbiome.[11] Studies aimed at slowing the progression of liver diseases via the modulation of the gut microbiome demonstrated that changes in the gut microbial ecosystem lead to disease

progression or regression.[12-14] Patients with chronic hepatitis B virus related liver cirrhosis have a reformed gut-liver axis and systemic inflammation that relate with severity of liver disease, gut barrier damage and modifications in the composition and function of gut microbiota.[15]

Endotoxins are among the best characterized pathogen-associated molecular patterns "recognized" by Toll-like receptor (TLR4).[16] Pathogen-associated (LPS) and damage-associated (high mobility group box 1 (HMGB1) are molecular patterns recognized by TLRs, a type I transmembrane glycoprotein. [17, 18] The TLR4 is part of the receptor complex that binds LPS. Toll-like receptor (TLR) plays an important role in the pathogenesis and advancement of several chronic liver diseases, including non-alcoholic fatty liver disease, CHBV, liver fibrosis and HCC.[19] The HBV-infected HepG2 cells can increase the expression of genes connected to cholesterol metabolism, and upsurge the hepatic cholesterol level via TLR2.[20, 21] The risk of HCC has been observed to be higher in CHBV patients with NAFLD compared to CHBV patients without NAFLD.[22] It has also been hypothesized that there is reduced bioavailability of entecavir and cytochrome enzymes involved in drug metabolism in fatty hepatocytes of CHBV patients.[23, 24] LPS is known to exert both acute and chronic inflammatory reactions, especially in the gut. The recent appraisal of the bidirectional communication between the liver and the gut (gut-liver axis), has supported a growing consent that human health is intimately related to the gut microbiota. Impaired intestinal barriers and alterations of the gut microbiota result in the release of gut-derived microbial antigens and their translocation into the liver. The gut mucus layer and tight junction proteins preserve the intestinal barrier. Chronic HBV infection causes a decrease in the thickness and the expression of tight junction proteins, leading to a compromised intestinal barrier. The association between gut microbiota adjustments and chronic liver diseases has received great attention recently.[25]

Accumulating evidence suggests that host metabolism plays a pivotal role in viral infections. The mechanisms by which CHBV infection perturbs hepatic lipid metabolism had revealed that HBV replication or expression causes widespread and varied changes in hepatic lipid metabolism, by initiating expression of some important lipogenesis proteins as well as upregulating fatty acid oxidation and bile acid synthesis.[26] Moreover, increasing studies found some potential targets to inhibit HBV replication by reducing or enhancing certain lipid metabolism-related proteins or metabolites and enzymes.[27]

Despite decades of progress with HBV therapy, a sterilizing cure with comprehensive extermination of intrahepatic covalently closed circular DNA (cccDNA) and integrated HBV DNA remains unachieved. Studies have analyzed the gut microbiome at each stage of HBV-induced liver diseases, but a consensus has not been reached on the microbial signatures across these stages and

how HBV upsets lipid metabolism in hepatocytes is yet to be fully appreciated. Indices such as EI; a measure of the log10 of the amount of endotoxin produced to HDL-c that binds and neutralizes them. Also, AIP a measure of log10 of TG to HDL-C is a predictive value for cardiovascular risk associated with endotoxin-related dyslipidemia. Then EI and AIP are novel measures that may serve as adjunctive markers in evaluating CHBV severity and progression. There is scarcity of data highlighting the relationship between dyslipidemia, AIP, EI, and LPS levels in CHBV infection. The study aims to evaluate the impact of gut microbiota dysbiosis on dyslipidemia, severity of CHBV and its progression to poorer outcomes.

Materials and Methods

Study design: This case-control study was conducted among patients with chronic HBV infection. Sixty subjects with CHBV and 40 apparently healthy age-matched participants without HBV infection who served as controls were enrolled for the study. The hepatitis B virus infected subjects were categorized based on the pathologic condition of the liver into those with chronic hepatitis B virus infection (CHBV, n=40), liver fibrosis (LF, n=20).

Study setting

The study was conducted among subjects attending Gastroenterology Clinic in Internal Medicine Department of a Tertiary Health Care Facility with diagnostic and treatment modalities for liver diseases from June 2022 to January 2023.

Sampling technique

The population consists of more than 135 eligible patients attending the clinic, after careful screening with inclusion and exclusion criteria and obtaining consent 60 patients were selected by systematic random method.

Study population

The study population consisted of adult patients (18-65 years) with confirmed diagnosis of CHBV attending the Tertiary Health Care Facility and apparently healthy aged-matched controls residing in the same geographic location were recruited into the study.

Inclusion criteria

Participants included those who were fasting, ages were within the selected age range and those without a history of smoking, alcohol and substance abuse, or gave consent and able to comply with study procedure.

Exclusion criteria

Individuals whose ages were outside the selected age range, those with a history of smoking, alcohol and substance abuse were excluded from the study.

Data collection tool

Socio-demographic data, (age, marital status, education, work), family and medical history of past illness, current medication use, social lifestyle (smoking habit, alcohol use, drug addiction, and substance abuse) were obtained from each participant using a well-structured interviewer

questionnaire by trained healthcare professionals, during clinic visits or hospital admissions. Liver fibrosis was confirmed by ultrasound scan.

Sample collection:

After an overnight fast, a standard venepuncture method was used to obtain 7 mL of blood from all the participants. Three milliliters of blood was dispensed into K_2EDTA samples bottle for platelet estimation and 4mls into plain bottles, allowed to clot and then centrifuged at 3 000 rpm for 5 mins at room temperature. The sera were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20 °C until analyses.

Study size:

Sample size was determined according to the method of Sullivan, using the formula $\frac{(Z_\alpha+Z_\beta)^2.\bar{p}(1-\bar{p})}{(p0-p1)2}$.[29] The power of 0.84 was calculated at beta error of 80%. Zβ= Beta error at 80% power is 0.84

Zα= Z value associated with 95% confidence level is 1.96, ie, (Alpha error at 95% level of confidence)

P0 = The anticipated probability of "exposure for people with the disease" (97%=0.97)

P1= is the proportion/anticipated probability of "exposure for people without the disease" 86% = 0.86 (estimated from the postulated odds ratio)

ṗ=mean proportion between cases and control group (p1+p2)/2 =(0.97+0.86)/2=0.91

d = difference to be detected (p0 – P1) = 0.97-0.86=0.11

The sample size of 60 patients arrived at, while 40 apparently healthy age-matched individuals who served as controls were selected for the study.

Ethical considerations

Ethical clearance was obtained from the University of Calabar Teaching Hospital. Informed consent was obtained from all participants enrolled in the study. Approval number UCTH/HREC/33/VOL.111/040. The research was conducted in compliance to the Helsinki declaration of 1975 concerning research involving human subjects and subsequent revisions. The right to withdraw from participation in the study at any point in time was respect all through the study.

Laboratory Methods

Determination of platelet count

Platelet count was determined by a 5 parts Sysmex XS-1000 haematology automatic analyzer. The Sysmex-XS-1000 can analyze and output the results for 32 parameters of blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five-part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise and precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte

populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells and are easily distinguishable in the DIFF scattergram.

Determination of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Serum ALT and AST were determined according to the method of Reitman and Frankel 1957.

Alanine aminotransferase was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.[29] The AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.[28]

Determination of serum alkaline phosphatase (ALP)

Serum ALP was determined by an optimized standard method according to the recommendation of the Deutsche Gesellschaft fur klinische Chemie. Using Diethanolamine buffered method (pH=10.0)

Estimation of Total Cholesterol and Triglyceride Concentration

Total cholesterol and Triglyceride levels were determined using the respective enzymatic colorimetric methods using test kits obtained from Randox Laboratories Company, UK

Determination of High-Density Lipoprotein Cholesterol Concentration

High density lipoprotein cholesterol was determined using precipitation/cholesterol enzymatic method.

Computation of Very Low-Density Lipoprotein Cholesterol Concentration

The VLDL-cholesterol concentration was calculated from the triglyceride concentration using the formula.

$$\text{VLDL} = \frac{\text{Triglyceride concentration}}{2.2}$$

Calculation of Low-Density Lipoprotein (LDL) Cholesterol Concentration

The LDL cholesterol concentration is calculated from the total cholesterol concentration, HDL-cholesterol concentration and the triglyceride concentration using the Friedewald formula:

LDL - C (mmol/L) = Total cholesterol – (HDL - C + VLDL-C)

Determination of serum lipopolysaccharide

Serum lipopolysaccharide was determined by sandwich ELISA method using a kit obtained from Sunlong Biotech CO., LTD., Zhejiang, Hangzhou, China.

Computation of Endotoxigenic index (EI)

Endotoxigenic index was computed as logarithm to base ten of the ratio of LPS to HDL-C Log10(LPS/HDL-C))

Computation of atherogenic index of plasma (AIP)

Atherogenic index of plasma was computed as logarithm to base ten of the ratio of TG to HDL (Log10(TG/HDL))

Statistical analyses

Results generated were presented as mean ±SD. Data were analyzed using the statistical package for social sciences (SPSS version 27.0, IBM, USA). Students's t-test, Analysis of variance (ANOVA) was used to test the variations within and among group means and Turkey-HSD post-hoc analyses was used for the comparison of multiple groups means. Receiver operator characteristic curve was used to compare the ability of some variable in predicting poorer outcomes in CHBV. Pearson's correlation was used to determine the associations between variables. The normality of data was determined using Shapiro-Wilk and Box and Whisker plots. Data not normally distributed were log-transformed. The confidence interval was set to 95% at $\alpha = 0.05$.

Results

Fifty per cent of the subjects with CHBV were males and fifty were females. Twenty per cent of subjects with liver fibrosis were males while 80% were females. Eighty five percent of the subjects were younger below 50 years while 15% were older 50 years or older

The comparison of age, liver enzymes, platelet count, lipid profile, LPS, AIP and EI in patients with chronic hepatitis B virus infection, liver fibrosis and control is shown in table 1. The liver enzymes, platelet count (PLT), TC, LDL-C, VLDL-C, TG, LPS, AIP and EI varied significantly (95% CI, $p<0.05$) among the groups. The comparison of age, liver enzymes, platelet count, lipid profile, LPS, AIP and EI in patients with CHBV with normal cholesterol level and CHBV with hypercholesterolemia is shown in table 2. The liver enzymes, LDL-C, VLDL-C, TG, LPS, AIP and EI were significantly higher in CHBV with hypercholesterolemia when compared with CHBV with normal cholesterol level, (95% CI, $p<0.05$). A correlation plot between AIP and EI in subjects with CHBV is shown in figure 1. A correlation plot between LPS against low density lipoprotein cholesterol in subjects with CHBV is represented in figure 2. A correlation plot between LPS against total cholesterol in subjects with CHBV is depicted in figure 3. The correlation plot between TG against LPS in subjects with CHBV is shown in figure 4. Box and Whisker plots of EI in subjects with CHBV and LF is shown in figure 5. Receiver operator characteristic curve of variables and their performance metrics is represented in figure 6. Overall model quality of the variables in the ROC is shown in figure 7.

Table 1, The Comparison of age, liver enzymes, platelet count, lipid profile, lipopolysaccharide, endotoxigenic index and atherogenic index of plasma in patients with chronic hepatitis B virus infection, liver fibrosis and control

Parameters	CHBV (n=37)	LF (n=13)	Controls(n=40)	Cal. F	P-val.	Effect sizes (ω^2)
Age (years)	38.45±9.27	41.70±9.06	41.40±9.07	1.255	0.290	0.002
ALT (IU/L)	12.03±8.59	16.90±8.99[a]	4.85±3.96	20.985	<0.001	0.132
AST (IU/L)	16.73±12.47	27.00±18.65[b]	10.35±7.66	12.035	<0.001	0.199
ALP (IU/L)	119.43±27.94	132.05±32.97[c]	108.93±21.76	5.100	0.008	0.076
PLT (10^9/l)	217.43±52.54	185.75±42.91	269.39±54.09[d]	20.244	<0.001	0.278
TC (mmol/L)	5.11±0.59	6.04±0.71[e]	4.87±0.66	22.510	<0.001	0.301
HDL (mmol/L)	1.78±0.16	1.85±0.22	1.70±0.29	2.797	0.066	0.035
LDL (mmol/L)	2.81±0.50	3.58±0.60[f]	2.68±0.40	24.005	<0.001	0.315
VLDL (mmol/L)	0.52±0.07	0.64±0.11[g]	0.50±0.08	17.269	<0.001	0.245
TG (mmol/L)	1.16±0.17	1.42±0.25[h]	1.12±0.18	17.470	<0.001	0.248
LPS (ng/ml)	225.72±21.97	325.14±25.64[i]	16.06±4.42	2337.91	<0.001	0.979
EI	2.10±0.05	2.25±0.05[j]	0.96±0.13	2066.50	<0.001	0.977
AIP	0.19±0.06[k]	0.12±0.08	0.18±0.09	5.776	0.004	0.087

Results expressed as mean ± SD, P<0.05 considered statistically significant, a, b, c, e, g, h, i, j & k significantly higher while d significantly lower in LF than in CHBV & controls, a, b, c, I, j & k were significantly higher while d significantly lower in CHBV than the controls, a, b, c, e, f, g, h, I, j &k were significantly higher while d significantly lower in CHBV than the controls. PLT = platelet count, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP=alkaline phosphatase, TC =Total cholesterol, LDL=low density lipoprotein cholesterol, HDL=high density lipoprotein cholesterol, VLDL= very low density lipoprotein cholesterol, TG= triglyceride, LPS= Lipopolysaccharide, EI=Endotoxigenic index, AIP=Atherogenic index of plasma

Table 2, Comparison of age, liver enzymes, platelet count, lipid profile, lipopolysaccharide, endotoxigenic index and atherogenic index of plasma between patients with chronic hepatitis B virus infection with normal and elevated levels of cholesterol

Parameters	TC ≤ 5.2 (n=28)	TC >5.2 (n=32**)**	Cal. T	P-Val.
Age (years)	38.75±9.98	40.22±8.66	0.610	0.544
ALT (IU/L)	9.93±2.40	16.90±11.14	3.245	0.002*
AST (IU/L)	13.18±4.47	26.25±18.83	3.583	0.001*
ALP (IU/L)	112.96±27.31	132.77±30.76	2.708	0.009
PLT (10^9/l)	218.74±44.15	196.44±54.07	1.707	0.093
HDL (mmol/L)	1.76±0.15	1.84±0.20	1.855	0.069
LDL (mmol/L)	2.56±0.29	3.51±0.53	8.405	<0.001*
VLDL (mmol/L)	0.49±0.05	0.62±0.10	5.821	<0.001*
TG (mmol/L)	1.09±0.13	1.37±0.23	5.992	<0.001*
LPS (ng/ml)	234.77±34.91	279.93±56.73	3.648	<0.001*
EI	2.12±0.07	2.17±0.08	2.363	0.022*
AIP	0.21±0.05	0.13±0.07	4.676	<0.001*

PLT=platelet count, ALT = alanine aminotransferase, AST = aspartate aminotransferase, ALP=alkaline phosphatase, TC =Total cholesterol, LDL=low density lipoprotein cholesterol, HDL=high density lipoprotein cholesterol, VLDL= very low-density lipoprotein cholesterol, TG= triglyceride, LPS= Lipopolysaccharide , EI=Endotoxigenic index, AIP=atherogenic index of plasma

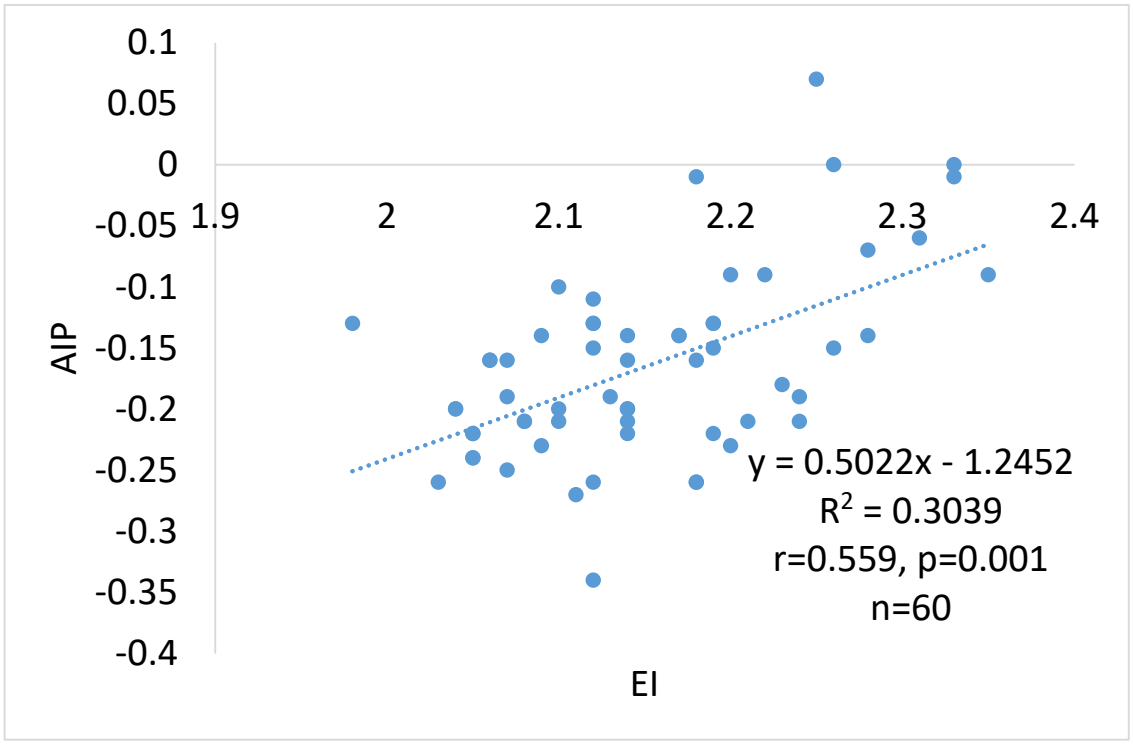


FIG. 1 Correlation plot between atherogenic index of plasma (AIP) against Endotoxigenic index (EI) in subjects with chronic hepatitis B virus

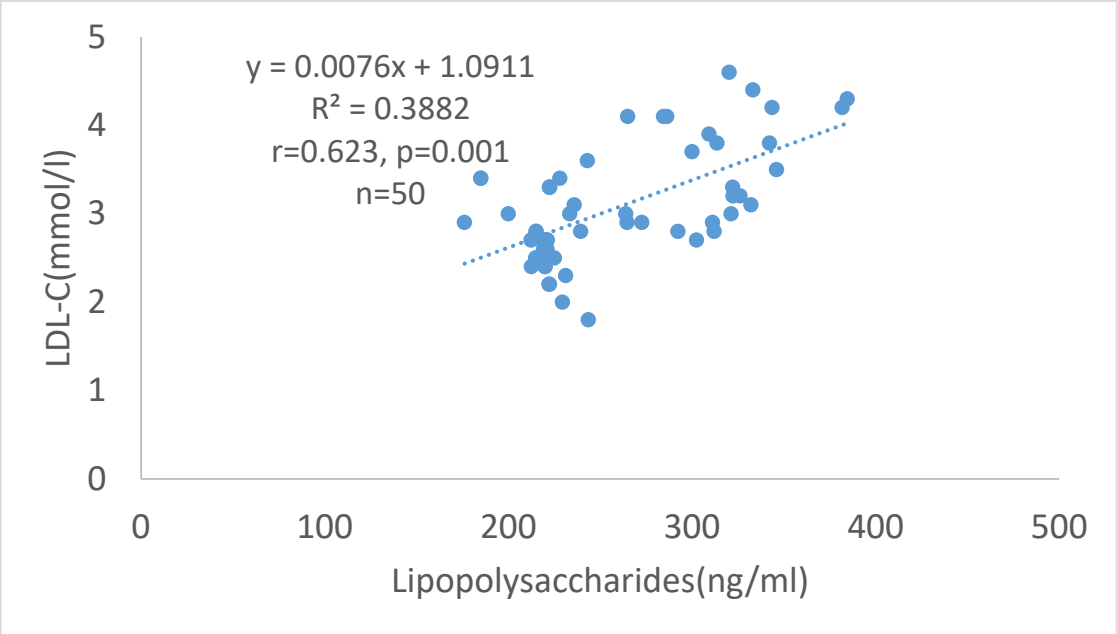


FIG. 2, Correlation plot between lipopolysaccharides against low-density lipoprotein cholesterol in subjects with chronic hepatitis B virus

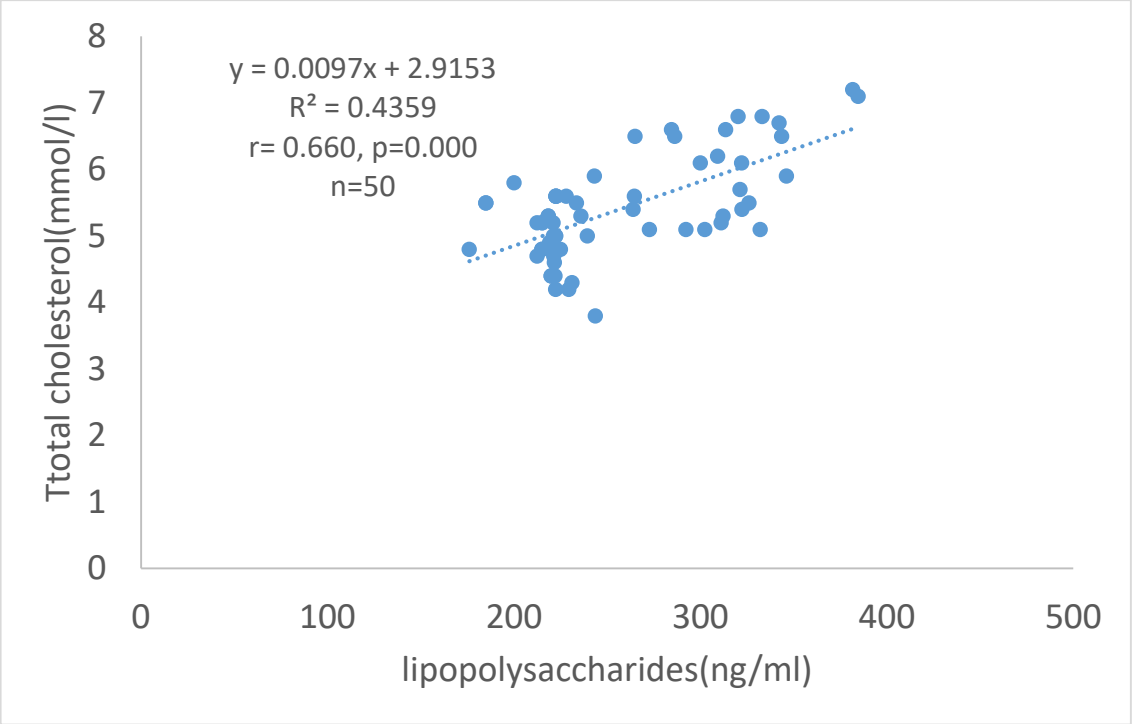


FIG. 3, Correlation plot between lipopolysaccharides against total cholesterol in subjects with chronic hepatitis B virus

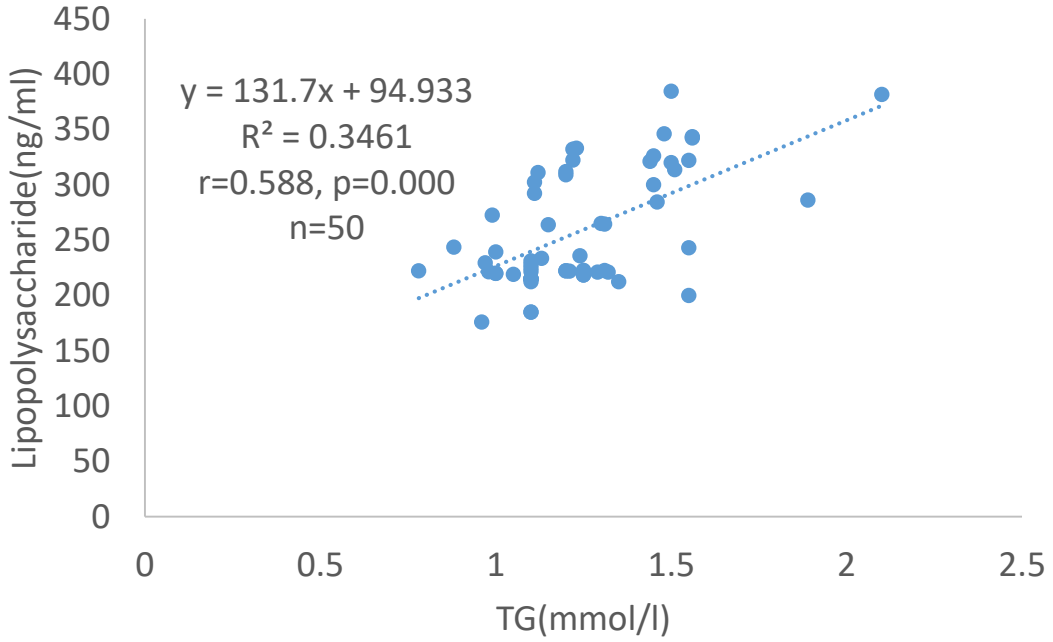


FIG. 4, Correlation plot between triglyceride against lipopolysaccharides in subjects with chronic hepatitis B virus

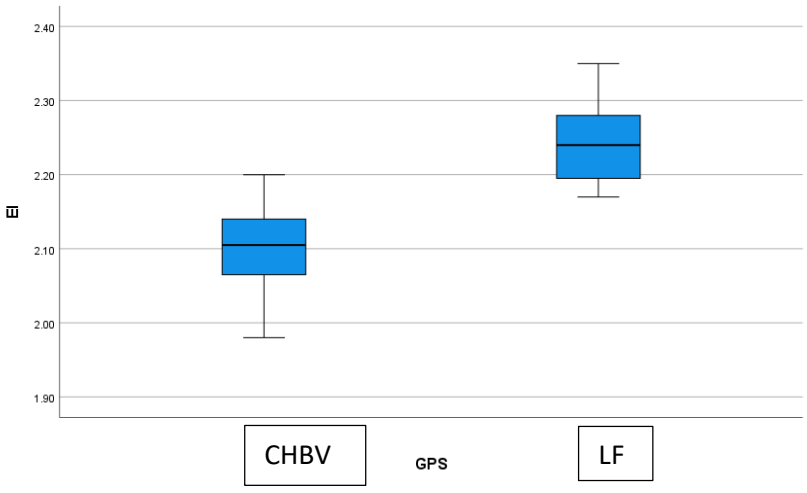
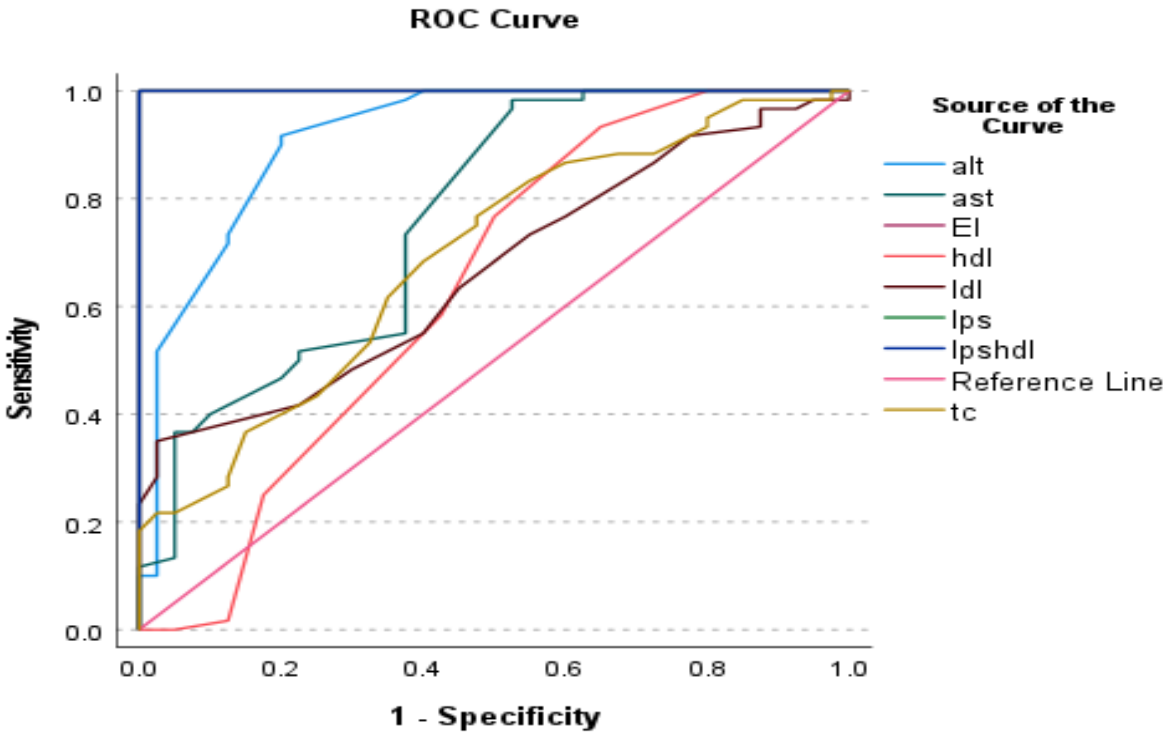


FIG.5 Box and whisker plot of endotoxigenic index in CHBV and LF



variables	AUC	S.E	P	95% C.I	Max K-S metric	Cutoff	Sen.	1-Spec.	spec
ALT	.916	.031	.000	.856-.977	.717	6.5000	92%	20%	80%
AST	.761	.050	.000	.663-.860	.458	7.5000	98%	52%	48%
LDL	.665	.054	.002	.560-.770	.325	3.2500	35%	3%	97%
LPS	1.000	.000	.000	1.000-1.000	1.000	100.17	100%	0%	100%
EI	1.000	.000	.000	1.000-1.000	1.000	1.5900	100%	0%	100%

FIG. 6 Receiver operator characteristic curve of variables and their performance metrics

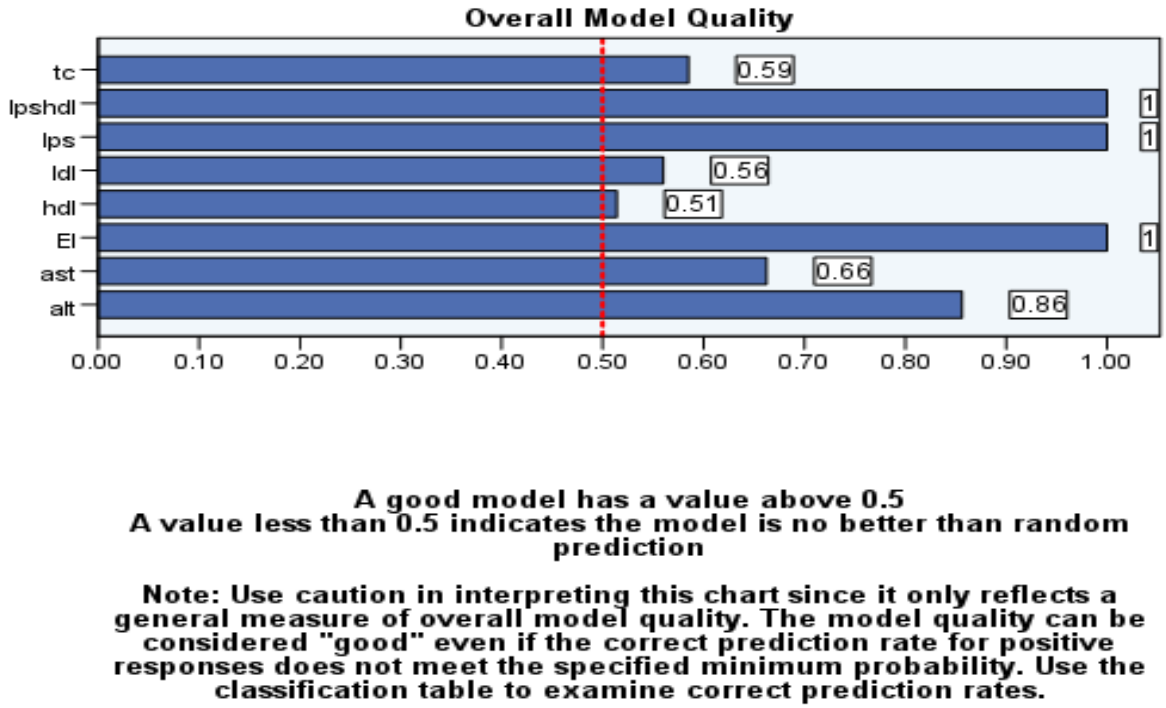


FIG.7, Overall model quality of the variables in the ROC

Discussion

Endotoxins (LPS), are potent inflammatory substances that trigger inflammation by binding to pattern recognition receptors on immune cells, resulting in the overproduction of pro-inflammatory cytokines. Elevated endotoxin levels in plasma due to "leaky gut," contributes to the pathogenesis of various chronic diseases such as CHBV. Chronic inflammation in HBV affects the integrity of hepatocytes membranes which may lead to necrotic and toxic hepatocytes death, the release of liver enzymes into circulation and altered hepatic lipid metabolism. Our study demonstrated significantly higher levels of ALT, AST and ALP, in patients with may suggest release of liver enzymes from the damaged hepatocytes into circulation. Furthermore, hepatocytes injury and compromised liver function due to fibrosis, obstruction of the draining network within the liver, nodular formation and intra-hepatic pressure may be responsible for the elevated levels of ALP in patients with LF compared with CHBV. These findings are in accordance with those previously reported,[30, 31] that HBV infection can alter levels of hepatic enzymes and compounds that may serve as predictors of the disease outcomes. The ALT levels are used to guide the initiation of antiviral therapy under current treatment recommendations for chronic HBV infection. Although serum ALT is not only derived from the liver, it is consistently present

in hepatocytes, and higher levels observed during the HBV infection are considered the most delicate marker for hepatocytes damage. However, in subjects with advanced liver damage and extensive loss of enzyme producing hepatocytes, the levels of these enzymes decreases towards normal levels. This must not be mistaken as a sign of recovery. In the case of recovery albumin levels will increase while the enzyme levels decrease. On the other hand, when the liver enzymes keep decreasing with albumin, this imply no recovery.

The significantly higher levels of TC, LDL-C, VLDL-C, TG, LPS, EI and AIP in patients with LF when compared with CHBV, may be attributed to HBV-related alteration in hepatic lipid metabolism and increased lipid absorption through the compromised gut barrier, as well as HBV hijack of cellular lipid biosynthesis pathways to produce the materials needed for its replication and persistence, leading to elevated level of various lipid components in these patients. This observation is similar to that previously reported[27] where it was established that HBV pathogenesis drives extensive and assorted alterations in hepatic lipid metabolism, by activation of some essential proteins involved in lipogenesis. Also, Li & Luo, observed in HepG2 cells that HBV replication increased the expression of low density lipoprotein receptor (LDLR) and hydroxymethylglutaryl coenzyme A reductase (HMGCR), leading to an increase in cholesterol uptake and synthesis.[32] Wang *et al.*, equally showed that in a mouse model with alcoholic fatty liver, HBV replication increased hepatic cholesterol deposition by enhancing the expression of the cholesterol synthesis-related genes SREBP2 and HMGCR.[33] Similarly, Slagle and Bouchard, observed that HBx is an essential regulatory protein that interacts with various proteins located in the cytoplasm, nucleus, and mitochondria.[34] Zhao *et al.*, also reported that HBx protein overexpression upregulates gene and transcriptional activation of Liver X receptors α and β (LXRα/β), sterol regulatory element-binding protein 1c (SREBP1c), CCAAT/enhancer-binding protein alpha (C/EBPα), and peroxisome proliferator-activated receptor gamma (PPARγ), which contribute to hepatic lipid synthesis.[35] Amponsah-Dacosta *et al., and Eworo et al.,* equally observed that dysbiosis of gut microbiota precedes the development of metabolic syndrome, obesity, buildup of fats in the cells of the liver due to metabolic syndrome factors like insulin resistance, dyslipidemia and dysregulation of cytokines and adipokines, leading to a band of fatty liver disorders in individuals with chronic liver disease.[36, 37] Similarly, Meena *et al.*, observed that hepatocytes receive fatty acids derived from lipolysis of adipose tissue or hydrolysis of fatty tissues, further oxidized to form ATP and acetyl-CoA, and synthesis of triglycerides.[38] Hepatitis B related liver injury and gut microbiota derived endotoxins may affect normal liver lipid metabolism while enhancing gut lipid absorption through the endotoxin-induced increased gut permeability (leaky gut), leading to increased lipid absorption and accumulation of lipids in the body. Targeting specific enzymes in lipid biosynthesis could

potentially disrupt viral replication and antigentowards improvement.[40] Mizutani, *et al.,* reported secretion. These observations are however not inthat the recovery of gut microbiota can promote the accordance with a study by Zhang *et al.,* whoregression of liver fibrosis. Gut dysbiosis produces reported that chronic HBV infection was associatedendotoxins which fuel a cycle of inflammation and with lower levels of serum total cholesterol andliver injury. Therapeutic strategies directed at triglyceride and a lower prevalence of hepaticmopping up endotoxins may limit progression of steatosis compared to non-HBV-infectedliver damage. Also, a previous study reported that subjects.[27]. HBV can have both pro-lipogenic andLPS causes secondary hepatic injury.[41] Also, Zhou anti-hyperlipidemic effects. The presence of*et al.,* observed that dysfunction of gut microbiota in hepatitis B Surface Antigen (Small HBsAg), canchronic hepatitis B infection influences disease disrupts lipid metabolism, leading to reduced overallpathogenesis and endotoxins helps in the activation lipid levels in infected individuals by suppressing theof innate immune response by recognizing TLRs, expression of apolipoprotein A-II and interferingespecially TLR2 and TLR4.[42] Bayram *et al.*, with cellular lipid biosynthesis pathways. Whiledemonstrated that the active receptor for Hepatitis B Virus X protein (HBx) promotes lipidlipopolysaccharide, CD14/TLR4/MD2 receptor accumulation and synthesis by activatingcomplex, on engagement causes the secretion of pro-transcription factors and lipogenesis genes.

The significantly higher levels of LPS, EI in patient with liver fibrosis and chronic hepatitis B virus when compared to the controls may suggest progressive changes in the gut microbiota composition following continued liver assault by viral replication. Alteration in gut microbiota conformation and consequent release of endotoxin leads to an increased lipid absorption from the gut. This finding is similar to that of Philips *et al.,* who reported that the gut microbiota alters as the HBV-related liver fibrosis is initiated and progresses, typified by a decrease in the ratio between “good” and “potentially pathogenic” microbial ecosystem.[39] Maciel-Fiuza *et al.,* observed that when the primary liver disease is controlled by means of antiviral agents, the gut microbiota dysbiosis reverses

inflammatory cytokines including tumor necrosis factor-α, interleukin 1, interleukin-6 and chemokines through the NF-κB signaling to mediate liver injury.[43] Lipopolysaccharides increases the production of cluster of differentiation 14 protein (CD14) through the TLR4 pathway, and reduces the relative gut epithelial resistance, increasing its permeability. LPS in the gastrointestinal tract downregulates the expression of several tight junction proteins (ZO-1 and closed protein) by increasing the penetrability of the intestinal mucosa, then entering the blood flow through the portal venous system. Increased intestinal permeability permits more LPS to reach the general circulation, and getting to different organs of the body, exhibiting a pro-inflammatory effect. Thus, lipopolysaccharides play a decisive role in steering

liver damage through the LPS-TLR4 signaling pathway. Similarly, a previous study reported higher level of LPS in subjects with LF compared to CHBV and control counterparts due to changes in the gut microbial bionetwork.[44].

The significantly lower levels of platelet count in patients with liver fibrosis and chronic hepatitis B virus infection when compared to the controls may imply progressive liver damage with impaired liver ability to synthesize proteins including thrombopoietin resulting in lower platelet count in these patients. This finding is in line with that of Yoshida, *et al.*, who reported that a decreasing platelet counts is observed in most chronic liver diseases pathogenesis, with thrombocytopenia mostly associated with hepatitis B and/or C.[45] Also, Yang, *et al.*, demonstrated that the cause of thrombocytopenia in liver disease including chronic HBV due to portal hypertension, hypersplenism, splenomegaly and reduced thrombopoietin production is closely linked with the extent of liver damage.[46] Also, a previous study reported thrombocytopenia in patients with CHBV and liver fibrosis compared to the control to be due to hypersplenism and enhanced destruction of platelets in the enlarged spleen as well as reduced thrombopoietin production from the failing liver.[47]

The LPS, EI and ALT demonstrated good performances in their area under the curve (AUC), maximum Kolmogorov-Smirnov metrics, sensitivity and specificity in predicting CHBV subjects with poorer outcomes. And may serve as additional markers in evaluating CHBV severity.

The HBV envelope itself is a lipid-derived structure acontaining viral surface proteins (HBsAg), which forms subviral particles in circulation. These lipid invesicles can act as antibodies neutralizing rafts as well as bind to immune cells to prevent a proper anti-viral T-cell response, thus allowing the virus to persist for years without being cleared. The balance between pro- and anti-inflammatory lipids is a key determinant of the severity of liver disease. Elevated levels of pro-inflammatory lipids and decreased levels of anti-inflammatory lipids (like HDL and some PUFAs) can worsen the inflammatory cascade, leading to a vicious cycle of liver cell damage, fibrosis, and , cirrhosis. Lipid fractions influence the fragile balance between the host's immune response and viral persistence. The immunomodulatory effects of lipid fractions are central to understanding the pathogenesis of chronic hepatitis B and offer new avenues for treatment. Dyslipidemia and dysbiosis influences ‘Endotoxigenic index’(EI) and Atherogenic Index of Plasma (AIP) which may be useful in assessing the severity of CHBV, net inflammatory status, risk to development of fatty liver disease and progression of liver fibrosis, cirrhosis and HCC in these subjects.

Summary: Endotoxins primarily drive inflammatory responses by fueling a continuous cycle of inflammation, liver cell damage, and fibrosis, finally contributing to the development of serious complications like cirrhosis and liver failure. However, it can also induce immunosuppressive effects, which may contribute to the immune tolerance and persistence of the HBV infection. This

complex interplay of endotoxin-mediated pro- and anti-inflammatory signals can create an environment that hinders the body's ability to clear the virus effectively. Therapeutic strategies that modulate endotoxin-induced inflammation response may be crucial in the clinical management of CHBV.

Limitation: Single-centre study and sample size.

Conclusion: This study suggests that gut dysbiosis, dyslipidemia, EI, and AIP may be useful adjunctive markers in evaluating CHBV severity and progression, but further longitudinal and mechanistic studies are needed.

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