

Original Research Article

Relationship between E-selectin, acid phosphatase, parasite density and temperature levels in *Plasmodium falciparum* malaria in Oye Local Government, Ekiti State, Nigeria

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Abstract

Purpose: To evaluate the potential of E-selectin and acid phosphatase as markers of disease severity.

Methods: A total of 168 patients comprising 84 malaria patients and 84 healthy patients were selected from three health facilities: General Hospital, Ilupeju, Itapa Ekiti, General Hospital Oye Ekiti and General Hospital, Ayede Ekiti, which collectively represent all public secondary hospitals within Oye Local Government Area, Ekiti State, Nigeria. Blood samples collected were stored in ethylene diamine tetracetic acid (EDTA) bottles and were tested for parasite density. Serum samples were used to determine E-selectin levels of the participants using enzyme-linked immunosorbent assay (ELISA). Acid phosphatase was determined using colorimetric methods. Axillary temperature was measured using a thermometer.

Results: Malaria infection was associated with significantly increased E-selectin and acid phosphatase levels compared with controls ($p < 0.05$). However, correlation analyses indicated no statistically significant relationship existed between E-selectin, acid phosphatase and parasite density. Similarly, only parasite density showed a significant correlation with temperature ($r = 0.272$, $p = 0.012$).

Conclusion: E-selectin and acid phosphatase increase in malaria infection; however, their role as predictors of disease severity is limited. This reveals that both markers are elevated in malaria infections, but neither E-selectin nor acid phosphatase correlates significantly with parasite density or body temperature, which are indicators of disease severity. This finding is crucial because it shows the diagnostic rather than prognostic potential of these markers.

Keywords: Endothelial cells, Cytoadherence, Sequestration, Leukocytes

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INTRODUCTION

Malaria is a life-threatening disease that develops when humans are infected with parasites from the *Plasmodium* family. These parasites are spread through the bites of female *Anopheles* mosquitoes. Currently, *P. falciparum*

accounts for approximately half of the world's malaria cases, with West Africa bearing the largest share [1]. Nigeria alone contributes roughly one-quarter of all global infections and deaths linked to malaria [1].

Endothelial selectin (E-selectin) belongs to the family of type 1 transmembrane glycoproteins and is structurally made up of about 610 amino acids in total [2]. During elongation, E-selectin is formed in endothelial cell lines obtained from umbilical vein and aortic tissues. E-selectin is involved in leukocyte adhesion. E-selectin is expressed on the luminal surface of endothelial cells, with expression markedly increased during vascular inflammation. E-selectin expression is upregulated during inflammation, primarily in response to cytokines such as interleukin-1 and tumor necrosis factor- α [3]. E-selectin in host tissues helps white blood cells stick together and enter tissues, suggesting that it contributes to malaria disease processes.

Malaria parasite density (MPD) is the quantification of the count of asexual parasites present in a defined volume of peripheral blood. It is often reported as per microliter of blood. Malaria parasite density is a valuable tool for predicting disease outcome, severity, and clinical response to antimalarial treatment. Sequestration and breakdown of infected as well as healthy red blood cells are the major means by which *Plasmodium* parasites induce malaria pathology in human hosts. Mature parasites release merozoites that invade red blood cells, where they multiply asexually, producing factors that are toxic to the red blood cell [4]. The more often the cycles of asexual multiplication occur within infected red blood cells, the more often the erythrocytes become filled with parasites at different stages of development.

Acid phosphatase is divided into serine/threonine-specific protein phosphatases or tyrosine-specific protein phosphatases [5]. Acid phosphatase catalyzes the reversible hydrolysis of phosphoanhydride bonds to yield inorganic phosphate. When parasites invade and egress from erythrocytes, tissue damage might occur, and parasite phosphatases and parasite release may induce host phosphatase responses. This might lead to a release of lysosomal enzymes from erythrocytes to initiate the host phosphatase response [6]. Fever helps synchronize parasite maturation stages, particularly via temperature fluctuations. Recurring fever episodes promote intraerythrocytic development of *P. falciparum* by activating a PfHsp90 multi-chaperone complex [7].

METHODS

Study site

The study was conducted in three health facilities: General Hospital, Ilupeju/ Itapa Ekiti,

General Hospital Oye Ekiti and General Hospital, Ayede Ekiti. which collectively represent all public secondary hospitals within Oye Local Government Area, Ekiti State.

Study design

The study was cross-sectional and only those who had symptoms of malaria and gave consent during their first visit were enrolled.

Ethical consideration

Ethical approval was obtained from the Hospital Management Board (HMB/AD/742/2025/09/032). The Author completed certified research ethics training through the CITI Program, and informed consent was obtained from all participants.

Sample collection

A total of 84 subjects and 84 controls were used for this study. Five millilitres of venous blood were collected and dispensed into serum separator tubes (SST) and EDTA bottles for each participant, respectively. The collected blood samples were used for malaria diagnosis and malaria parasite density determination. Following a 5-minute centrifugation at 1500 revolutions per minute (RPM), plasma and serum were aliquoted and stored at -20 °C until analysis.

Malaria diagnosis

Malaria screening of all participants was carried out using rapid diagnostic test kit (RDT). Thick and thin blood films were prepared and air-dried. The thin blood film was fixed with absolute alcohol. A 3 % Giemsa stain was added to the slides for 45 minutes. It was rinsed and air-dried. The slides were viewed using light microscope.

Parasite density determination

Parasite counting was carried out using thick blood films stained with Giemsa. Microscopic examination was performed with a light microscope using the $\times 100$ oil-immersion objective. White blood cells and malaria parasites were counted simultaneously during examination.

Parasite counting was performed by examining either 200 or 500 white blood cells. When 100 or more parasites were observed within 200 white blood cells, counting was discontinued and the result was recorded as parasites per 200 white blood cells. When fewer than 100 parasites were observed, counting was continued up to 500 white blood cells and the result was recorded as

parasites per 500 white blood cells. All parasites and white blood cells present in the final microscopic field were included in the count.

Measurement of E-selectin using enzyme-linked immunosorbent assay (ELISA)

E-selectin concentrations were measured in serum samples using a commercial ELISA kit (Abcam, Cambridge, UK) following the manufacturer's instructions. Samples were diluted 1:10, and absorbance readings were compared to a serially diluted standard curve. Duplicate measurements for each sample and control were averaged, and background was corrected using the zero standard. Concentrations were calculated from the log-log standard curve and adjusted for the dilution factor. Optical density readings were taken at 450 nm within 15 minutes using an ELISA reader (Techan, China).

Acid phosphatase measurement

Serum acid phosphatase was assayed using a commercial kit from Agappe Diagnostics (Kerala, India) following the manufacturer's protocols. Enzyme activities were determined spectrophotometrically based on the formation of a colour reaction product. The serum acid phosphatase concentrations were read with the Genrui semi-auto chemistry analyser (WP21A).

Statistical analysis

Data were analysed using IBM SPSS Statistics version 23. Descriptive statistics were used to summarize the study variables, while inferential statistics were applied to test the research hypotheses. Differences between groups were assessed using Student's t-test, and associations between variables were evaluated using Pearson's correlation coefficient. Statistical significance was defined as $P < 0.05$.

RESULTS

Table 1: Comparative analysis of the demographic, biochemical and clinical parameters between malaria subjects (n 84) and healthy control group (n = 84)

Variable	Group	Mean±SD	P-value
Age (Years)	Malaria subjects	17.76±14.59	0.000*
	Control	29.14±18.41	
E- selectin (ng/mL)	Malaria subjects	309.62±357.82	0.000*
	Control	63.23±39.49	
Acid phosphatase (µg/L)	Malaria subjects	5.61±1.27	0.000*
	Control	3.07±0.83	
Parasitemia (Parasite/µL)	Malaria subjects	42862.01±6524.13	-
	Control	0	
Body temperature (°C)	Malaria subjects	37.17±1.31	0.05
	Control	36.84±0.43	

Study group comprised 84 subjects who tested positive for malaria infection; the study group's mean age was 17.76 ± 14.59 years. The healthy control group comprised 84 subjects with a mean age of 29.14 ± 18.41 years. There was a significant difference in ages between the study group and control group (Table 1). The mean E-selectin level in the malaria-infected group (309.62 ± 357.82) was higher than the mean control group (63.23 ± 39.49), with $p < 0.05$. There was significant statistical difference in E-selectin between the study group and control group (Table 1). Pearson correlation between E-selectin and parasite density was weak and negative ($r = -0.090$, $p = 0.416$), suggesting no significant linear relationship (Table 2). Malaria-infected individuals exhibited significantly higher levels of mean acid phosphatase (5.61 ± 1.27) relative to mean controls (3.07 ± 0.84), with a highly significant difference ($p < 0.05$) (Table 1). A weak positive correlation existed between acid phosphatase and parasite density ($r = 0.114$, $p = 0.301$), which was not statistically significant (Table 2).

In Table 2, the correlation between E-selectin and acid phosphatase was weak and not statistically significant ($r = 0.118$, $p = 0.286$). Parasite density demonstrated a statistically significant correlation with body temperature ($r = 0.272$, $p = 0.012$), while E-selectin ($r = 0.031$, $p = 0.782$) and Acid phosphatase ($r = 0.058$, $p = 0.597$) showed no statistically significant associations (Table 3).

DISCUSSION

E-selectin is upregulated in malaria infection, likely reflecting endothelial activation resulting from inflammation induced by *Plasmodium falciparum*. This finding supported Dos-Santos research that Plasma levels of soluble E-selectin were increased in subjects presenting with *falciparum* and *vivax* malaria compared to non-malarial controls [8].

Table 2: Correlation between E- selectin, parasite density and acid phosphatase in malaria subjects

Variable	E-selectin	Parasite density	Acid phosphatase
E-selectin	1	-0.090	0.118
P-value	-	0.416	0.286
Parasite density	- 0.090	10.416	0.114
P-value	-		0.301
Acid phosphatase	0.118	0.114	1
P-value	0.286	0.301	-

Table 3: Correlation between biomarkers and temperature in malaria subjects (n = 84)

Variable	Pearson correlation	P-value
E- selectin vs Temperature	0.031	0.782
Parasite density vs Temperature	0.272*	0.012
Acid phosphatase vs Temperature	0.058	0.597

E-selectin levels do not increase in proportion to parasite load and may instead reflect general immune activation rather than parasitaemia severity. This finding supported Yeo *et al*, who reported that E-selectin levels increased in severe malaria cases but were inversely correlated with parasitaemia, suggesting that endothelial activation may occur independently or precede high parasite levels [9]. The findings of this research were inconsistent with those of John *et al* who reported that elevated parasite density was positively correlated with increased levels of circulating E-selectin [10]. Hemolysis and macrophage activation during malaria increased serum acid phosphatase. This finding agrees with Yogi *et al*, who carried out clinical cross-sectional study and reported that serum acid phosphatase was significantly elevated in persons affected by malaria [11].

Although acid phosphatase (ACP) is elevated in infected individuals, it does not correlate strongly with parasitemia, suggesting that other factors influenced its level during malaria infections. Kapoor *et al* [12] also found that serum acid phosphatase was significantly elevated in malaria patients. Acid phosphatase was discussed as a potential marker, but it does not provide a direct association with parasite density [12]. The weak correlations between E- selectin and Acid phosphatase suggest that both markers are elevated in malaria; they may be regulated by different biological mechanisms or pathways, such as endothelial activation versus immune cell activity. These biomarkers were mostly studied independently in malaria cases against healthy controls or other biomarkers. D'Souza *et al* reported an independent increase in acid phosphatase in malaria-infected subjects [13]. Given the elevated levels of E-selectin and acid phosphatase in infected individuals, both E-selectin and acid phosphatase showed potential as biomarkers for identifying malaria presence. However, E-selectin and acid phosphatase lack significant correlation with parasite density,

limiting their capacity to indicate disease severity. Ejezie and Ezedinachi reported in Calabar, Nigeria, that parasite density rose with temperature [14]. Salinas [36] in Peru reported the same findings. Sowunmi *et al* [15] observed that a statistically significant positive relationship existed between parasitemia and body temperature ($r = 0.26$; increase of ~350,000 parasites/ μL associated with a 1°C rise above 37.5°C). Although D'Souza *et al* [13] reported that acid phosphatase levels were increased in malaria cases, no consistent positive association with temperature was observed. Browner and Haber worked on serum phosphate and not acid phosphatase enzyme, which showed a strong inverse relation with body temperature in malaria patients [16].

CONCLUSION

The significantly higher levels of E-selectin and acid phosphatase in malaria-infected subjects were evaluated against those of healthy control subjects, which suggested that these biomarkers may be useful indicators of malaria infections. E-selectin and acid phosphatase elevation reflect the biological response to *Plasmodium falciparum* infection, where E-selectin indicates endothelial activation and inflammation, and acid phosphatase reflects lysosomal activity due to immune response or hemolysis. These markers could be incorporated into diagnostic panels to help confirm malaria, especially in complex or atypical clinical presentations. Although E-selectin and acid phosphatase levels were elevated in malaria cases, they did not significantly correlate with parasite density or body temperature, which is an indicator of disease severity. The implication is that these biomarkers may not be suitable for predicting how severe the malaria infection is. E-selectin and acid phosphatase cannot reliably predict clinical outcomes or guide decisions around hospitalization or intensive treatment.

The absence of significant correlation between the studied markers and temperature underlines the need to identify or combine multiple biomarkers to improve diagnostic accuracy and prognostic assessment. Future studies should explore multi-biomarker approaches or investigate other molecules (e.g., cytokines, lactate, or C-reactive protein) alongside E-selectin and acid phosphatase to enhance clinical utility. Sample size should also be increased to detect true differences when they exist and to reduce the influence of outliers.

DECLARATIONS

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Ethical approval

It was obtained from the Hospital Management Board, Ado Ekiti, Ekiti State (no. HMB/AD/742/2025/09/032).

Use of Artificial Intelligence

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and analysed during the study are available from the corresponding author upon request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the four authors named in this article, and the authors will bear all liabilities related to claims in the content of this article.

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