British Journal of Medicine & Medical Research
16(1): 1-7, 2016, Article no.BJMMR.23751
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Association between Clinical Malaria and Blood Lipids in North Eastern India

Synrang Batngen Warjri[1], Tony Ete[1*], Animesh Mishra[2], Bhupen Barman[1], Jaya Mishra[3], Star Pala[4], Taso Beyong[1] and Neel Kanth Issar[1]

[1]Department of General Medicine, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India.
[2]Department of Cardiology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India.
[3]Department of Pathology, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India.
[4]Department of Community Medicine, North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences, Shillong, Meghalaya, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author SBW conceptualised and designed the study, monitored data collection for the whole trial, analysed the data, wrote the first draft of the manuscript and revised the paper. Author TE conceptualised and designed the study, interpretation of data and revised the paper. Author AM supervised the study, interpretation of the data and revised the paper. Author BB managed the study protocols, acquisition of data and revised the paper. Author JM managed the data acquisition, statistical analysis and revised the paper. Author SP managed the statistical analysis, interpretation of data and revised the paper. Author TB managed the literature searches, data collection and revised the paper. Author NKI managed the collection of data and revised the paper. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/23751
Editor(s):
(1) Nurhan Cucer, Erciyes University, Medical Biology Department, Turkey.
Reviewers:
(1) Najamus Sehar, Graduate Staten Island University, USA.
(2) Francis W. Hombhanje, Divine Word University, Papua New Guinea.
(3) Jorge Fraga Nodarse, Institute of Tropical Medicine Pedro Kouri, Cuba.
(4) Innocent Onyesom, Delta State University, Abraka, Nigeria.
Complete Peer review History: http://sciencedomain.org/review-history/14819

Original Research Article

Received 19th December 2015
Accepted 31st March 2016
Published 30th May 2016

ABSTRACT

Background: Changes in lipid profile are seen in many patients infected with malaria parasite. The malaria parasite causes hepatocellular damage and disturbs lipid handling by the liver. Inside

**Corresponding author: E-mail: tonyete14@gmail.com;*

hepatocytes and erythrocytes the parasite replicates rapidly scavenging cholesterol and lipids required for its growth and metabolism from the host. It also requires host lipids for detoxification of free heme to form the malarial pigment, haemozoin. The important question is whether these changes are characteristic for malaria infection or are they simply part of an acute phase reaction? This study analyzes the correlation between malaria infection and derangements in lipid profiles.

Materials and Methods: This study comprised of 29 confirmed malaria cases, and 29 subjects in apparent good health, without the infection were included as control cases. Malaria cases were confirmed using rapid antibody-based diagnostic card tests that detect histidine-rich protein 2 (HRP2) or lactate dehydrogenase antigens in finger-prick blood samples followed by microscopic confirmation of malaria parasite. A 12 -hour fasting lipid profile was estimated by enzymatic method on day 2. Data obtained were statistically analyzed using Student's *t* Test, assuming $p<0.05$ as significant. All issues related to ethics were taken care of during the whole course of study.

Results: As compared with control subjects, patients with malaria showed low HDL (16.48±6.490 mg/dL versus 41.38±15.110 mg/dL), low LDL (70.45±22.720 mg/dL versus 104.46±27.353 mg/dL), low cholesterol (103.52±35.331 mg/dL versus 169.45±34.040 mg/dL) and elevated triglycerides (214.24±109.365 mg/dL versus 131.15±30.813 mg/dL). The observations show a statistically significant difference in HDL, LDL, cholesterol and triglycerides between malaria patients and control subjects ($p<0.05$).

Conclusion: These results show a characteristic pattern of derangements of lipid profile in malaria. Further studies are required to understand the diagnostic, prognostic and therapeutic implications of these derangements.

Keywords: Malaria; lipids; triglycerides; cholesterol.

1. INTRODUCTION

Malaria is a mosquito-borne infectious disease caused by a protozoan parasite belonging to the genus — *Plasmodium*. It is widely endemic in tropical and subtropical parts of the world where it continues to cause significant morbidity and mortality. Patients with malarial infection show a wide range of metabolic derangements including changes in serum lipid profile. These changes in serum lipid profile and their possible correlation with malarial infection has been reported in various studies. Changes in serum lipid profile are seen in many other conditions including postsurgical [1], burn injury [2], malignancy [3] and acute myocardial infarction [4]. Serum lipid derangements are also seen in patients with other parasitic infections including leishmaniasis, toxoplasmosis and helminthes [5]. Gallin et al. [6] reported changes in serum lipid profile in gram-negative bacilli infection. With such diverse causes of lipid derangements, the question arises as to whether these serum lipid changes are specific for malarial infection or are they simply part of an acute phase reaction? A recent systematic review and meta-analysis of serum lipids and lipoproteins in malaria conducted by Visser et al. [7] showed a significant change in lipid profile in malaria patients which, according to the analysis, were characteristic for malaria. These included low serum total cholesterol, low serum high density lipoprotein (HDL) and a low serum low density lipoprotein (LDL) in malaria.

The exact mechanisms resulting in these derangements in serum lipid profile in patients infected with the malaria parasite is still poorly understood. The several probable biological mechanisms that can explain these changes involve a complex interplay between the parasite and the host. The malarial sporozoites are introduced into the blood stream of a human being following the bite of an infected female *Anopheles* mosquito. The sporozoites then travel to the liver where they invade the hepatocytes causing liver injury. Within the hepatocytes the sporozoites divide and mature to form merozoites which are released into the bloodstream. These merozoites then infect red blood cells where they further replicate. In both phases — the hepatic phase and the erythrocytic phase — the intracellular malarial parasite requires a huge amount of nutrients as it rapidly multiplies. The malaria parasite, however, is unable to completely synthesize all of its organic nutrients, including sterols, required for its own growth and replication through its own biosynthetic pathways, and in order to maintain viability the parasite has to import these nutrients from the host cell through its enveloping membrane, the parasitophorous vacuolar

membrane (PVM) [8]. A study by Labaied et al. [9] noted that *Plasmodium* can divert and salvage cholesterol from the host cells as it replicates inside the liver cells. Another study by Imrie et al. [10] reported that in the absence of serum, HDL in low concentration is essential for the maintenance of *P. falciparum* growth in *invitro* culture. In higher concentrations, however, HDL is toxic to the parasite within the infected erythrocytes, causing abnormal maturation and death of trophozoites. The malaria parasite is also able to extract cholesterol directly from the blood via a receptor mediated endocytosis pathway [11]. These diversions of cholesterol and lipids from the host by the *Plasmodium* parasite as it rapidly replicates further contribute to the alterations in the plasma lipid profile. Another contributing mechanism leading to alteration in lipid profile is the fact that the host lipids are required by the malaria parasite for the formation of haemozoin, the malaria pigment [12]. Inside the red blood cells the growing malaria parasite progressively consumes and degrades intracellular proteins, including haemoglobin, leading to the formation of free heme. Heme is toxic to the malaria parasite and it is detoxified by lipid-mediated crystallization to biologically inert haemozoin.

Malaria parasite-induced hepatocellular damage leads to abnormal lipid handling by the liver and hence, unable to maintain homeostasis of lipid and lipoprotein metabolism. This causes derangements in plasma lipid profile.

This study, therefore, intends to study the effect of malarial infection on serum lipid profile, and to in addition characterize the pattern of derangements in serum lipid profile induced by malarial infection amongst patients in North Eastern India.

2. MATERIALS AND METHODS

2.1 Selection of Cases and Controls

This study enrolled 29 confirmed malaria patients admitted in Medicine Department of North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences Hospital, Mawdiangdiang, Shillong, between March, 2014 and February, 2015. Twenty nine (29) participants who were without any clinical or laboratory evidence of malarial infection were included as control subjects. Patients excluded from the study include those who were hypertensive, diabetics, suffering from renal diseases, liver disease, obstructive jaundice, cancer, obesity, alcoholism and persons living with human immunodeficiency virus (HIV) infection. The age range of participants was between 18 to 40 years. Clinically suspected malaria cases were confirmed using rapid antibody-based diagnostic card tests (Alere Trueline™ Rapid Diagnostic Kit, Bio Standard Diagnostic, Haryana, India) that detect histidine-rich protein 2 (HRP2) or lactate dehydrogenase antigens in finger-prick blood samples. Confirmation by microscopic detection of malaria parasite was also done.

2.2 Collection and Processing of Blood Specimen

Five milliliters (5 ml) of venous blood was obtained from the anterior cubital vein by sterile venipuncture procedure using 5ml disposable, sterile syringe. The sample was obtained after a 12 hour fasting period for all controls. For all malaria cases, sample was taken on day 2 after a 12 hour fasting period. The blood was collected in di-potassium ethylene-diamine-tetracetic acid (K_2EDTA) tubes and stored at 5^{o}C until required for analyses.

2.3 Microscopic Detection of Malaria Parasite

Thick blood films were made from EDTA blood sample and stained with Giemsa's staining technique. The slides were observed under microscopy using ×100 objectives lenses under oil immersion.

2.4 Biochemical Analysis of Prepared Serum

After centrifugation, serum was decanted and serum HDL, total cholesterol, LDL and triglycerides were estimated using the Beckman Coulter AU chemistry analyzer (USA). HDL and LDL were measured by homogenous enzymatic direct assay [13,14]. Serum cholesterol was measure by CHOD-PAP (cholesterol oxidase – phenol and aminophenazone) enzymatic method as described by Allain et al. [15]. Serum triglycerides were measured by GPO/POD (glycerol phosphate oxidase /peroxidase) enzymatic method [16].

2.5 Statistical Analysis

Descriptive analysis was done and for continuous data, *t*-Test was used to compare

experimented and control groups. A *p*- value of < 0.05 was considered significant. The IBM SPSS 22 (Statistical Package for Social Sciences) was used for statistical analysis.

3. RESULTS

The results obtained are documented in Tables 1-3. Table 1 show the haematological and biochemical characteristics of both the malarial infected patients and control subjects, while Table 2 shows the data on the changes in serum lipid profile induced by malarial infection. The impact of malarial causative parasite strain (*vivax* or *falciparum*) on serum lipids are shown in Table 3.

Malarial infection significantly increased serum urea and liver enzymes —possible indications of renal and hepatic dysfunction.

Data (Table 2) provides evidence which indicates that malarial infection among patients in North Eastern India significantly reduced (*P*<0.05) HDL, LDL and total cholesterol, amounts in serum. These changes show that malarial infection among Indians affects lipid homeostasis.

Data (Table 3) show that the species of *Plasmodium (vivax* or *falciparum)* does not significantly impact serum lipids.

4. DISCUSSION

The results of this study are in agreement with the reports of several other workers who studied the impact of malaria infection on serum lipid profile. Data show characteristic pattern of derangements that include lower levels of HDL, LDL, total cholesterol and higher levels of triglycerides in malaria patients as compared with those for healthy subjects (controls). These changes were all statistically significant with a p-value of < 0.05 (Table 2). Our study found no significant difference in the pattern of lipid derangements induced by the parasite species (*P. vivax* versus *P. falciparum)*. A recent systematic review and meta-analysis of serum lipids and lipoproteins in malaria conducted by Visser et al. [7] similarly showed a statistically significant decrease in serum total cholesterol, serum HDL and serum LDL in malaria patients as compared with reference values obtained from healthy and asymptomatic control subjects. The study also showed increased levels of triglycerides in malaria patients as compared with control values. The study found that the increase in triglycerides in malaria patients was statistically significant when compared with healthy control subjects. The results of this present study strengthen the argument that the pattern of derangement of lipid profile seen in malaria is characteristic and specific for the disease. This is further supported by studies

Table 1. Haematological and biochemical characteristics of recruited subjects

Recruited subjects/patients		
	With malarial	**Without malarial**
Haemoglobin (gm %)	10.20±2.45	12.36±1.01
TLC (cells/cmm)	7250.00±3594.58	6826.92±1616.55
Platelets (X 10^6 cells/cmm)	2.11±2.33	2.62±0.71
Urea (mg/dl)	63.74±77.93*	17.12±9.01
Creatinine (mg/dl)	1.82±2.16	1.01±0.16
SGOT (U/L)	86.00±135.13*	28.08±12.85*
SGPT (U/L)	60.25±82.2*	27.65±10.70*

*Values are expressed as Mean ± SD for n=29 per group. *Significantly higher (P<0.05) than comparable control value. Abbreviations: TLC – total leukocyte count, SGOT – serum glutamic oxaloacetic transaminase, SGPT – serum glutamic pyruvic transaminase*

Table 2. Changes in serum lipids induced by malarial infection in humans

Changes in serum lipids in human subjects		
	With malaria	**Without malaria**
HDL (mg/dL)	16.48±6.49*	41.38±15.11
LDL(mg/dL)	70.45±22.72*	104.46±27.35
Total Cholesterol(mg/dL)	103.52±35.33*	169.54±34.04
Triglyceride(mg/dL)	214.24±109.37*	131.15±30.81

Data are written as Mean ± SD for n=29 subjects per group. HDL- High Density Lipoprotein, LDL – Low Density Lipoprotein

Table 3. Impact of *Plasmodium* species (*vivax* or *falciparum*) on serum lipid among Indians

Changes in serum lipids in human subjects		
	***P. vivax* (n=9)**	***P. falciparum* (n=20)**
HDL	15.78±5.72	16.80±6.93
LDL	75.56±25.94	68.15±21.43
Total Cholesterol	112.22±38.65	99.60±34.04
Triglyceride	222.56±92.30	210.50±118.29

Values are expressed as Mean ± SD for "n" number per group

which show a relation between the level of derangements of lipids and the level of parasitaemia. Mohanty et al. [17] analyzed the serum triglycerides in 60 patients with *falciparum* malaria and in 83 healthy controls. The malaria patients included 37 severe cases and 23 mild cases. It was found that the mean triglyceride level in the severe group (2.53±1.29 mmol/l) was significantly higher than in the mild malaria group (1.72±0.57 mmol/l).

The implications of these findings are manifold. First, the characteristic pattern of derangement in lipid profile in malaria may possibly serve as an aid to the clinical diagnosis of malaria especially in the absence of a positive blood film. In this regard, it is essential to understand that lipid derangements also occur in other infectious diseases. Hence, studies that compare lipid profile derangements between malaria and other infectious diseases are crucial to understanding the diagnostic potential of these lipid derangements in malaria. Visser et al. [7] in their meta-analysis and review of studies that included comparisons between malaria patients and control subjects suffering from other febrile diseases suggested that the observed changes in total cholesterol, HDL and LDL concentrations were more pronounced and statistically significant during malaria than in other febrile diseases. The study concluded that these changes were characteristic and specific for malaria.

Second, there is a relation between the extent of lipid derangements and the severity of malaria infection. Al-Omar et al. [18] in their study which included 200 malaria patients and 200 age-matched healthy blood donors found that there was a significant inverse correlation between parasite count and serum cholesterol level. Higher levels of parasitaemia were associated with lower serum cholesterol levels. In another study by Parola et al. [19] it was found that the serum triglyceride levels were significantly higher in patients with severe *falciparum* malaria than in mild *falciparum* malaria. They suggested that hypertriglyceridaemia may be used as an indicator of severity in *falciparum* malaria.

Finally, these findings may have a possible therapeutic implication. Reis et al. [20] found that chloroquine when combined with lovastatin prevented cognitive impairment in a murine model of cerebral malaria. Statin treatment prevents neuroinflammation and blood brain barrier dysfunction in experimental cerebral malaria in murine model. It concluded that statins may be a valuable adjuvant therapeutic agent for prevention of cognitive impairment in patients with cerebral malaria. This therapeutic effect of statins may, however, be due to their powerful pleiotropic effects that are independent of their cholesterol-lowering properties.

Nevertheless, this study was limited by the small sample size. With only 29 cases, it is difficult to analyze the effect of malaria infection on lipid profile based on smaller subgroups such as age, sex, hepatic dysfunction or species of malaria parasite. This study only compared malaria patients with asymptomatic healthy controls. Comparing the lipid derangements between malaria patients and symptomatic controls due to other infectious diseases could have enriched our understanding and contribute to the emerging facts.

5. CONCLUSION

In conclusion, our study further strengthens the findings in other similar studies detailing the characteristic pattern of derangements in lipid profile in malaria patients. Malaria infection, both *P. vivax* and *P. falciparum* infections, causes derangements in lipid profile that are characterized by low serum total cholesterol, low HDL, low LDL and high triglyceride levels. These derangements may be of diagnostic, prognostic or therapeutic value. Further studies are warranted to fully understand the implications and clinical application of these findings.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Akgun S, Ertel NH, Mosenthal A, Oser W. Postsurgical reduction of serum lipoproteins: Interleukin-6 and the acute-phase response. J Lab Clin Med. 1998; 131:103–8.
2. Coombes EJ, Shakespeare PG, Batstone GF. Lipoprotein changes after burn injury in man. J Trauma. 1980;20:971–5.
3. Budd D, Ginsberg H. Hypocholesterolemia and acute myelogenous leukemia. Association between disease activity and plasma low-density lipoprotein cholesterol concentrations. Cancer. 1986;58:1361–5.
4. Rosenson RS. Myocardial injury: The acute phase response and lipoprotein metabolism. J Am Coll Cardiol. 1993;22:933–40.
5. Bansal D, Bhatti HS, Sehgal R. Role of cholesterol in parasitic infection. Lipid Health Dis. 2005;4:10-16.
6. Gallin JI, Kaye D, O'Leary WM. Serum lipid in infection. N Engl J Med. 1969;281: 1081-1086.
7. Visser BJ, Wieten RW, Nagel IM, Grobusch MP. Serum lipids and lipoproteins in malaria - a systematic review and meta-analysis. Malaria Journal. 2013;12(1):442.
8. Lingelbach K, Joiner KA. The parasitophorous vacuole membrane surrounding *Plasmodium* and Toxoplasma: An unusual compartment in infected cells. J Cell Sci. 1998;111(Pt 11):1467–1475.
9. Labaied M, Jayabalasingham B, Bano N, Cha SJ, Sandoval J, Guan G, et al. *Plasmodium* salvages cholesterol internalized by LDL and synthesized de novo in the liver. Cell Microbiol. 2011; 13:569–586.
10. Imrie H, Ferguson DJ, Carter M, Drain J, Schiflett A, Hajduk SL, et al. Light and electron microscopical observations of the effects of high-density lipoprotein on growth of *Plasmodium falciparum in vitro*. Parasitology. 2004;128:577–584.
11. Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. Receptor mediated endocytosis: Concepts emerging from the LDL receptor system. Annu Rev Cell Biol. 1985;1:1–39.
12. Fitch CD, Cai GZ, Chen YF, Shoemaker JD. Involvement of lipids in ferriprotoporphyrin IX polymerization in malaria. Biochim Biophys Acta. 1999; 1454:31–37.
13. Warnick G, Nauck M, Rifai N. Evolution of methods for measurement of high-density lipoprotein cholesterol: From ultracentrifugation to homogeneous assays. Clin Chem. 2001;47:1579-1596.
14. Esteban-Salán M, Guimón-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-Usandizaga P, Amoroto-Del-Río E. Analytical and clinical evaluation of two homogeneous assays for ldl-cholesterol in hyperlipidemic patients. Clinical Chemistry. 2000;46(8 pt 1):1121-1131.
15. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin. Chem. 1974;20:470-475.
16. Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin. Chem. 1973;19:476-482.
17. Mohanty S, Mishra SK, Das BS, Satpathy SK, Mohanty D, Patnaik JK, et al. Altered plasma lipid pattern in *falciparum* malaria. Ann Trop Med Parasitol. 1992;86:601–606.
18. Al-Omar IA, Eligail AM, Al-Ashban RM, Shah AH. Effect of *falciparum* malaria infection on blood cholesterol and platelets. Journal of Saudi Chemical Society. 2010; 14:83–89.
19. Parola P, Gazin P, Patella F, Badiaga S, Delmont J, Brouqui P. Hypertriglyceridemia as an indicator of the severity of *falciparum* malaria in returned travelers: A

clinical retrospective study. Parasitol Res. 2004;92:464–466.

20. Reis PA, Estato V, da Silva TI, d'Avila JC, Siqueira LD, Assis EF, et al. Statins decrease neuroinflammation and prevent cognitive impairment after cerebral malaria. PLoS Pathogens. 2012;8(12):e1003099.
DOI: 10.1371/journal.ppat.1003099

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14819