

Review paper

**Temporal Variation of lipids in patients with uncomplicated malaria due to
*Plasmodium falciparum***

Abstract

Changes in lipid profile are commonly reported in adult patients with malaria, but only a few studies have evaluated such lipid abnormalities in uncomplicated falciparum malaria cases from the South America. This is a prospective study designed to evaluate transient lipid abnormalities in adults with uncomplicated falciparum malaria as well as to assess if the parasite count is correlated with the lipid levels at admission. A total of 60 adult males were included in the study, of which 30 had the slide-confirmed infection by *P. falciparum*. Serial blood samples were collected at admission and on days 3, 14 and 43. Triglycerides, total cholesterol, high-density lipoprotein (HDL-c), and low-density lipoprotein (LDL-c) were measured by colorimetric methods. The levels of total cholesterol, LDL and HDL were significantly lower in malaria cases compared to healthy controls, but the levels of triglycerides were significantly higher in these patients. The lipid levels were not associated with the parasite count. In conclusion, there are significant lipid abnormalities in the adults with low levels of *P. falciparum* infection and mild signs and symptoms of the disease, which are not associated with parasitemia at admission.

Key-words: malaria, lipids, infectious disease, cholesterol

1. Introduction

Malaria by *P. falciparum* is still a relevant public health issue worldwide. In the Brazilian Amazon basin, approximately 15,000 cases occurred in 2015[1]. Repeated episodes of the disease are common in this area conferring to native's a partial immunity, so only a few cases progress to severe illness with cerebral or renal impairment. Overall, patients have low parasite density and mild signs and symptoms [2-4]; however, laboratory abnormalities are

26 often reported in the acute phase of the disease, such as anemia, thrombocytopenia,
27 hypoglycemia, and of the lipid profile. [5-7].

28 The latter is common in the acute or chronic phases of infectious or inflammatory diseases
29 [8,9]. Low levels of total cholesterol, high-density lipoproteins (HDL-c) and low-density
30 lipoproteins (LDL-c) accompanied by high levels of triglycerides and very low-density
31 lipoproteins (VLDL-c) characterize the lipid changes in malaria. Such abnormalities are
32 transient; occur in the most prevalent species of *Plasmodium* as well as in complicated and
33 non-complicated cases [10-15].

34 Despite the high occurrence of malaria in Amazon basin, only a few studies reported lipid
35 changes in this endemic setting. Evaluate the lipid abnormalities in the course of malaria in
36 areas with high transmission rates is relevant since a long-term change in lipid profile can
37 directly influence the risk of coronary disease and other related diseases [13-15]. Moreover,
38 genetic ancestry influences the lipid profile of admixed population as that from South
39 American that has different contributions of European, African and Amerindian ethnic
40 ancestries [16-18]. Thus, the present study aims to evaluate the temporal changes in lipid
41 profile of adults from Brazilian Amazon basin with uncomplicated malaria by *P. falciparum*
42 and to assess if the parasite count correlates with the lipid levels at admission.

43 **2. Material and methods**

44 **Ethical statement**

45 The study was submitted to Plataforma Brasil under protocol CAAE 2 07199612.0.0000.0018
46 and approved under the number 261.593/2013. Informed written consent was obtained from
47 all participants.

48 **Study site and participants**

49 This was a prospective study carried out in the municipality of Anajas in the Marajo
50 Island. Anajas has an area of 6,912 Km² and 27,540 inhabitants. The economy is based on

51 agriculture practices, timber extraction, and fishing. The municipality accounts for 17.4% of
52 malaria cases in the state of Para and has a constant annual incidence of cases above 50/1,000
53 inhabitants. The municipality has seven health public facilities, including an ambulatory for
54 the diagnosis and treatment of malaria.

55 Participants were randomly recruited among those who searched for attendance at Malaria
56 Ambulatory of the municipality with signals and symptoms suggestive of malaria. Two
57 groups of male adults were included in the study: (1) case: male adults with slide-confirmed
58 infection by *P. falciparum* and (2) control: male adult with a negative diagnosis of malaria
59 after microscopic examination of three blood smears. Exclusion criteria included patients
60 with mixed malaria or signs and symptoms of severe malaria (jaundice, renal or pulmonary
61 impairment, severe anemia, altered levels of consciousness), parasitemia above 4%, chronic
62 and other parasitic diseases, overweight and underweight individuals assessed by the body
63 mass index. In the control group, those who have reported a history of malaria infection
64 within the previous twelve months were also excluded.

65 **Treatment and follow-up**

66 Patients with malaria were treated with mefloquine and artesunate according to the
67 recommendation of the World Health Organization [19]. The clinical staff of Malaria
68 Ambulatory was responsible for prescribing and dispensing the anti-malarial drugs. The
69 patients received specific instructions about the administration on each day and were strongly
70 recommended to complete the course of treatment. Data regarding the clinical signals and
71 symptoms and the history of malaria, including the last episode of the disease, were carefully
72 recorded. All patients were requested to return for follow-up on days 3, 14 and 43 or at any
73 time that signs and symptoms suggestive of malaria occurred. A parasite count was
74 performed at each visit.

75 **Blood sampling and laboratory analysis**

76 Serial blood samples were collected in both groups recruited for the study on Days 0, 3, 14
77 and 43. Samples were collected after a fasting of 12 h. Triglycerides, total cholesterol, HDL
78 and LDL were measured by colorimetric methods using Merck-Diagnostic Kits, following
79 the good laboratory practices. All blood samples were analyzed in triplicate. The normal
80 ranges of each lipid followed the recommendations of the Brazilian Cardiology Society [20].

81 **Microscopic examination**

82 Parasite counts were done every 24 hours in Giemsa-stained thick films until clearance.
83 Blood films were examined microscopically using 100X (oil immersion) objectives. Parasite
84 density was expressed as the number of parasites per microliter of blood. This was derived
85 from the number of parasites per 200 white blood cells in a thick film, considering a total
86 white blood cell count of 8,000. The limit of detection of parasites was 40/ μ L [21].

87 **Data analysis**

88 Data are presented as a mean and confidence interval. The Student t-test was used to compare
89 lipid levels between cases and controls on D0. A univariate analysis was used to compare
90 lipid levels amongst days of blood sampling in patients with malaria. The Pearson coefficient
91 of correlation was used to estimate the correlations between parasitemia with triglycerides,
92 total cholesterol, and fractions on D0. Data were analyzed using SPSS software, Release21
93 (IBMinc, Chicago, IL, EUA). The accepted significance level was 5%.

94 **3. Results**

95 A total of 60 male adults met the criteria for the inclusion in the study. The cases of malaria
96 corresponded to 50% (n=30) of the participants. All cases reported previous episodes of the
97 disease. Moreover, all cases showed low parasite count and mild signals and symptoms. The
98 baseline characteristics of participants are shown in Table 1.

99
100

101 Table 1. Baseline characteristics of participants

Characteristic	Cases (n=30)	Controls (n=30)	p-value
Age, years	33(11)	34(12)	0.521
Parasitaemia at admission, geometrical mean	3178 (7.1)		
Previous episodes of disease, number of cases (%)	60	0	<0.0001
	48 (20)		
Parasite clearance, hours			
Fever clearance, hours	24(12)		
Erythrocytes at admission, $\times 10^6/\mu\text{l}$	4.29(0.79)	4.7(0.82)	0.836
Hematocrit at admission, %	36.4(6)	39 (5)	0.331
Hemoglobin at admission, g/dl	11.9(2)	12(2)	0.9
Platelets at admission, $\times 10^3/\mu\text{l}$	136.9(61.1)	240.1(48.2)	<0.001

102

103 The mean total cholesterol level of the cases on D0 was 116 mg/dl (95% CI 103–129 mg/dl),

104 on D3 was 118 mg/dl (95% CI 108–127 mg/dl), on D14 was 144 mg/dl (95% CI 135–152

105 mg/dl) and on D43 was 157 mg/dl (95% CI 147–168 mg/dl). The univariate analysis showed

106 significant changes in cholesterol levels amongst days of the study, with the lowest values on

107 days 0 and 3 ($F=14.95$, $p<0.001$). The healthy controls showed total cholesterol mean level of

108 174 mg/dl (95% CI 162–186 mg/dl), which was significantly high than the cases on D0

109 ($t=6.652$, $p<0.0001$). Although the mean total cholesterol level increased in the follow-up of

110 the cases, they were still below the controls on Day 43 ($t=2.181$, $p=0.033$). On D0, there was

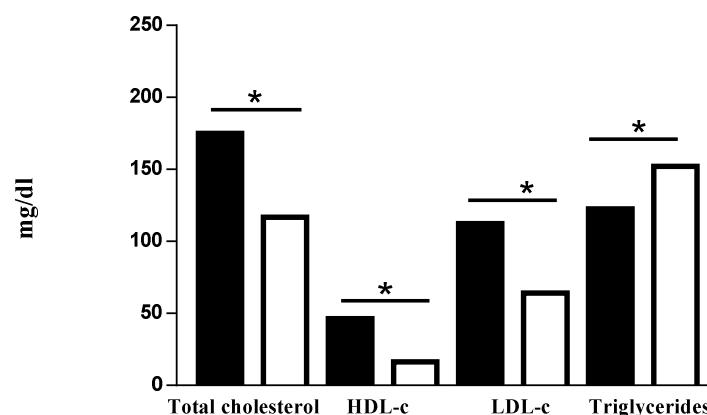
111 no significant correlation between the parasitemia and the total cholesterol ($r=0.2$, $p=0.209$).

112 The mean HDL-c level of the cases on Day 0 was 16.3 mg/dl (95% CI 11.9–20.6 mg/dl), on
 113 Day 3 was 17.4 mg/dl (95% CI 14–20.8 mg/dl), on Day 14 was 30 mg/dl (95% CI 27.3–32.4
 114 mg/dl) and on Day 43 was 35 mg/dl (95% CI 31–37.2 mg/dl). The univariate analysis showed
 115 significant changes in HDL-c levels amongst days of the study with the lowest values on
 116 Days 0 and 3 ($F=28.69$, $p<0.001$). The healthy controls have an HDL-c mean level of 46.3
 117 mg/dl (95% IC 41–51mg/dl), which was significantly high than the cases on D0 ($t=8.99$,
 118 $p<0.0001$). The values of HDL-c of the cases increased in the follow-up, but they were still
 119 below the controls on Day 43 ($t=4.12$, $p=0.001$). At admission, there was no significant
 120 correlation between parasitemia and HDL-c levels ($r=0.0734$, $p=0.699$).

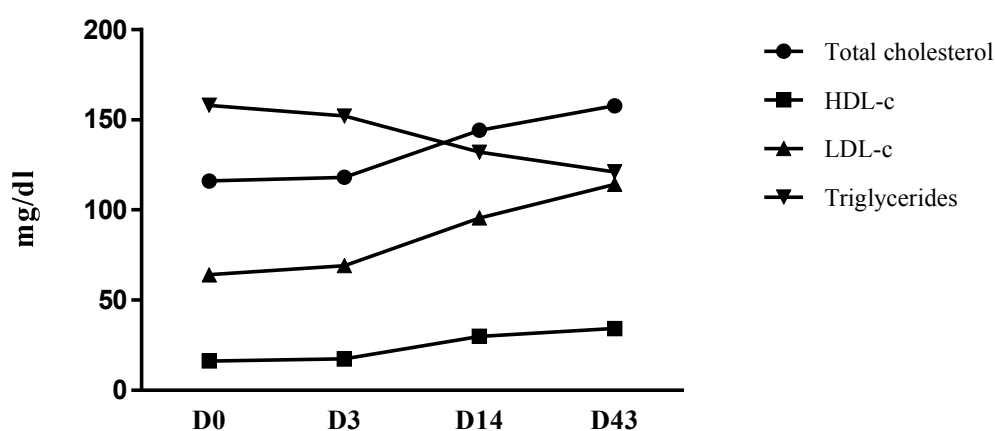
121 The mean levels of LDL-c of cases on Day 0 was 64 mg/dl (95% CI 48–80 mg/dl), on Day 3
 122 was 69 mg/dl (95% CI 55–82 mg/dl), on Day 14 was 95 mg/dl (95% CI 86–105mg/dl) and on
 123 Day 43 was 114 mg/dl (95% CI 104–125 mg/dl). The univariate analysis showed a significant
 124 difference amongst days of the study, with the lowest values on Days 0 and 3 ($F=14.54$,
 125 $p<0.0001$). The healthy controls have an LDL-c mean level of 112 mg/dl (95% CI 102–121
 126 mg/dl), which was significantly higher than the cases on D0 ($t=5.25$, $p<0.0001$). However, the
 127 LDL-c mean levels were similar to the healthy control on Day 43 ($t=0.2914$, $p=0.7718$). On
 128 D0, there was no significant correlation between parasitemia and LDL-c levels ($r=0.1804$;
 129 $p=0.3402$).

130 The mean level of triglycerides of the cases on D0 was 158 mg/dl (95% CI 135–181 mg/dl),
 131 on Day 3 was 152 mg/dl (95% CI 131–173 mg/dl), on Day 14 was 132 mg/dl (95% CI= 112–
 132 153 mg/dl) and on Day 43 was 122 mg/dl (95% CI 102–140 mg/dl). The univariate analysis
 133 showed a significant difference in triglyceride levels amongst days of the study ($F=2.741$;
 134 $p=0.0464$), with the highest values on Days 0 and 3. The healthy controls had a mean level
 135 of 122 mg/dl (95% IC= 98–146mg/dl), which was significantly lower than the cases on D0

136 (t= 1.908, p=0.0615). On D0, there was no significant correlation between the parasitemia
137 and the triglycerides levels (r= -0.2619; p=0.1622). (Figure 1 and Figure 2)



138
139 Figure 1. Lipid levels in patients with uncomplicated falciparum (■) malaria at admission, and in the
140 control group (□).
141 *p<0.05



143
144 Figure 2. Temporal variation of lipid profiles in patients with uncomplicated falciparum malaria

145 4. Discussion

146 The temporal variation of lipid levels was examined in patients with uncomplicated malaria
147 by *P. falciparum* from the South America. At admission, the levels of total cholesterol, HDL-
148 c, and LDL-c were lower in malaria cases than in healthy controls. On the other hand, the
149 levels of triglycerides were high in malaria cases compared to healthy controls. These data

confirm that patients with malaria from the South America have significant changes in lipid levels in the acute phase of the disease, as well as the lipids abnormalities found are similar to uncomplicated falciparum malaria cases from the Africa and the Asia [10-15].

Changes in lipid and lipoprotein levels occur in most patients with active infections caused by parasites, but the exact mechanism is unclear [8,9]. In malaria, such alterations are due to the Plasmodium, to human-related factors, or both. [10,13,14,22, 23]. Plasmodium does not have any biological pathway for sterol synthesis for their metabolic requirements, such as membrane or hemozoin formation, and the cholesterol is obtained from the exogenous and endogenous pathways of the human host [24,25].

Abnormalities in triglycerides levels are probably due to the increase of VLDL production resulting from the excessive mobilization of free fatty acids from adipose tissue in response to stress due to the disease as well as to the impairment of the lipoprotein lipase system mediated through the levels of the tumor necrosis factor. Regard to the total cholesterol and cholesterol-rich lipoproteins, the most significant impact of malaria was on HDL-c. The probable causes are the increased rate of HDL-C removal by Plasmodium, activation of the reticuloendothelial system leading to non-specific uptake of HDL-c in the phagocytic cell and oxidation of lipoproteins due to redox imbalance caused by the disease and by antimalarial drugs. [26-32].

Studies evaluating transient changes in lipid profiles of malaria patients have shown inconclusive results, ranging from a few days to one year[10,12,15]. In the study, the levels of lipids changed differently after the clearance of parasites, as for instance, triglycerides levels in malaria patients were similar to healthy controls on Day 3. LDL-c levels in malaria cases increased in follow-up and were similar to healthy controls only on Day 43. On the other hand, the total cholesterol and HDL-c levels increased slowly compared to LDL-c. In

fact, the total cholesterol and HDL-c levels were significantly lower than to the healthy controls on Day 43.

These changes in lipid profiles in the follow-up can be related to both the clearance of parasites and the improvement of the nutritional status of patients in the convalescence period [9,10,12,30]. Moreover, there were no significant correlations between the parasitemia at admission and the lipids levels. A plausible explanation is the low parasite density at admission in the study.

The data of the current study are relevant in the clinical spectrum of uncomplicated falciparum malaria cases, since they confirm the occurrence of transient changes in lipid levels, with highest abnormalities in the high-density lipoprotein levels. Finally, there are no significant correlations between parasitemia at admission and lipids levels.

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