ORIGINAL RESEARCH

Co-Morbidly of Malaria and Typhoid Perturbs Lipid Homeostasis in Humans
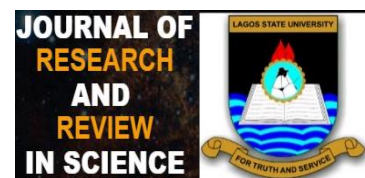
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Abstract:
Introduction: Malaria and typhoid diseases have remained endemic in low-income countries, including Nigeria.
Aims: This study investigated the impact of malaria concurrently occurring with typhoid on plasma, erythrocytes, and lipoproteins lipid profile.
Materials and Methods: Cholesterol, triacylglycerol (TAG) phospholipids (PLs), and non-esterified fatty acids (NEFAs) were determined spectrophotometrically in controls and patients presenting at the Out-Patient Clinic of the State Hospital, Abeokuta, Nigeria.
Results: The presence of either or both parasitic infections provoked dyslipidemia when compared with the controls. Dyslipidaemia was characterised by significant $(P < 0.05)$ decreased plasma, erythrocytes, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol; however very LDL (VLDL) cholesterol increased. While hypertriglyceridemia was observed in plasma, hypotriglyceridemia was observed in the erythrocytes of the patients. In HDL, hypertriglyceridemia was observed in malaria-infected patients whereas hypotriglyceridemia was observed in typhoid-infected and co-infection subjects. Malaria and/or typhoid induced phospholipidemia in plasma and erythrocytes, but provoked decreased HDL-phospholipids (PLs) only in malaria-infected patients. Malaria and/or typhoid elicited decreased LDL+VLDL-PLs. While increased plasma NEFAs concentration was observed in malaria-infected patients; malaria and co-infection resulted in decreased erythrocytes NEFAs. Malaria and/or typhoid caused decreased cholesterol/phospholipids molar ratio in plasma, erythrocytes, and HDL.
Conclusion: The findings of this study indicate that parasitic protozoa and bacterial infections produce a plethora of effects on lipid metabolism, ranging from up-/down-regulation of certain lipid metabolites. These may be early biochemical events in the induction of atherosclerosis by parasitic infections.
Keywords: Co-morbidly, malaria, typhoid, lipid profile, lipoproteins

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Parasitic protozoa and bacterial infections constitute major global health problems and are responsible for some of the most devastating and prevalent diseases of humans worldwide. Among the many parasitic infections, malaria stands out as the most significant human parasitic disease. It remains an important cause of morbidity and mortality in the tropical world [1]. Despite the progress made to combat malaria, there were 228 million episodes of malaria globally and 409,000 deaths in 2019; 91% and 19% occurred in Africa and Nigeria, respectively [2]. About 50% of the Nigerian population experiences malaria annually [3]. Worldwide, Nigeria has the highest malaria-induced death [3].

Typhoid, another human parasitic disease, is an acute generalized infection of the reticuloendothelial system. It is a food and water-borne disease caused by Salmonella enterica subspecies enterica serotype Typhi (Salmonella typhi). It is a serious public health problem in low-income countries, causing an estimated 14.3 million illnesses and 116,800 deaths worldwide each year [4]. The three main diseases caused by Salmonella are typhoid fever (elicited by S. Typhi), gastroenteritis (elicited S. Typhimurium and S. enteritidis), and invasive disease (elicited by S. choleraesuis).

Malaria and typhoid remain a threat to many people in Sub Saharan Africa for several reasons: (1) the elevated poverty, deterioration in public health services, compounded by HIV/AIDS and increasing resistance of malaria parasites to chloroquine, (2) the lack of potable water and widespread misuse of the Widal agglutination test for diagnosing typhoid fever [5].

Malaria and typhoid fever often present with similar symptoms especially in the early stages of typhoid [6]. Thus it is very common to see patients undergoing both typhoid and malaria treatments even if their diagnosis has not been confirmed. There are more typhoid cases in areas of drug resistance than malaria and a cross-reaction between malaria parasites and salmonella antigens may cause false-positive Widal agglutination test [7].

Epidemiologic researches suggest a correlation between infection and atherosclerosis [8]. Hypercholesterolemia and hypertriglyceridemia were noted in both uncomplicated and complicated malaria [9]. While divers' infectious agents are seen in the arterial wall and atheromatous lesions, it is ambiguous how other infections (like Salmonella typhi and *P* falciparum) are linked to atherosclerosis [8].

Both malaria and typhoid infections are endemic in Nigeria [10]. There is a dearth of data on the lipid changes related to the comorbidity of malaria and typhoid. Any consideration of malaria and typhoid parasites must begin with an examination of the host erythrocytes and their plasma milieu. The lipid composition of these two compartments governs the availability of lipids and lipid precursors to the parasites. Hence the major objective of this study is to seek to understand the impact of malaria co-morbidly occurring with typhoid on some aspects of lipid metabolism and their association with indices of cardiovascular disease.

2. MATERIAL AND METHODS

2.1 Chemicals and reagents

Sodium hydroxide, potassium hydroxide, sodium chloride, manganese chloride, potassium cyanide, ferric cyanide, ferric chloride dehydrate, sodium hydrogen carbonate, potassium hexacyanoferrate, trichloroacetic acid, Triton X-100, and Giemsa stain were products of Aldrich Sigma Chemical Company (St. Louis, MO, USA). Sulphuric acid, hydrochloric acid, chloroform, isopropanol, ethanol, ammonium thiocyanate, and glacial acetic acid were purchased from British Drug Houses (Poole, UK). Heparin was procured from Rotex Medical, Trittau, Germany. Diagnostic kits for Widal bacteria test, cholesterol, and triacylglycerol, were products of Cromatest® Diagnostics, Joaquim Costal, Montgat, Barcelona, Spain. Norm-jet needles and syringes were products of Norm-Jet Inc., Tuttlingen, Germany. Heparinized tubes were products of Sterling Products, Essex, England. Except otherwise stated all other chemicals and reagents were of analytical grade and were obtained from British Drug Houses (Poole, UK).

2.2 Subjects

Informed consent was obtained from the patients. Control subjects (n = 61) who were relatives of the patients, staff, and students of the Federal University of Agriculture, Abeokuta, Nigeria, were recruited in the study at the same period with the patients. Control subjects (n = 61) and patients (n = 364) presenting at the Medical Out-Patient Clinic of the State Hospital, Abeokuta, Nigeria, with a diagnosis of falciparum malaria, typhoid, and co-infections of typhoid and malaria, were recruited for this study. The institution-approved research number for the study is FUNAAB050050.

2.3 Determination of Malaria Status using Thick and Thin-Film Methods

Blood samples were obtained from the controls and patients (before treatment with anti-malaria and anti-typhoid drugs) from the antecubital vein (after an overnight fast) for Giemsa stained thick and thin blood film microscopy for malaria parasites examination as described by Ohanu (2019) [11].

2.4 Determination of Typhoid Status using Widal Reaction

Typhoid status was carried out as described by Ohanu (2019) [11].

2.5 Determination of Packed Cell Volume

Packed cell volume was determined as described by Ohanu (2019) [11].

2.6 Determination of Haemoglobin

Haemoglobin content determination followed the method described by Fairbanks and Klee (1987) [12].

2.7 Preparation of Serum and Erythrocytes Suspension

Part of the blood samples that were collected was expelled into heparinized tubes. The blood samples were centrifuged at 5,000 rpm for 10 min. The plasma which was the clear supernatant removed and was used for the estimation of serum enzymes. The buffy coat was equally removed by careful suction to obtain the erythrocytes as described by Adesanoye et al. (2013) [13].

2.8 Determination of Plasma Lipid Profile

Plasma concentrations of total cholesterol and triacylglycerol were determined with commercial kits (Cromatest® Diagnostics, Joaquim Costal, Montgat, Barcelona, Spain). HDL cholesterol and triacylglycerol were determined in plasma with the same commercial kits for total cholesterol and triacylglycerol after very-low-density lipoproteins (VLDL), and LDL were precipitated with the heparin-MnCl2 solution as described by Akamo et al., (2017) [14]. Total phospholipids in plasma and HDL were extracted with the chloroform-methanol mixture (2:1, v/v) as described by Folch et al., (1957) [15]. Phospholipids content was then determined as described by Stewart, (1980) [16]. The concentrations of Very Low-Density Lipoprotein (VLDL)-cholesterol and LDL-cholesterol were calculated by a modification of the Friedwald formula [17]. Coronary Risk Index (CRI=TC/HDL-C) and Atherogenic Index (AI=LDLC/ HDLC) were then calculated for each subject. Plasma non-esterified fatty acids determination followed the method described by Plummer (1978) [18]. Sodiumdiethyldithiocrbamate is used as a chromogen to determine the amount of copper in the chloroform which is the equivalent amount of the non-esterified fatty acids determined spectrophotometrically

2.9 Determination of Erythrocytes Lipid Profile

Since the Folch extraction [15] formed lipid extracts that were exceedingly colored, a better method to extract erythrocytes lipids was engaged by using chloroform: isopropanol (7:11, v/v) as described by Rose and Oklander (1965) [19]. After which erythrocytes cholesterol, triacylglycerol, phospholipids, and non-esterified fatty acids followed the same methods as described for plasma lipids above.

2.10 Statistical Analysis

Data were expressed as the mean ± SEM of five replicates in each group. Analysis of Variance

(ANOVA) was carried out to test for the level of homogeneity among the groups. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). A p-value of less than 0.05 was considered statistically significant. All the statistics were carried out by SPSS (Statistical Package for Social Sciences) software for Windows version 20 (SPSS Inc., Chicago, Illinois, USA). Graphs were plotted using GraphPad Prism 8 Software (GraphPad Software Inc., San Diego, USA).

3. RESULTS

Some demographic and clinical characteristics of the subjects are depicted in Table 1. Their ages ranged between 15 and 68 years. In malaria and typhoid + malaria subjects, the highest parasite density was observed in the female subjects. While no significant ($P > 0.05$) difference was observed in the mean packed cell volume (PCV) and hemoglobin (Hb) values of typhoid male subjects when compared with their control counterparts, PCV and Hb values of other parasite-infected subjects decreased significantly ($P <0.05$) when compared with their control counterparts. The decrease was more marked in malaria females when compared with control and other parasite-infected subjects. Quantitatively, PCV and Hb of the parasite-infected subjects were between 11% and 26% lower than their control counterparts.

Table 1: Demographic and clinical characteristics of the subjects.

	Age (year)	PCV (%)	Hb (g/dL)	Malaria parasite count (/µl)
CM (n=30)	36.17±13.34[a]	42.70±5.31[a]	14.21±1.69[a]	0000.00±0000.00[a]
MM (n=60)	32.67±10.18[a]	34.93±5.12[c]	11.71±1.71[c]	8550.00±1213.27[b]
TM (n=60)	33.47±11.34[a]	41.43±4.26[a]	13.78±1.33[a]	0000.00±0000.00[a]
MTM (n=61)	35.62±15.38[a]	36.33±5.41[c]	12.16±1.80[c]	8475.41±1074.04[b]
CF (n=31)	36.68±10.68[a]	39.10±4.96[b]	13.12±1.73[b]	0000.00±0000.00[a]
MF (n=61)	34.72±11.21[a]	30.34±3.55[e]	10.19±1.18[e]	9016.39±1372.25[c]
TF (n=60)	33.52±12.61[a]	35.33±3.06[c]	11.82±1.02[c]	0000.00±0000.00[a]
MTF (n=62)	32.76±12.69[a]	32.21±4.39[d]	10.85±1.44[d]	8854.84±1143.00[bc]

CM, control male; MM, malaria male; TM, typhoid male; MTM malaria + typhoid male; CF, control female; MF, malaria female; TF, typhoid female; a MTF, and malaria + typhoid female; PCV, packed cell volume; and Hb, hemoglobin. Values are mean ± standard deviation. Values within the same column with different superscripts are significantly different at $P < 0.05$.

Figure 1 depicts the effect of malaria and typhoid on the investigated lipid profiles in the plasma and erythrocytes. The presence of either or both parasitic infections resulted in significant ($P <0.05$) alterations in the plasma lipid profiles (Figure 1 a-e) of the subjects. In the plasma of the parasite-infected subjects, there was a significant ($P <0.05$) decrease in the cholesterol concentrations (Fig. 1a). The decrease was more marked in the co-infected males when compared with control and other parasite-infected subjects. Quantitatively, plasma cholesterol values of the parasite-infected subjects were between 25% and 36% lower than their control counterparts. While the presence of either or both infections did not affect plasma TAG contents (Fig. 1b) of typhoid female subjects ($P > 0.05$), plasma TAG of other parasite-infected subjects increased significantly ($P <0.05$) when compared with the control. Plasma TAG levels of the subjects were between 1.2 to 1.78 times higher than that of the control, with the highest levels observed in malaria (male and female). Also in the

plasma of the parasite-infected subjects, there was a significant $(P <0.05)$ increase in the phospholipids (PLs) concentrations (Fig. 1c). Plasma PLs concentration was between 1.22 to 1.55 times higher than that of the control, with the highest values observed in malaria-infected males. While plasma non-esterified fatty acids (NEFAs) concentrations (Fig. 1d) in typhoid subjects were not significantly $(P > 0.05)$ different from control, plasma NEFAs concentration of malaria and co-infection increased significantly $(P <0.05)$ when compared with the control. In the plasma of these subjects, NEFAs values were between 1.2 to 1.6 times higher than that of the control, with the highest levels observed in malaria (male and female) subjects. The plasma cholesterol: phospholipids molar ratio (Fig. 1e) decreased significantly $(P <0.05)$ when compared with the controls. The malaria male had the lowest value which was 59% lower than the control counterpart. Overall, the plasma cholesterol: phospholipids molar ratios of the parasite-infected subjects were between 40% and 60% lower than their control counterparts.

The presence of either or both parasitic infections elicited marked $(P <0.05)$ alterations in the erythrocytes lipid profiles (Figure 1 f-j) of the subjects. In the erythrocytes of the parasite-infected subjects, there was a significant $(P <0.05)$ decrease in the cholesterol concentration (Fig. 1f). The decrease was more marked in malaria males when compared with control and other parasite-infected subjects. Quantitatively, the RBC cholesterol values of the parasite-infected subjects were between 31% and 41% lower than their control counterparts. While erythrocytes TAG concentration (Fig. 1g) in co-infection females was not significantly $(P > 0.05)$ different from control, TAG concentration was reduced in other parasite-infected subjects, with typhoid females having the least TAG value. In contrast to erythrocytes TAG, there was a significant $(P <0.05)$ increase in the erythrocytes phospholipids (Fig. 1h). In the erythrocytes of the subjects, phospholipids concentration was between 1.22 to 1.7 times higher than that of the control, with the highest values observed in typhoid females. While erythrocyte NEFAs levels (Fig. 1i) in typhoid subjects were not significantly $(P <0.05)$ different from control. The erythrocyte NEFAs levels were between 13% and 26% lower than that of the control with the lowest levels observed in malaria males. The erythrocytes cholesterol: phospholipids molar ratio (Fig. 1j) decreased significantly $(P <0.05)$ when compared with the controls. The typhoid-infected subjects had the lowest value which was 60% lower than the control counterpart $(P <0.05)$. The erythrocytes cholesterol: phospholipids ratios of the parasite-infected subjects were between 37% and 60% lower than their control counterparts.
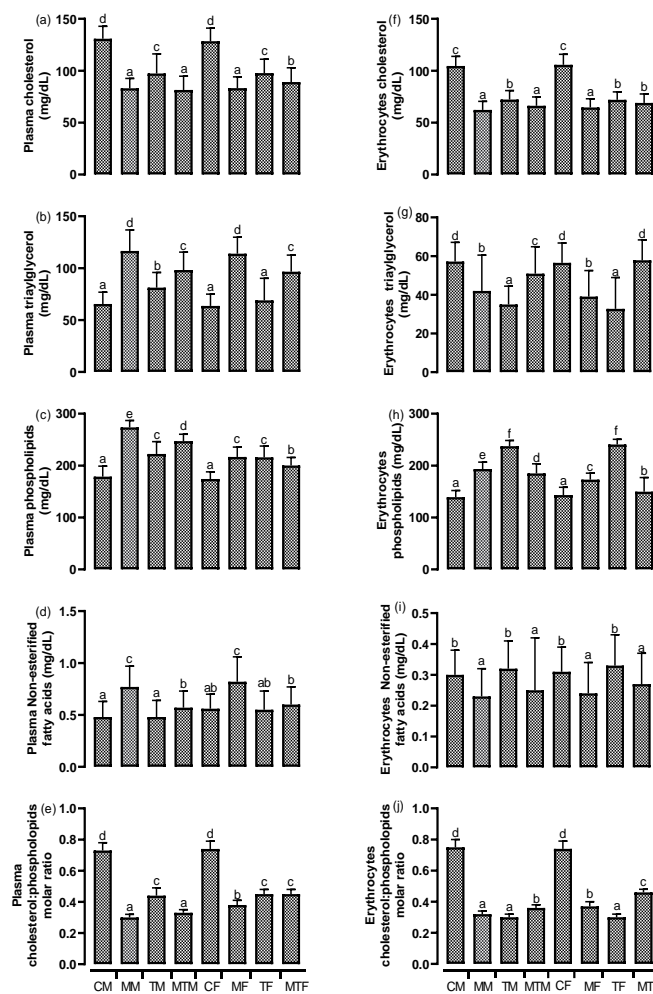

Figure 1: (a-j). Effects of malaria, typhoid and co-infection on plasma, and erythrocytes lipid profiles.CM, control male; MM, malaria male; TM, typhoid male; MTM malaria + typhoid male; CF, control female; MF, malaria female; TF, typhoid female; a MTF, and malaria + typhoid female. Bars represent mean ± standard deviation. Bars with different letters are significantly different at $P < 0.05$.

The lipoprotein lipid contents of both control and parasite-infected subjects are summarized in Figure 2. HDL responded to the presence of parasite infection with a significant $(P <0.05)$ decrease in cholesterol level (Fig. 2a). The decrease was more marked in malaria males when compared with control and other parasite-infected subjects. Quantitatively, HDL cholesterol values of the parasite-infected subjects were between 26% and 45% lower than their control counterpart. A marked $(P <0.05)$ hypertriglyceridemia (Fig. 2b) was observed in the malaria subjects, while hypotriglyceridemia was observed in typhoid infection. There was no significant difference $(P > 0.05)$ in HDL phospholipids levels (Fig. 2c) between control and parasite-infected subject except in malaria males and malaria females where a slight decrease in phospholipids was observed. The HDL cholesterol: phospholipids molar ratio (Fig. 2d) decreased in parasite-infected subjects. A significant $(P <0.05)$ reduction in LDL cholesterol (LDL-C) (Fig. 2e) of the parasite-infected subjects was observed. The decrease was more marked in co-infected males when compared with the control and other parasite-infected

subjects. LDL–C values of the parasite-infected subjects were between 30% and 54% lower than their control counterparts. While VLDL–C (Fig. 2f) was not significantly (*P* > 0.05) different in the typhoid females when compared with controls, values obtained for the other parasite-infected subjects were significantly (*P* <0.05) higher than controls. Either or both parasitic infections significantly (*P* <0.05) increase LDL+VLDL-TAG values (Fig. 2g). The LDL+VLDL-TAG concentrations were between 1.5 and 2.8 times higher than that of the control, with the highest values observed in co-infected males. In contrast to LDL+VLDL-TAG, LDL+VLDL–PLs (Fig. 2h) decreased in the parasite-infected subjects (*P* <0.05). The decrease was more marked in co-infected females when compared with control and other parasites-infected subjects. Quantitatively, LDL+VLDL–PL values of the parasite-infected subjects were between 9% and 25% lower than their control counterparts.
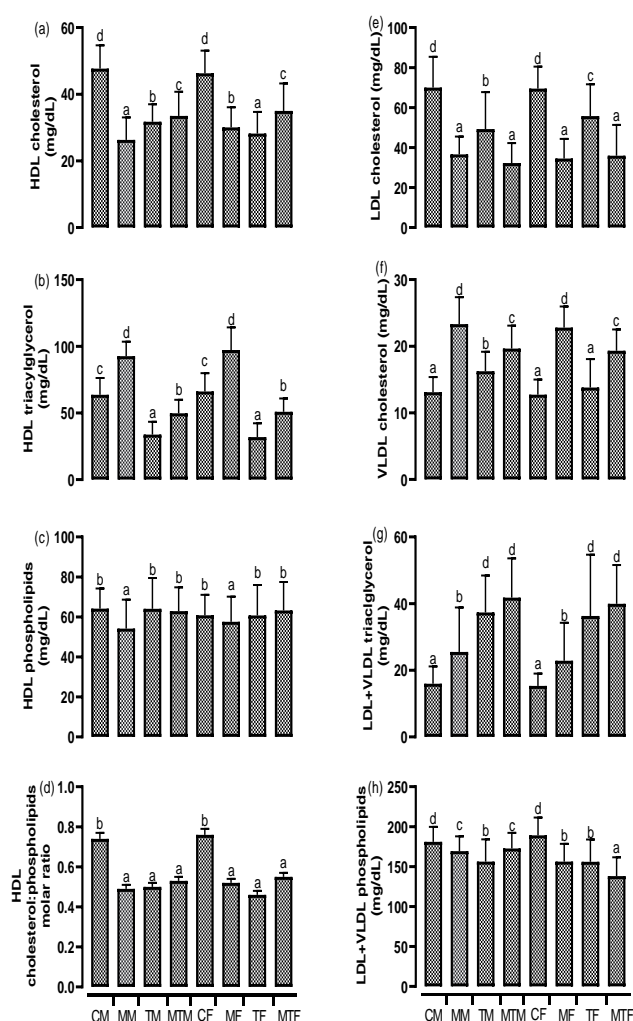

Figure 2: (a-h). Effects of malaria, typhoid, and co-infection on lipoprotein lipid profiles.CM, control male; MM, malaria male; TM, typhoid male; MTM malaria + typhoid male; CF, control female; MF, malaria female; TF, typhoid female; a MTF, and malaria + typhoid female Bars represent mean ± standard deviation. Bars with different letters are significantly different at *P* < 0.05.

Figure 3a depicts the coronary risk index (CRI, which is the molar ratio of total cholesterol/HDL cholesterol)

in the controls and parasite-infected subjects. While CRI in both sexes of the co-infection patients resulted in a significant (*P* <0.05) decrease others show significant (*P* <0.05) increases in the CRI when compared with the control groups. The highest (1.3 fold increase) was observed in typhoid females. However, co-infection (males and females) significantly reduced the ratio (*P* <0.05). While no statistically significant (*P* > 0.05) difference was noted in the mean LDL cholesterol/HDL cholesterol molar ratio (Fig. 3b) of malaria males, malaria females; typhoid infection led to about 1.4 fold increase in this ratio in the female subjects. However, malaria females and co-infection (both sexes) significantly (*P* <0.05) reduced this ratio.
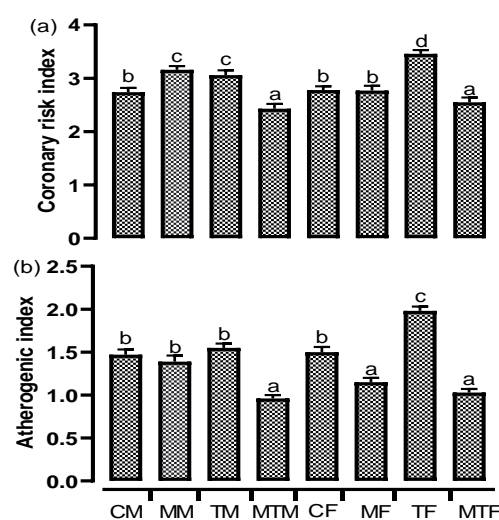

Figure 3: (a-b) Effects of malaria, typhoid, and co-infection on coronary and atherogenic indices.CM, control male; MM, malaria male; TM, typhoid male; MTM malaria + typhoid male; CF, control female; MF, malaria female; TF, typhoid female; a MTF, and malaria + typhoid female. Bars represent mean ± standard deviation. Bars with different letters are significantly different at *P* < 0.05.

Correlations, as calculated by Pearson's method, revealed significant direct linear and inverse linear relationships among the parameters (Table 2). A significant positive associations were noted between parasite density and plasma triacylglycerol (r=0.675, *P<0.01*), plasma phospholipids (r=0.389, *P<0.01*), plasma non-esterified fatty acids (r=0.383, *P<0.01*), HDL triacylglycerol (r=0.531, *P<0.01*), and VLDL cholesterol (r=0.675, *P<0.01*). However, a significant negative associations were noted between parasite count and packed cell volume (r=-0.559, *P<0.01*), heamoglobin (Hb) (r=-0.554, *P<0.01*), plasma cholesterol (r=-0.569, *P<0.01*), plasma cholesterol/phospholipids molar ratio (r=-0.573, *P<0.01*), erythrocytes cholesterol (r =-0.539, *P<0.01*). Plasma cholesterol also correlated positively with erythrocytes cholesterol (r=0.842, *P<0.01*). Taken together, among the subjects, it was observed that there were more significant negatives than positive correlations among the parameters, irrespective of

whether either or both parasitic infections were present.

Table 2: Positive and negative associations among parameters in the subjects

Parameters	Correlation coefficient (r)	P-value
PD vs.plasma triacylglycerol	0.675	0.001
PD vs.plasma phospholipids	0.389	0.001
PD vs.HDL triacylglycerol	0.531	0.001
PD vs.VLDL cholesterol	0.675	0.001
PD vs.plasma NEFAs	0.383	0.001
Plasma cholesterol vs. RBCs cholesterol	0.842	0.001
PD vs.packed cell volume	- 0.559	0.001
PD vs.Haemoglobin	- 0.554	0.001
PD vs.plasma cholesterol	- 0.569	0.001
PD vs.plasma cholesterol/ phospholipids ratio	- 0.573	0.001
PD vs.RBC cholesterol	- 0.539	0.001
PD vs.RBC phospholipids	- 0.389	0.001
PD vs.RBC cholesterol/ phospholipids ratio	- 0.211	0.001
PD vs.HDL cholesterol	- 0.229	0.001
PD vs.LDL cholesterol	- 0.616	0.001
PD vs.LDL CRI	- 0.351	0.025
PD vs.RBC NEFAs	- 0.342	0.001

PD (Parasite density); vs (versus); NEFAs (non-esterified fatty acids);CRI (coronary risk index)

4. DISCUSSION

The outcome of this present research specifies that acute malaria, typhoid, and typhoid plus malarial infections certainly perturb the metabolism of lipids in plasma, erythrocytes, and lipoproteins of the host organism. These alterations were echoed as up-/down-regulation of the amounts of the major lipids (cholesterol, triacylglycerol, phospholipids, and non-esterified fatty acids).

Studies on fatty acid constituents have revealed that the capability of plasmodium to perform saturation or desaturation reactions of aliphatic chains, chain lengthening, and shortening, is restricted [20]. Furthermore, Plasmodium-infected erythrocytes are unable to synthesize fatty acids de novo from acetate during the erythrocytic cycle [20, 21]. The parasite growth requires exogenous lipids, derived from either the host erythrocytes or from the surrounding plasma. The reports of Holz (1977) [21] and van Schaijk et al. (2014) [20] are similar to the findings in this study, malaria and co-infection remarkably $(P < 0.05)$ lowered erythrocytes NEFAs levels. In contrast to erythrocytes NEFAs, there was an increase in plasma NEFAs of parasite-infected subjects. To sustain usual equanimity, the organism must sustain physiological levels of metabolites in its varied metabolic process. An alteration of these concentrations could have severe physiological penalties for the organism [22]. In the current research, we noted increases in plasma NEFAs and phospholipids and decreases in

cholesterol of both plasma and erythrocytes of parasite-infected subjects. NEFA in plasma is produced from the hydrolysis of triacylglycerol and then release into the plasma. A considerable level of this hydrolysis ensues in the adipose tissue and is catalysed by a triacylglycerol lipase dissimilar to the lipoprotein lipase which forms outside the adipose tissue cell. Increased plasma NEFAs suggest increased mobilization of these lipids from the adipose tissue [23]. Hence, the observation of increased plasma NEFAs in the parasite-infected subjects suggests a parasite-induced intensification of triacylglycerol hydrolysis ensuing into NEFAs by the triacylglycerol lipase and the associated amplified deployment of the NEFAs into the plasma. The physiological penalties of these increased plasma NEFAs could be varied and could be seen from the metabolic functions of the NEFAs.

The main source of energy in many tissues such as the liver, heart, kidney, muscle, lung, testis, brain, and adipose tissue are NEFAs, even though the brain cannot extract NEFAs from the blood [22]. NEFAs are also the source of triacylglycerol biosynthesis in the liver [24]. So, elevated plasma NEFAs in the parasite-infected subjects suggest that the hepatic take uP of these lipids was reversed by parasitic infection, hence negotiating energy synthesis in this organ. Elevated plasma NEFAs, as revealed from this study as a result of the presence of either or both parasitic infections, would anticipate further physiological penalties. Captivating experimental results from animal studies have revealed a direct correlation between elevated plasma NEFAs and pathologic diseases and disorders including diabetes mellitus, obesity, and cardiovascular diseases [23, 25].

Cholesterol and phospholipids are parts of the plasma membranes of living things. Cholesterol equally serves as the precursor of steroid hormones; phospholipids serve as an emulsifying surrogate to stabilize the proper colloidal state of the cytoplasm [22]. Furthermore, phospholipids equally participate in transporting hydrophobic components into and out of the cells [22]. Cholesterol and triacylglycerol have been extensively investigated in the plasma during many metabolic dysfunctions because they participate in vascular disarray. Nevertheless, in contrast with plasma cholesterol quantification, undersized consciousness has been given to plasma phospholipids, together with lipid parameters of erythrocytes and lipoproteins in various pathologies. We observed that either or both parasitic infections resulted in hyperphospholipidemia in plasma and erythrocytes of the patients whereas PLs concentrations decreased in HDL of malaria-infected patients. Hyperphospholipidaemia is characterized by the availability of non-esterified fatty [26]. The results from this investigation show that the above-stated mechanisms might be responsible for the observed hyperphospholipidemia especially in the plasma. LDL+VLDL PLs concentrations also decreased in either and co-infections. Furthermore, while increased cholesterol concentration was observed in VLDL of malaria-infected subjects, there was a depletion of plasma, erythrocytes, HDL, and LDL cholesterol of either or both parasitic infected subjects. Most extra-hepatic tissues, even though having a demand for

cholesterol, have little activity of the cholesterol biosynthetic pathway. Their cholesterol demands are furnished by LDL, which is embodied by receptor-mediated endocytosis [27].

A key function of HDL cholesterol is to promote reverse cholesterol transport by mopping superfluity cholesterol from peripheral tissues then esterification through lecithin: cholesterol acyltransferase (LCAT) and convey cholesterol to the liver and steroidogenic organs for ensuing biosynthesis of bile acids and lipoproteins and ultimate removal from the body [28]. This function of HDL has been revealed to be accountable for its atheroprotective potentials. HDL cholesterol also controls the interchange of proteins and lipids between several lipoproteins [29]. Furthermore, HDL supply the part of the protein necessary to trigger lipoprotein lipid which delivers fatty acid that can be oxidized by the β–oxidation pathway to produce energy [30]. Fundamentally, HDL can inhibit the oxidation of LDL and the atherogenic consequences of oxidized LDL on account of its antioxidant potential. Nonetheless, it has been communicated that infections are linked to a reduction in HDL cholesterol levels [28]. The mechanism for the infection-induced reduction in HDL cholesterol concentrations has not been resolutely accepted. However, an incessant low level of HDL cholesterol in chronic infection indicates that this change may be unpleasant since results from epidemiologic researches have revealed a pronounced peril of coronary artery disease in an individual with low HDL cholesterol concentration. The observation of decreased HDL-C and LDL-C levels and the increase in total cholesterol/HDL cholesterol in malaria and typhoid and LDL/HDL cholesterol ratio in co-infection in this study suggests that reverse cholesterol transport in these subjects was affected by parasitic infections. Either or both parasitic infections resulted in decreased cholesterol/phospholipids molar ratio in plasma, erythrocytes, and HDL. This molar ratio is an index of membrane fluidity. An increase in this molar ratio suggests decreased fluidity, and deformability, and ultimately impairs the systemic movement, especially that of erythrocytes [31].

Triacylglycerol functions as energy reservoirs in animals. In contrast to cholesterol, hypertriglyceridemia was observed in plasma. Our observation of increased plasma TAG in malaria patients agrees with the observation of other researchers [32, 33]. The observed parasite-induced plasma hypertriacylglycerolemia in this study could be the result of either elevated VLDL biosynthesis or reduced VLDL elimination [34]. In HDL, hypertriglyceridemia was observed in malaria-infected patients whereas hypotriglyceridemia was observed in typhoid-infected subjects. The hypertriglyceridemia was more pronounced in HDL and plasma of malaria-infected patients. The amount of LDL+VLDL-TAG also increased in and co-parasitic infections.

The mechanisms required in lipid changes related to malaria remain uncertain [35]. They may be partially host-associated i.e. associated with the acute-phase reaction. The acute phase response is correlated with modification in lipid metabolism including a moderate rise in serum TAG and VLDL, but a decrease in HDL and LDL cholesterol [35, 36]. Nevertheless, a differential take-uP of HDL particles by P. falciparum has been hypothesized [37] and further revealed by in vitro investigations [38]. From the present data, it appears that acute malarial and typhoid infection is accompanied by proatherogenic changes in plasma lipoprotein profile that comprises an increase in VLDL cholesterol, slightly increased VLDL TAG, decrease in HDL cholesterol, and increase in HDL TAG.

5. CONCLUSION

Taken together, the findings of this study indicate that parasitic protozoa and bacterial infections produce a plethora of effects on lipid metabolism, ranging from up-/down- regulation of certain lipid metabolites spectrum of the host. These may be early important biochemical events in the induction of atherosclerosis by parasitic infections.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed following the ethical standards laid down in the 1964 Declaration of Helsinki. The institution-approved research number for the study is FUNAAB050050.

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