



# Comparative Evaluation of the Serum Micronutrients in Malaria-infected Patients and Healthy Subjects

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## Authors' contributions

This work was carried out in collaboration between both authors. Author MTP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author CMM managed the analysis of the study. Authors MTP and CMM managed the literature searches. Both authors read and approved the final manuscript.

## Article Information

DOI: <https://doi.org/10.9734/ajrid/2025/v16i4433>

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://pr.sdiarticle5.com/review-history/131946>

Original Research Article

Received: 02/01/2025

Accepted: 04/03/2025

Published: 08/03/2025

## ABSTRACT

**Background:** Malaria, an endemic infection within tropical regions across the globe, is known to have a high level of malnutrition burden.

**Aim:** To determine the serum level of micronutrients in healthy subjects and malaria-infected people.

**Methods:** The study used a cross-sectional approach to sample 400 subjects with random selection techniques. 200 were selected from malaria-infected subjects while 200 subjects were taken from healthy subjects. Thick and thin film smears were viewed microscopically to

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confirm positive and negative subjects. An automated analyzer was used to measure micronutrients.

**Results:** All control subjects were significantly higher ( $p < .0001$ ) than the malaria-infected patients for calcium, magnesium, phosphate and iron. Micronutrients that showed a significant comparison of means using one-way analyses of variance were iron ( $p < .0001$ ), phosphate ( $p = .0023$ ), and calcium ( $p < .0001$ ). The post hoc analyses showed that for the iron parameter, subjects within the age range of 1 to 10 years ( $10.4 \pm 1.4 \mu\text{mol/L}$ ) exhibited significantly lower serum levels compared to 21-30 years ( $14.2 \pm 1.4 \mu\text{mol/L}$ ,  $p < .0001$ ), 31-40 years ( $14.6 \pm 1.2 \mu\text{mol/L}$ ,  $p < .0001$ ), and  $\geq 41$  ( $12.8 \pm 1.4 \mu\text{mol/L}$ ,  $p < .0089$ ). Similarly, people within the age range of 11 to 20 years ( $11.6 \pm 2.1 \mu\text{mol/L}$ ) showed significantly lower serum levels of iron compared to those people within the age ranges of 21 to 30 years ( $14.2 \pm 1.4 \mu\text{mol/L}$ ,  $p = .0039$ ) and 31 to 40 years ( $14.6 \pm 1.2 \mu\text{mol/L}$ ,  $p = .0007$ ). All the correlations between the age and the malaria infection showed significant correlations for all the micronutrients with p-values of  $< .0001$ . On the contrary, the healthy subjects only had significant correlations for calcium and phosphate. Calcium showed significant correlations for both control and healthy subjects but with moderate correlations with malaria-infected people having a positive correlation ( $r = 0.6823$ ), while healthy subjects had negative correlation ( $r = -0.6753$ ).

**Conclusion:** Micronutrients such as iron, phosphate, calcium and magnesium were significantly higher in healthy subjects than malaria-infected subjects. This implies that these nutrients could play a crucial role as a likely risk factor for the infection.

**Keywords:** Serum micronutrient; cytokines; malaria infection; clinical studies.

## 1. INTRODUCTION

Malaria is a serious tropical infectious disease with an estimated 263 million current infections and 597,000 annual mortalities. Africa has a disproportionately high burden of this infection with over 94% of the infection (World Health Organization, 2024). Malaria in humans is caused by five species of the *Plasmodium* parasite, with *P. falciparum* and *P. vivax* being the most dangerous. *P. falciparum* is the most lethal and widely found across Africa, while *P. vivax* is the predominant species in many regions outside sub-Saharan Africa (Mbanefo and Kumar, 2020). The other species capable of infecting humans include *P. malariae*, *P. ovale*, and *P. knowlesi* (Abdulkareem et al., 2017, Benzecry et al., 2016). Previous studies have shown consistent evidence associating micronutrient deficiencies and malaria incidence. These reduced levels of micronutrients such as zinc, copper, and iron have been linked with the competition by *Plasmodium* species with the host (Dinga et al., 2024).

Micronutrient deficiency is associated with several infections due to their in the immune system's function. Deficiency in micronutrients has been traced to developing nations especially sub-Saharan Africa (Bailey et al., 2015). It has been discovered that the reverse is also the case whereby deficiency in certain micronutrients

predisposes patients to malaria incidence, while malaria itself has been associated with malnutrition and possibly micronutrient deficiencies (Muriuki et al., 2021, Bailey et al., 2015). Micronutrients are essential elements required by organisms in varying quantities throughout life to orchestrate a range of physiological functions to maintain health. Calcium (Ca), iron (Fe), magnesium (Mg), zinc (Zn) and inorganic phosphate (P) are essential trace elements, and their plasma levels change during most infections. It was also proposed that some trace element concentrations change in malaria patients (Gombart et al., 2020). However, it is not yet known whether the causes of these changes are a result of specific deficiencies from imbalances in the defense strategies regulated by acute-phase proteins.

Although required in very small amounts, elements such as Ca, Mg, P and Fe are vital for maintaining health (Dinga et al., 2024). Also referred to as macro and micro-minerals, these elements are part of enzymes, hormones and cells in the body. However, data about significant variations of these micronutrients in the course of infections in developing countries should be kept under surveillance. This is most important in places where problems of malnutrition and infection are common. There is a paucity of data on the serum level of micronutrients in malaria-infected patients. Owing to the numerous benefits of micronutrients in the improvement of

human immunity and health, little has been established on the association between parasitic infection and micronutrient levels. Hence, this study aimed to assess the serum level of micronutrients in malaria-infected subjects in comparison to those of healthy subjects and ascertain the relationship between these nutrients and other variables.

## 2. MATERIALS AND METHODS

### 2.1 Study Site

The location of the study was Rivers State University. The university is located at latitude 4° 47' 54" N to 4° 48' 55" N and longitude 6° 59' 23" E to 6° 58' 57" E. It is approximately 1.77 km<sup>2</sup>.

### 2.2 Study Design

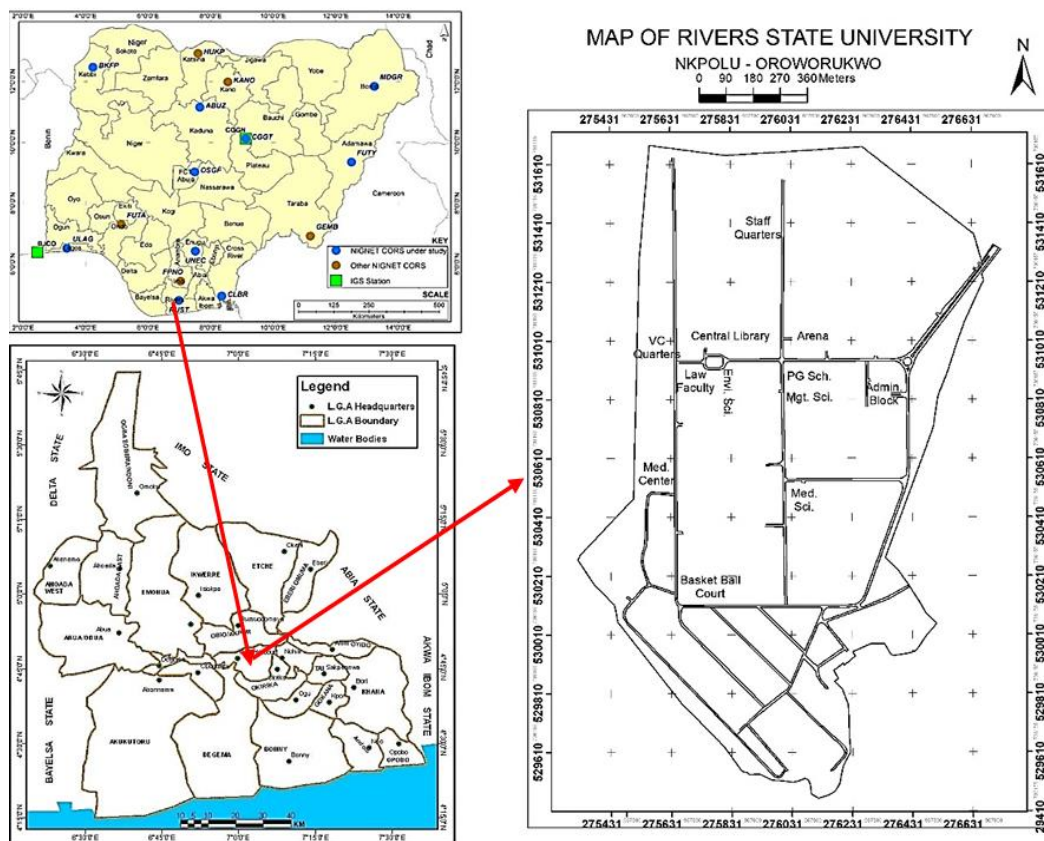
The study adopted a cross-sectional study approach where all the sampling was done at a single time point between December 2022 to November 2023.

### 2.3 Inclusion and Exclusion Criteria

Individuals who have other forms of infection or illness were not eligible for the study. People on medications were also not eligible for the study. The age included were people between 1 to 60 years. Only people who had malaria infection were eligible for the study adopted as an exposure condition.

### 2.4 Sample Size and Study Population

Varied prevalence of malaria infection has been published by several papers: 42% (Onosakponome et al., 2022), and 2.4% (Elekima et al., 2024). We used a prevalence of 2.4% determined by Elekima et al. 2024. Using Cochran's equation of sample size =  $\frac{Z.Z.p.q}{e.e}$ , the appropriate sample size given the specified combination of precision, confidence and variability was 36. However, for a more robust sample size representation, the sample size adopted by our study was 200 each for the control subjects and malaria-infected subjects.



**Fig. 1. Map of Rivers State University**  
(adapted from Pepple et al., 2024)

## 2.5 Sample Collection

Blood samples of 5 mL were collected into EDTA bottles from each control and malaria-infected subject. Samples for micronutrient analysis were spun at 3000 revolutions per minute for 5 minutes. The plasma was collected into plain bottles and stored for future analyses.

## 2.6 Evaluation of Malaria Infection

Malaria parasites were detected by microscopy technique using thick and thin films according to the technique described by Obisike et al. (2024).

## 2.7 Laboratory Determination of Micronutrients

The plasma level of micronutrients was analyzed using the atomic absorption spectrophotometer described in Olaniyi and Arinola (2007).

## 2.8 Data Analyses

The results were presented as Mean $\pm$ SD and bar charts. The statistical package used was GraphPad Prism. The statistical tools used for the analysis were one-way analysis of variance (ANOVA), Chi-square, and t-test. The statistical significance was considered at a 95% confidence interval.

## 3. RESULTS

### 3.1 Impact of Malaria Infection on Serum Micronutrient Level

Figs. 2A to D show the comparative levels of some serum micronutrient levels in healthy subjects and malaria-infected patients. All the healthy subjects showed significantly higher serum levels of micronutrients ( $p < .0001$ ) than the malaria-infected patients for calcium, magnesium, phosphate and iron.

### 3.2 Age-Dependent Serum Level of Micronutrients in Malaria-Infected Patients

Table 1 depicts the trends in the amounts of micronutrients across different age groups of the sampled population. Overall observation noted is an increment in the serum levels of these micronutrients with age. Those that showed significant comparison of means using one-way analyses of variance were iron ( $p < .0001$ ),

phosphate ( $p = .0023$ ), and calcium ( $p < .0001$ ). More specifically, the post hoc analyses showed that for the iron parameter, subjects within the age range of 1 to 10 years ( $10.4 \pm 1.4 \mu\text{mol/L}$ ) exhibited significantly lower serum levels compared to 21-30 years ( $14.2 \pm 1.4 \mu\text{mol/L}$ ,  $p < .0001$ ), 31-40 years ( $14.6 \pm 1.2 \mu\text{mol/L}$ ,  $p < .0001$ ), and  $\geq 41$  ( $12.8 \pm 1.4 \mu\text{mol/L}$ ,  $p < .0089$ ). Similarly, people within the age range of 11 to 20 years ( $11.6 \pm 2.1 \mu\text{mol/L}$ ) showed significantly lower serum levels of iron compared to those people within the age ranges of 21 to 30 years ( $14.2 \pm 1.4 \mu\text{mol/L}$ ,  $p = .0039$ ) and 31 to 40 years ( $14.6 \pm 1.2 \mu\text{mol/L}$ ,  $p = .0007$ ). For the phosphate micronutrient, only two groups showed significant comparison: 1-10 years ( $0.68 \pm 0.12 \mu\text{mol/L}$ ) and  $\geq 41$  years ( $1.02 \pm 0.40 \mu\text{mol/L}$ ,  $p = .0058$ ) as well as 11-20 years ( $0.71 \pm 0.12 \mu\text{mol/L}$ )  $\geq 41$  years ( $1.02 \pm 0.40 \mu\text{mol/L}$ ,  $p = .0143$ ).

### 3.3 Correlation of Age and the Micronutrients in Malaria-Infected Patients and Healthy Subjects

Table 2 represents the correlation between age and micronutrients for malaria-infected subjects and healthy subjects. While all the correlations between age and malaria infection showed significant correlations for all the micronutrients with p-values of  $< .0001$  for each micronutrient, the healthy subjects only had significant correlations for calcium and phosphate. Interestingly, only the calcium showed significant correlations for both control and healthy subjects but with moderate correlations with the malaria-infected people having a positive correlation ( $r = 0.6823$ ), while healthy subjects had a negative correlation ( $r = -0.6753$ ).

### 3.4 Trend in Micronutrients

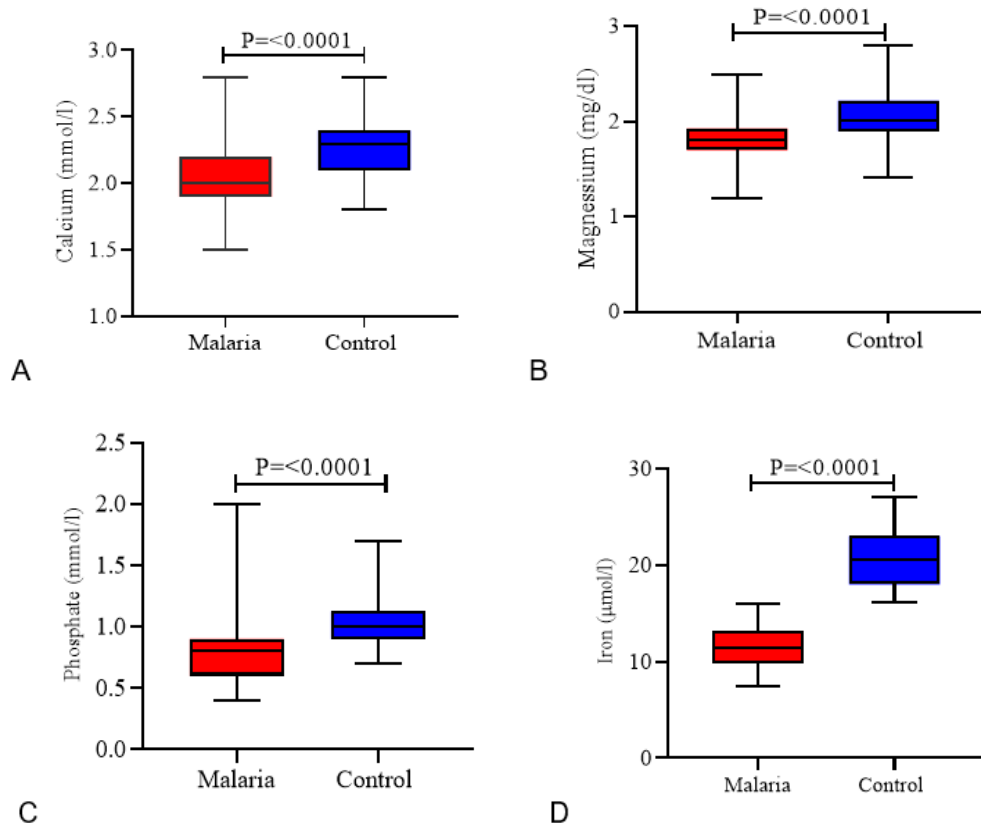
Figs. 3A to D represent the trend in the micronutrients using ages. The graphs show that all the nutrient levels were higher in healthy subjects compared to malaria-infected subjects. Notably, two micronutrients, calcium and phosphate, after the age of 30 years, had equal serum levels. In iron and magnesium, there was a gradual age-dependent increase in the serum level of these micronutrients.

### 3.5 Effect of Sex on Micronutrients in Malaria-Infected Patients and Healthy Subjects

Figs. 4A to D showed a sub-characterization of the micronutrient serum level in male and female

patients. This sex-based comparison helps to further provide more specific comparative serum levels of the micronutrients. The micronutrient

serum levels were higher in control than malaria-infected patients for both males and females.



**Fig. 2. Serum Level of micronutrient in malaria-infected subjects and controls**  
Bars represent mean  $\pm$  SD of 200 subjects each for control and malaria-infected subjects.

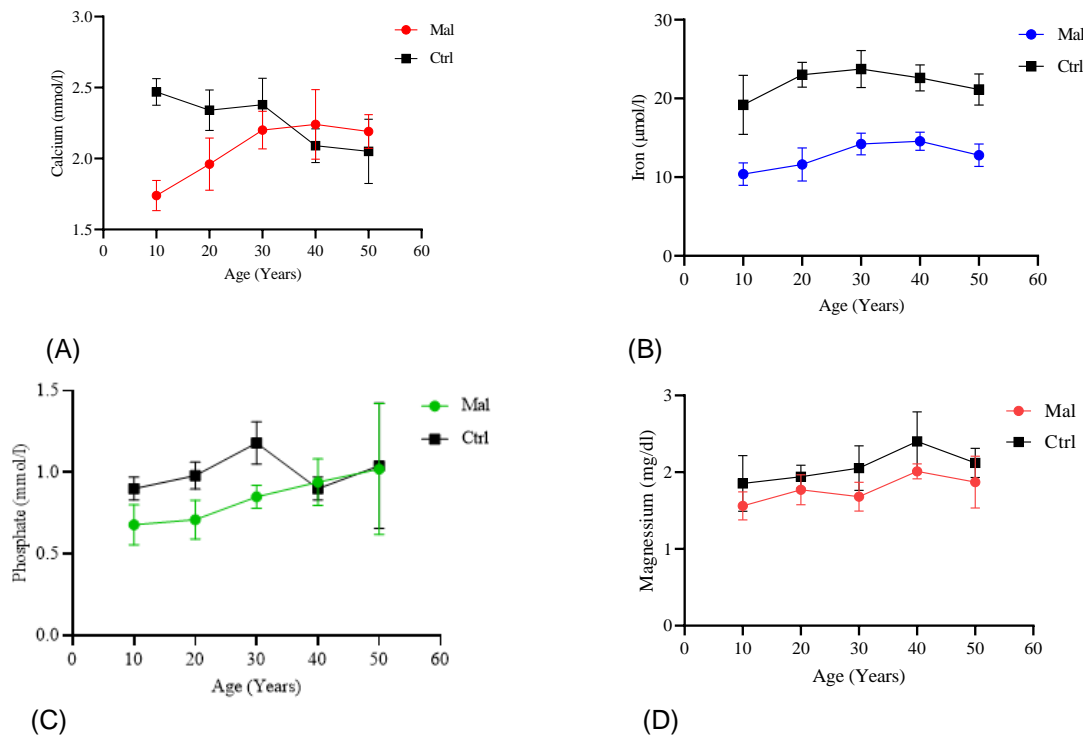
**Table 1. Trends in the serum level of micronutrients across different ages**

Age groups (Years)	Fe ( $\mu$ mol/L)	P ( $\mu$ mol/L)	Mg ( $\mu$ mol/L)	Ca ( $\mu$ mol/L)
1-10	10.4 $\pm$ 1.4	0.68 $\pm$ 0.12	1.85 $\pm$ 0.4	1.74 $\pm$ 0.11
11-20	11.6 $\pm$ 2.1	0.71 $\pm$ 0.12	1.94 $\pm$ 0.15	1.96 $\pm$ 0.18
21-30	14.2 $\pm$ 1.4	0.85 $\pm$ 0.71	2.05 $\pm$ 0.29	2.20 $\pm$ 0.13
31-40	14.6 $\pm$ 1.2	0.94 $\pm$ 0.14	2.40 $\pm$ 0.39	2.24 $\pm$ 0.25
$\geq 41$	12.8 $\pm$ 1.4	1.02 $\pm$ 0.40	2.12 $\pm$ 0.19	2.19 $\pm$ 0.12
p-value	<.0001	.0023	.0692	<.0001
<b>Tukey's multiple comparisons</b>				
1-10 vs. 11-20	.4064	.9976	.9896	<b>.0372</b>
1-10 vs. 21-30	<b>&lt;.0001</b>	.3717	.8125	<b>&lt;.0001</b>
1-10 vs. 31-40	<b>&lt;.0001</b>	.0561	.0535	<b>&lt;.0001</b>
1-10 vs. $\geq 41$	<b>.0089</b>	<b>.0058</b>	.6094	<b>&lt;.0001</b>
11-20 vs. 21-30	<b>.0039</b>	.5654	.9705	<b>.0186</b>
11-20 vs. 31-40	<b>.0007</b>	.1157	.1320	<b>.0041</b>
11-20 vs. $\geq 41$	.4233	<b>.0143</b>	.8629	<b>.0265</b>
21-30 vs. 31-40	.9827	.8685	.3615	.9828
21-30 vs. $\geq 41$	.2569	.3717	.9962	>.9999
31-40 vs. $\geq 41$	.0882	.9101	.5635	.9612

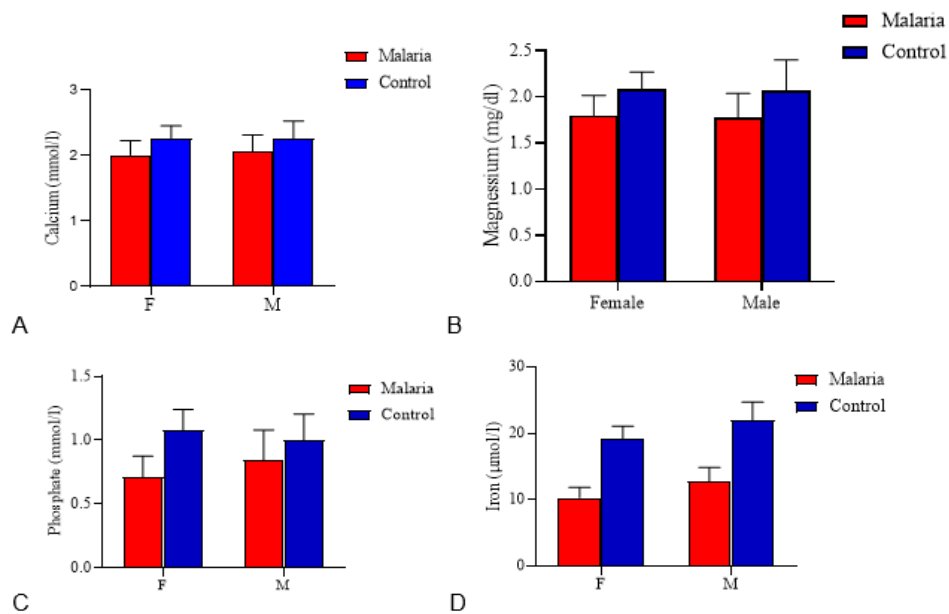
**Table 2. Correlation of subject age and the serum level of micronutrients**

	Calcium		Magnesium		Phosphate		Iron	
	r	p	r	p	r	p	r	p
<b>Malaria</b>	0.6823	<b>&lt;.0001</b>	0.4458	<b>&lt;.0001</b>	0.5890	<b>&lt;.0001</b>	0.4340	<b>&lt;.0001</b>
<b>Control</b>	-0.6753	<b>&lt;.0001</b>	0.2602	.0680	0.3169	<b>.0249</b>	0.1721	.2321

*p-value in bold showed significant*



**Fig. 3. Trends in micronutrient serum levels in malaria-infected and healthy subjects**  
Each point shows the Mean and Standard Deviation



**Fig. 4. Level of micronutrients based on sex**

#### 4. DISCUSSION

Malaria is an endemic infection in many tropical regions of the world but very few studies have attempted to characterize the population micronutrient status of people infected with malaria. The current study varies from previous studies by considering both children and adult populations while previous investigations considered either of these populations (Benzacry et al., 2016, Dinga et al., 2024). Also, this study introduces some new micronutrients which have been overlooked such as phosphate, calcium and magnesium. Instead, most studies focus on iron since it is the major cause of anemia (Dinga et al., 2024). The reasons for their deliberate focus on iron in malaria-infected patients stem from its direct interference in red cell formation which results in their destruction during malaria infection pathogenesis (Spottiswoode et al., 2014).

This study demonstrated significantly higher serum levels of iron, calcium, magnesium and phosphate parameters in healthy subjects compared to malaria-infected subjects. These results suggest that malaria has a negative impact on the serum level of these micronutrients in the body. These results align with previous studies on the reduction of some micronutrient serum levels (Tyagi et al., 2017). Lower serum levels of these micronutrients could also be attributed to inadequate intake, impaired intestinal absorption and renal wasting (Barbagallo et al., 2021). The low serum level of iron in malaria-infected subjects has been associated with key roles of iron in the electron transport chain of prokaryotes (Wunderlich et al., 2024). Hence, the absence of iron serves as a limiting factor to the growth of these microorganisms.

In some studies, iron deficiency has been attributed to having protective effects in children and pregnant women (Gwamaka et al., 2012). Maternal iron deficiency has even resulted in a more than 5-fold decrease in placental malaria (Kabyemela et al., 2008). Similarly, several studies have suggested that external supplementation of iron exacerbates malaria (Gwamaka et al., 2012). These studies further buttress the findings in this study with low serum iron levels in malaria-infected patients (Wolley et al., 2024). Studies have shown that this low iron level is a compensatory mechanism that offers protection for the host against the infection (Spottiswoode et al., 2012, 2014, Muriuki et al., 2021). On the one hand, our study used serum

iron content in the subjects to determine the serum iron levels. Iron is tightly regulated hence, there would be a need for advanced methods that measure the available iron levels present in the tissues (functional iron), iron meant for cellular requirement (transport irons) and iron stores (Pfeiffer and Looker, 2017).

The calcium level was significantly higher in healthy subjects than in malaria-infected patients. This is similar to the pattern in calcium levels observed in Asaolu and Igbaakin (2009). This low of calcium in malaria-infected patients could be due to the excretion of calcium in the urine. It is worth mentioning that the level of calcium in our study was lower than those obtained in Asaolu and Igbaakin's (2009) study.

Reduction in magnesium levels was noted in malaria-infected patients compared to healthy people. This has also been noted in other studies to suggest possible depletion of the nutrient in response to malaria infection (Abdelsalam, 2016, Baloch et al., 2011). The geographical differences in the levels of magnesium are striking, as African studies show varying results for magnesium levels, ranging from reduced levels to no significant change. On the other hand, studies from Asia consistently found elevated magnesium levels in malaria patients (Kotepui et al., 2024). These discrepancies suggest that regional factors, such as differences in nutrition or genetics, may play a role in influencing these outcomes.

The lower phosphate level noted in our study is also corroborated by Harper and Browner (2007), who observed that 59% of patients had low phosphate levels. Normal adults are supposed to have a phosphate level of 0.8 to 1.5 mmol/L (Manghat et al., 2014). This range was obtained for the healthy subjects in our study, however, the phosphate levels in malaria-infected subjects were below 1 mmol/L. This low phosphate level may impact the energy levels as the phosphate is the fundamental energy currency of the cell through adenosine triphosphate (Suen et al., 2020). These results also resonate with the moderately significant correlations noted with the phosphate.

The reduction in these micronutrients may be attributed to haemolysis, increased oxidative stress, and impaired absorption due to gastrointestinal disturbances associated with malaria infection (Wolley et al., 2024, Gomes et al., 2022, Okagu et al., 2022). Given the essential roles of these minerals in physiological

functions, their depletion may exacerbate malaria morbidity, potentially worsening the clinical outcomes in affected individuals.

Significant correlations were observed between age and all micronutrients in malaria-infected patients ( $p < 0.0001$ ), while healthy subjects only showed significant correlations for calcium and phosphate. Interestingly, calcium exhibited opposing correlations: a positive correlation in malaria-infected patients ( $r = 0.6823$ ) and a negative correlation in healthy controls ( $r = -0.6753$ ). This suggests that while malaria-infected individuals experience a compensatory increase in calcium levels with age, healthy individuals may experience a decline due to physiological ageing (Veldurthy et al., 2016). Such findings emphasize the need for age-specific nutritional interventions to address micronutrient deficiencies in malaria-endemic regions. This association shows that some micronutrients such as calcium could exhibit age-dependent changes in levels among malaria-infected and healthy individuals. These new findings differ from normal literature that suggests that low calcium levels are common in the elderly due to the high prevalence of vitamin D deficiency (Gallagher et al., 2012). As our current study only considered people between the ages of 1 to 60 years, it excluded the geriatric population, this could explain the reason for the deviation from existing literature. Hence, an improvement that could be made to advance this study is to expand the scope to include the elderly population.

The trends in micronutrient levels across different age groups, as observed in Table 1, reveal a general increase in micronutrient levels with advancing age. Notably, iron, phosphate, and calcium levels displayed significant variation across age groups, with younger individuals (1-10 years and 11-20 years) exhibiting significantly lower iron levels compared to adults aged 21-40 years ( $p < 0.0001$  for multiple comparisons). This observation aligns with the increased susceptibility of children to iron deficiency in malaria-endemic regions (Clark et al., 2014, Muriuki et al., 2021, Shankar et al., 2022). The significantly lower phosphate levels in younger age groups compared to those aged  $\geq 41$  years ( $p = 0.0058$  and  $p = 0.0143$ ) suggests that younger malaria-infected individuals may have a greater risk of phosphate depletion, which could impair energy metabolism and immune function (Suen et al., 2020). The age-dependent increase in calcium levels may be attributed to improved dietary intake and metabolic adaptation over

time, compensating for the depletion caused by malaria infection. An important limitation of the current research is the neglect of the fact that malarial infection has different species of *Plasmodium*. There are currently 5 known species of *Plasmodium*, namely *P. falciparum*, *P. ovale*, *P. knowlesi*, *P. malariae* and *P. vivax*. These different types could modulate the immunological system in different fashions. So far only the effects of two species of *Plasmodium*, *P. vivax* and *P. falciparum* on the level of micronutrients have been explored (Abdulkareem et al., 2017, Benzecry et al., 2016). This calls for further research into the effect of the other species. A comprehensive look into other risk factors that could affect the levels of micronutrients needs to be explored. For instance, Infants, children, pregnant women, and the elderly have different micronutrient needs and are more vulnerable to deficiencies. Socioeconomic and food security, medication, and medical conditions could also impact the level of micronutrients.

## 5. CONCLUSION

The study's findings suggest that malaria infection is associated with significantly lower serum levels of key micronutrients, including iron, calcium, phosphate, and magnesium. Micronutrients such as iron, phosphate, calcium, and magnesium were significantly higher in healthy subjects than in malaria-infected subjects. Only calcium showed significant moderate correlations with age, with a positive correlation in malaria-infected patients and a negative correlation in healthy subjects.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## CONSENT

As per the university standards, Participants' written consent has been collected and preserved by the authors.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENTS

The authors wish to thank the Department Medical Microbiology of Rivers State University for offering their facility for this study.



## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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