

Original research article**A study of lipid parameters in malarial patients attending a tertiary health care centre in Mangaluru****ABSTRACT**

Aim of the study : To estimate the lipid parameters among *Plasmodium vivax* and mixed malaria (*Falciparum* and *Vivax*) infected patients.

Study design: This was a prospective observational and comparative study.

Place and Duration of Study : The Present study was undertaken in the departments of medicine and biochemistry at A.J. Institute of Medical Sciences and Research (AJIMS), Mangaluru, Karnataka between Dec 2017 and May 2018 .

Methods: It was a Prospective observational comparative study. A total of 100 patients (50 *P.Vivax* and 50 Mixed Malaria cases) were consecutively taken in the study. The lipid profiles of the cases were compared with that of 100 healthy volunteers (control group). Data was collected and analysed.

Results: Serum total cholesterol, HDL and LDL levels were significantly low ($P<0.001$) in cases and serum TG and VLDL levels were higher in cases ($P<0.001$) than in control. There were no significant changes in mean serum lipids profiles between *P.Vivax* and Mixed Malaria groups.

Conclusion: The derangement in lipid profiles in *falciparum* malaria was characteristic and specific for the disease. Characteristic changes were lower HDL, LDL and total cholesterol levels and higher TG and VLDL levels in comparison to control groups. These findings may be of diagnostic and prognostic value.

Keywords: *Malaria infection, Mixed Malaria, Lipid profiles*

Introduction

Malaria is an infectious disease that is caused by mosquito, which are basically haemoprotozoal organisms belonging to the Apicomplexa phylum and *Plasmodium* genus. Malaria shows high morbidity and mortality rates in the tropical as well as subtropical regions. Among all *Plasmodium* species causing malaria, *Plasmodium falciparum* is the most pathogenic form. The other forms are *Plasmodium vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*.

Nearly 698 million people all over the world fall under the high risk category as per the World Malaria Report of 2017. Among this figure, India alone accounted for 6% of the cases, followed by 6% of mortality rate, and 51% of *P. vivax* cases throughout the world. The 2017 report also stated 1.31

million malaria cases and 23990 deaths in India [1]. Another study by Murray et al (2012) stated that the deaths due to malaria in India was 46, 800 in the studied year (2010). [2]. However, the cases of malaria caused by *P. falciparum* accounts for 30–90% of all infectious diseases occurring in the forest areas in case of ethnic tribes; and less than 10% of all cases occur in indo-gangetic plains, North and north-western states, as well as south of Tamil Nadu. [3]. Unrestrained development of urban areas, drought, migration issues and lax control efforts have been some of the major contributing factors of all malarial cases in India.

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) [4]. A study by Labaied et al. [5] noted that Plasmodium can divert and salvage cholesterol from the host cells as it replicates inside the liver cells. Imrie et al. [6] stated that for *in vitro* culturing of *P. Falciparum*, the conditions of low HDL concentration with no addition of serum has to be maintained. In case of high HDL concentrations, it shows toxicity towards the growth and development of the parasite within the infected erythrocytes, which can lead to death or abnormal maturation of trophozoites. The malaria parasite is also able to extract cholesterol directly from the blood via a receptor mediated endocytosis pathway [7]. 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 [8]. Inside the erythrocytes, the parasite feeds on the intracellular proteins, including haemoglobin, and degrades it, thus releasing 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.

The study was conducted in the Indian city of Mangaluru (formerly known as Mangalore), located on the shores of Arabian Sea. Due to rapid development in construction activities in the city, this has led to many upsurging events such as chloroquine resistance in *P. falciparum* and the city becoming endemic for malaria. Mangaluru has been one of the first cities in India to adopt the artemisinin-based combination treatment for *P. falciparum*. This study aims at evaluating the effect of malarial infection on serum lipid profile, and in addition, characterizes the pattern of derangements in serum lipid profile induced by malarial infection amongst patients in Mangaluru.

2. MATERIALS AND METHODS

2.1 Selection of Cases and Controls

The Present study was undertaken in the departments of medicine and biochemistry at A.J. Institute of Medical Sciences and Research (AJIMS), Mangaluru, Karnataka between Dec 2017 and May 2018 after approval of institutional ethics committee.

70 Study design: This was a prospective observational and comparative study. This study enrolled 100
71 confirmed malaria patients (50 confirmed cases of P.Vivax and 50 confirmed cases of Mixed malaria
72 (P.Vivax + Falciparum) admitted in Medicine Department of AJIMS.

73 As control, age and sex-matched apparently one hundred healthy participants who were without any
74 clinical or laboratory evidence of malarial infection were included as control subjects. Patient's
75 consent was sort for through an informed consent form.

76 Patients excluded from the study include those who were hypertensive, diabetics, suffering from renal
77 diseases, liver disease, obstructive jaundice, bacterial infections, other viral and parasitic infections,
78 cancer, obesity, alcoholism, H/O drug intake like oral contraceptive pills, steroid, statins and human
79 immunodeficiency virus (HIV) infection.

80 The age range of participants was between 18 to 50 years. Clinically suspected malaria cases were
81 confirmed using rapid antibody-based diagnostic card tests (Alere Trueline™ Rapid Diagnostic Kit,
82 Bio Standard Diagnostic, Haryana, India) that detect histidine rich protein 2 (HRP2) or lactate
83 dehydrogenase antigens in finger-prick blood samples. Confirmation by microscopic detection of
84 malaria parasite was also done.

85 **2.2 Sample Collection**

86 Collection and Processing of Blood Specimen : Following aseptic precautions five millimetres (5 ml) of
87 fasting venous blood sample was collected from each subject on the second day of visiting hospital as
88 baseline sample after the patient has been clinically diagnosed for malaria infection.

89 4 ml of blood sample was collected on a plain vacutainer to assay lipid profile and 1 ml of blood
90 sample was dispensed into di-potassium ethylenediaminetetracetic acid (K2EDTA) vaccutainer
91 bottles for malaria parasite detection on thick blood film.

92 The blood sample was obtained after a 12 hour fasting period for all controls.

93 **2.3 Microscopic Detection of Malaria Parasite**

94 Thick blood film was made from EDTA blood sample and stained with Giemsa's staining technique for
95 malaria parasite detection; observed under microscopy using x40 and x100 objectives lenses, the
96 procedure was described by Monica Cheesbrough [9]. Standard operational procedures were
97 prepared for identification of malaria species and for the detection of malaria parasitemia and smears
98 were examined by a senior medical laboratory scientist. The slide was considered negative when
99 there were no parasites in 100 HPF.

100 **2.4 Biochemical Analysis of Prepared Serum**

101 Biochemical Analysis of Prepared Serum After centrifugation, serum was decanted and serum HDL,
102 total cholesterol, LDL and triglycerides were estimated using the Beckman Coulter AU chemistry
103 analyser (USA). HDL and LDL were measured by homogenous enzymatic direct assay [10, 11].

Serum cholesterol was measure by CHOD-PAP (cholesterol oxidase – phenol and aminophenazone) enzymatic method as described by Allain et al. [12]. Serum triglycerides were measured by GPO/POD (glycerol phosphate oxidase /peroxidase) enzymatic method [13]. LDL concentrations were determined by Friedewald equation. (14). VLDL concentrations were estimated using the assumption that in fasting subjects, the VLDL cholesterol to total plasma triglyceride (TG) ratio is relatively fixed at 1:5.

2.5 Statistical Analysis

Data obtained were analysed using Microsoft excel for statistical analysis to obtain the following descriptive statistics: mean, standard deviation (SD), standard error of the mean (SE) and range. Significant test was done by ANOVA Analysis. A p value of < 0.05 was considered significant. Bonferroni correction was used to reduce the chances of obtaining false-positive results.

RESULTS

Table 1: Age Distribution between Cases and Controls

Groups	N	Age (in years)		ANOVA F	P
		Mean	Std. Deviation		
Vivax	50	29.20	11.90	11.940	<0.001 HS
Vivax + Falciparum	50	31.82	14.29		
Control	100	38.21	9.49		
Total	200	34.36	12.07		

Table 2: Sex Distribution between Cases and Controls

Gender	Group		Total
	Cases	Controls	
Female	13 13.0%	0 0%	13 6.5%
Male	87 87.0%	100 100.0%	187 93.5%
Total	100 100.0%	100 100.0%	200 100.0%

This study was concluded on 100 laboratory confirmed cases of malaria patients of P.Vivax (50) and Mixed malaria- P.falciparum+Vivax (50). There were 100 healthy volunteers taken as control group.

Table 1 here shows the mean ages of patients in P.Vivax group was 29.20±11.90, Mixed Malaria group was 31.82±14.29 and control group was 38.21±12.07 respectively.

Table 2 shows the sex distribution among cases (males-87% and females 13%) and controls (males-87% and females 13%).

Table 3 :showing the mean value for total cholesterol, HDL and LDL was significantly lower in case group 95.21±22.24 mg/dl, 13.24±7.01 mg/dl and 64.85±16.50 mg/dl respectively then in control group of 182.73±17.14 mg/dl ,44.11±7.30 ,and 112.38±17.37 mg/dl respectively (P <0.05). But, serum

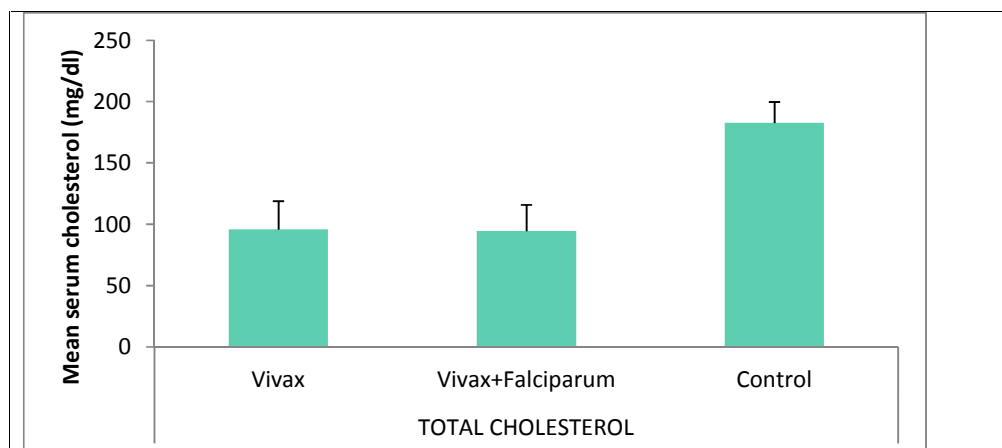
triglyceride and VLDL were significantly higher in case group 146.78 ± 38.59 mg/dl and 29.35 ± 7.27 mg/dl respectively than in control group 132.40 ± 15.51 mg/dl and 24.68 ± 3.68 mg/dl respectively ($P < 0.05$).

Table 3: Comparison of Lipid parameters in cases and controls

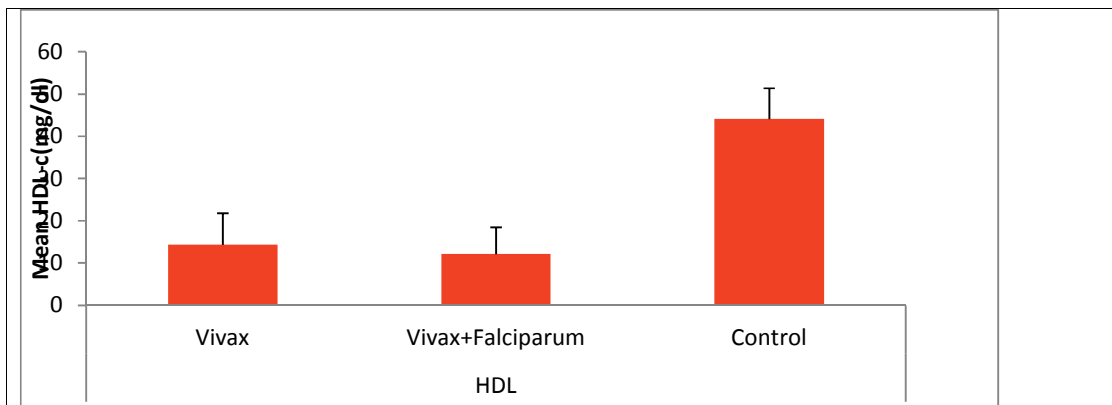
Parameters		N	Mean	Std. Deviation	95% Confidence Interval for Mean		t value	p
					Lower Bound	Upper bound		
Total cholesterol	Cases	100	96.21	22.247	90.80	99.62	31.16	<0.001 HS
	Control	100	182.73	17.142	179.33	186.13		
Triglycerides	Cases	100	146.78	38.598	139.12	154.44	3.46	0.001 HS
	Control	100	132.40	15.510	129.32	135.48		
HDL	Cases	100	13.24	7.014	11.85	14.63	30.49	<0.001 HS
	Control	100	44.11	7.300	42.66	45.56		
LDL	Cases	100	64.85	16.503	61.58	68.12	19.84	<0.001 HS
	Control	100	112.38	17.373	108.93	115.83		
VLDL	Cases	100	29.35	7.727	27.82	30.88	5.46	<0.001 HS
	Control	100	24.68	3.684	23.95	25.41		

The mean values of lipid parameters in the 3 groups (Vivax, mixed malaria and controls) are shown in graphs 1-5

Graph 1: Serum cholesterol levels among the study subjects

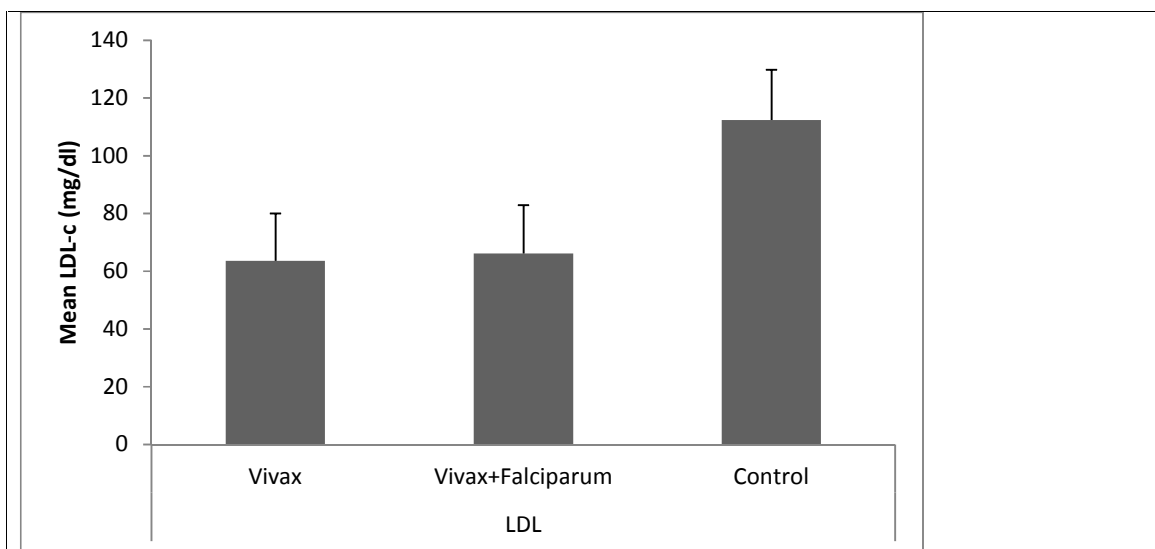


Graph 2: Serum HDL cholesterol levels among the study subjects



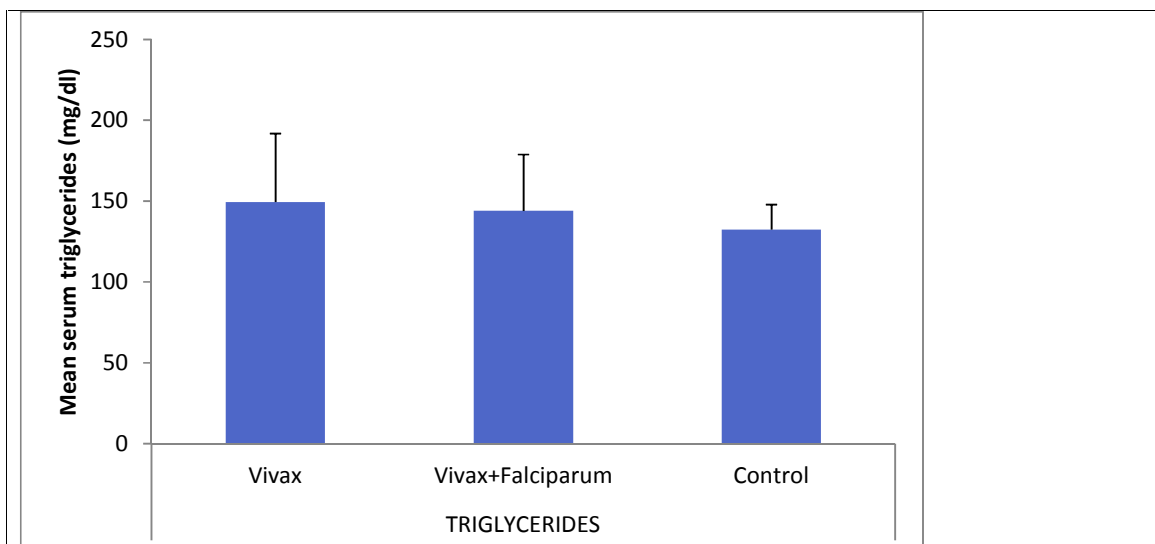
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141 **Graph 3: Serum LDL cholesterol levels among the study subjects**



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143 **Graph 4: Serum triglycerides levels among the study subjects**



Graph 5: Serum VLDL cholesterol levels among the study subjects

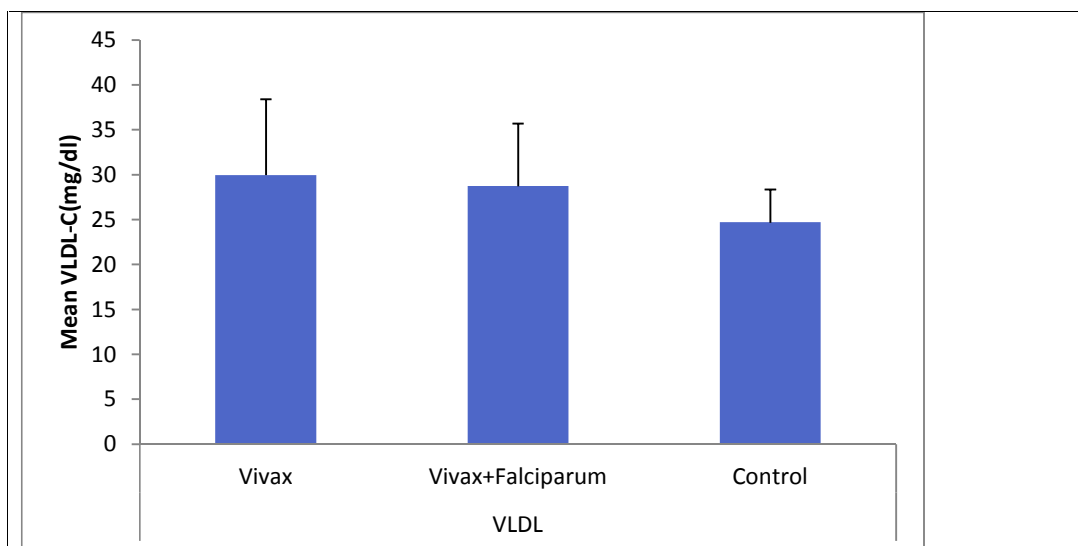


Table 4: Comparison of Lipid and other biochemical parameters in Vivax and Mixed Malaria

Parameters		N	Mean	Std. Deviation	95% Confidence Interval for Mean		t value	p
					Lower Bound	Upper bound		
Total cholesterol	vivax	50	95.84	23.251	89.23	102.45	.282	.779
	Vivax+falciparum	50	94.58	21.412	88.49	100.67		
Triglycerides	vivax	50	149.54	42.203	137.55	161.53	.713	.477
	Vivax+falciparum	50	144.02	34.833	134.12	153.92		
HDL	vivax	50	14.34	7.515	12.20	16.48	1.580	.117
	Vivax+falciparum	50	12.14	6.360	10.33	13.95		
LDL	vivax	50	63.56	16.387	58.90	68.22	-.780	.437
	Vivax+falciparum	50	66.14	16.683	61.40	70.88		
VLDL	vivax	50	29.96	8.442	27.56	32.36	.788	.433
	Vivax+falciparum	50	28.74	6.972	26.76	30.72		

Discussion

In this study, total cholesterol was significantly lower in pv. malaria and mixed malaria in comparison with the non-malarial control group. In line with the present findings, a study by Djoumessi [15] carried out a comparison study between the malaria patients with low cholesterol levels with that of the normal healthy control group.

In the current study, HDL, and LDL levels were found to be low, and VLDL and triglycerides levels were found to be high in case of pv. malaria and mixed malaria as compared to healthy group. Visser et al. [16] similarly showed similar statistically significant results in terms of decrease in serum total cholesterol, HDL and LDL in malaria patients as compared to reference values obtained from asymptomatic control subjects. The study also showed increased levels of triglycerides in malaria

patients as compared with control values which were in accordance with this study. The current study strengthens the argument that the pattern of derangement of lipid profile seen in malaria is characteristic and specific for the disease.

The serum LDL and HDL levels were found to be low in case of patients with moderate malarial infection as stated by Chikezie and Okpara [17]. The results were suggestive of the fact that low LDL levels, which are basically oxidized lipoproteins, have a significant effect in determining the pathogenesis of malaria. Also, the results were in line with the present studies. A significant decrease was observed in HDL, VLDL and total cholesterol levels in the malaria infected group when compared to that of the healthy group [18]. The reduced level of total cholesterol might be due to the reduction in HDL levels, which results in oxidative modification. This in turn increases the intracellular oxidative stress levels in parasitized erythrocytes of patients affected with malaria in addition to extracellular stress resulting out from haemolysed RBCs, which again leads to lipoprotein degradation. . Similar results were reported by few researchers who stated an increase in LDL and HDL levels and a slight increase in TG levels [19, 20, 21].

Sibmoo et al. [22] stated that in comparison to control, serum VLDL and HDL levels were high in malaria patients. It was also noticed that oxidized LDL was found to increase in the endothelial expression of adhesion molecules. The prevalence of malaria parasite infection in male is more as considered to females, which was in line with a previous study [23]. This was because the exposure of the body is more in case of males in case of high environmental temperature, and thus, it increasing their chances of getting bitten. This study also states better immunity in females, which is due to the genetic and hormonal factors [23].

The current study states that acute malaria case shows decrease in HDL, LDL, TG and total cholesterol levels as compared to control.

The implications of this would include the characteristic pattern of derangement in lipid profile in malaria may possibly serve as an aid in diagnosing malaria, especially in the absence of positive blood film. In this regard, it is essential to understand that lipid derangements also occur in other infectious diseases. Visser et al. [16] in their meta-analysis and review of studies included comparison between patients with malaria and control group suffering from other febrile diseases, which suggested that the observed changes in total cholesterol, HDL and LDL concentrations were in increased state and found to be statistically significant in case of malaria as compared to other febrile diseases. The study concluded that these changes were characteristic and specific for malaria.

There is a relation between the extent of lipid derangements and the severity of malaria infection. Al-Omar et al. [24] found a inverse correlation between parasite count and serum cholesterol level. Severe parasitaemia was associated with lower serum cholesterol levels. In another study by Parola et al. [25] it was found that the serum triglyceride levels were found to be high in falciparum malaria patients as compared to mild condition. They suggested that hypertriglyceridemia may be used as an indicator of severity in falciparum malaria.

These findings may have a possible therapeutic implication. Reis et al. [26] found that chloroquine when combined with lovastatin prevented cognitive impairment in the murine model of cerebral malaria. Treatment with statin was found to prevent neurological inflammation and blood brain barrier dysfunction in case of cerebral malaria in murine model. Statins might prove to be one of the valuable adjuvant therapeutic agents used in prevention of cognitive impairment in cerebral malaria patients. This therapeutic effect of statins may, however, be due to their powerful pleiotropic effects that are independent of their cholesterol-lowering properties.

The current study stated that acute malaria infection leads to production of unique perturbation and modifications in the plasma lipoprotein metabolism and plasma lipid profiles.

Nevertheless, this study was limited by the small sample size. 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. A detailed study needs to be performed on Plasmodium biology's core features to understand the disease better. Developing lipidomic, transcriptomic and proteomic profiles of malaria would pave the path towards understanding the metabolism of the casual agents which underlie its molecular aetiology. This will further lead to development of novel treatment approach.

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. A reduction in the levels of lipoproteins may lead to feeling of weakness that indicates high number of parasites in the patient's body. This is because to replicate faster these parasites feed on the lipoproteins of the host cells, which leads to further increase in its surface area and volume of internal membranes. Decrease in some of the lipids might lead to onset of severe malarial infection.

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.

References

1. WHO. World Malaria Report, 2017. WHO. Available at <http://www.who.int/malaria/publications/world-malaria-report-2017/en/>
2. Murray CJL et al. Global malaria mortality between 1980 and 2010: a systematic analysis. The Lancet. 2012; 379(9814):413-431. Doi: 10.1016/S0140-6736(12)60034-8. Available at [http://www.lancet.com/journals/lancet/article/PIIS0140-6736\(12\)60034-8/fulltext](http://www.lancet.com/journals/lancet/article/PIIS0140-6736(12)60034-8/fulltext)
3. Ashwani Kumar, Neena Valecha, Tanu Jain, Aditya P. Dash. Burden of Malaria in India: Retrospective and Prospective View. Am J Trop Med Hyg. 2007; 77(6_Suppl):69-78. Full Text at http://www.ajtmh.org/cgi/reprint/77/6_Suppl/69
4. 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.
5. 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.
6. 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.

- 248 7. Goldstein JL, Brown MS, Anderson RG, Russell DW, Schneider WJ. Receptor mediated
249 endocytosis: Concepts emerging from the LDL receptor system. *Annul Rev Cell Biol.* 1985; 1:1–39.
- 250 8. Fitch CD, Cai GZ, Chen YF, Shoemaker JD. Involvement of lipids in ferriprotoporphyrin IX
251 polymerization in malaria. *Biochim Biophys Acta.* 1999; 1454:31–37.
- 252 9. Monica Cheesbrough. *Discrete Laboratory Practice in Tropical Countries Part 1*, Cambridge
253 Second Editions. Published by Press Syndicate of the University of Cambridge, (2005) chp. 5, page
254 247 -258.
- 255 10. Warnick G, Nauck M, Rifai N. Evolution of methods for measurement of high-density lipoprotein
256 cholesterol: From ultracentrifugation to homogeneous assays. *Clin Chem.* 2001; 47:1579-1596.
- 257 11. Esteban-Salán M, Guimón-Bardesi A, de La Viuda-Unzueta JM, Azcarate-Ania MN, Pascual-
258 Usandizaga P, Amoroto-Del-Río E. Analytical and clinical evaluation of two homogeneous assays for
259 ldl-cholesterol in hyperlipidemic patients. *Clinical Chemistry.* 2000; 46:1121-1131.
- 260 12. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum
261 cholesterol. *Clin. Chem.* 1974; 20:470-475.
- 262 13. Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. *Clin.*
263 *Chem.* 1973; 19:476-482.
- 264 14. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density
265 lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;
266 18:499-502.
- 267 15. Djoumessi S: Serum lipids and lipoproteins during malaria infection. *Pathol Biol*, (1989) 37: 909-
268 911.
- 269 16. Visser BJ, Wieten RW, Nagel IM, Grobusch MP. Serum lipids and lipoproteins in malaria: a
270 systematic review and meta-analysis. *Malaria J.* 2013; 12(1):442.
- 271 17. Chikezie, P.C and Okpara, R.T. Serum Lipid Profile and Hepatic Dysfunction in Moderate
272 *Plasmodium Falciparum* Infection, *Global Journal of Medical research Diseases* (2013) Volume 13
273 Issue 4 Version 1.0 pg 14-20.
- 274 18. Ogbodo, S. O., Ogah, O., Obu, H.A., Shu, E.N. and Afiukwa, C. Lipid and lipoprotein levels in
275 children with malaria parasitaemia. *Current Paediatric Research.* (2008) 12 (1 & 2), 12-17.
- 276 19. Nilsson-Ehle I, Nilsson-Ehle P: Changes in plasma lipoproteins in acute malaria. *J Intern Med*,
277 (1990) 227: 151-155.
- 278 20. Mohanty S, Mishra SK, Das BS, Satpathy SK, Mohanty D, Patnaik JK, Bose TK: Altered plasma
279 lipid pattern in falciparum malaria. *Ann Trop Med Parasitol*, (1992) 86: 601-606.
- 280 21. Faucher, Jean-François & Ngou-Milama, Edouard & Anoumou Missinou, Michel & Ngomo,
281 Raphaël & Kombila, Maryvonne & G Kremsner, Peter. (2002). The impact of malaria on common lipid
282 parameters. *Parasitology research.* 88. 1040-3. 10.1007/s00436-002-0712-6.
- 283 22. Sibmooh, N., Yamanont, P., Krudsood, S., Leowattana, W., Brittenham, G., Looareesuwan, S. and
284 Udomsangpetch, R. Increased fluidity and oxidation of malarial lipoproteins: relation with severity and
285 induction of endothelial expression of adhesion molecules. *Lipids in Health and Disease.* (2004) 3, 15.
- 286 23. Zuk M, McKean KA. Sex differences in parasite infections: patterns and processes. *Inter J*
287 *Parasitol*; (1996) 26: 1009-23.

- 288 24. Al-Omar IA, Eligail AM, Al-Ashban RM, Shah AH. Effect of falciparum malaria infection on blood
289 cholesterol and platelets. J Saudi Chem Soc. 2010; 14(1):83–89.
- 290 25. Parola P, Gazin P, Patella F, Badiaga S, Delmont J, Brouqui P. Hypertriglyceridemia as an
291 indicator of the severity of falciparum malaria in returned travellers: A clinical retrospective study.
292 Parasitol Res. 2004; 92:464–466.
- 293 26. Reis PA, Estado V, da Silva TI, d'Avila JC, Siqueira LD, Assis EF, et al. Statins decrease
294 neuroinflammation and prevent cognitive impairment after cerebral malaria. PLoS Pathogens. 2012;
295 8(12):e1003099.