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Assessment of Serum Iron, Manganese and Cu/Zn Ratio in the Course of *falciparum* Malaria among Ivorian Patients (Côte d'Ivoire)

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Authors' contributions

This work was carried out in collaboration between all authors. Author KKI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MGM and BI managed the analyses of the study. Authors MGM and DAJ managed the literature searches and contributed to write the final draft. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Objectives: The question of interaction between endemic malaria infection and nutritional status of the host is always controversial and their relationship remains difficult to establish. Furthermore, the nutritional requirements of the host include some trace elements that are also essential for malaria parasite. The aim of this work was to assess serum titers of iron, manganese and Cu/Zn ratio, an indicator of oxidative stress during *Falciparum* malaria among ivorian patients.
Design & Methods: The study was conducted between January and June 2013 among 61 malaria infected subjects and 57 uninfected controls aged 8months to 45years. These were previously evaluated about their dietary habits during diagnosis by thick and thin blood smear. For each patient, serum titres of Fe, Zn, Cu and Mn were determined using atomic absorption spectrometry followed by determination of Cu/Zn ratio which is an

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indicator of oxidative stress.
Results: The mean of iron titers was of 0.75±0.10mg/L in malaria infected patients compared to 0.94±0.09mg/L in controls. Serum iron progressively decreased as parasitæmia increased, with the lowest mean value (0.46±0.06mg/L) observed when parasite density was >40000/µL. The serum variations of zinc, copper and manganese were not significant. However, the serum titers of zinc and copper in both groups studied were lower than the references; while manganese titer was high.
Conclusion: This study shows that *Falciparum* malaria decreases the serum iron and results in significantly lower iron at high parasitæmia. However, there was no significant variation in oxidative stress with parasitæmia. The fact that serum titers of zinc and copper in both the malaria infected subjects and the control group were lower than the reference levels raises the question of bioavailability and insufficient nutritional intake of these micronutrients, which may be a subject of further study.

Keywords: Côte d'Ivoire; Falciparum malaria; serum; trace elements.

1. INTRODUCTION

In malaria-endemic areas, particularly in sub-Saharan Africa, the fight against malaria is a growing concern and lies partly in the multi-resistance of parasite to various antimalarial drugs currently available. This resistance is sometimes the result of poor compliance to treatment [1], causing point mutations [2] leading to repeated therapeutic failures, and often impedes the management of malaria. Thus in Côte d'Ivoire, malaria remains a public health problem where nearly 43% of visits to hospitals are due to that disease. The most vulnerable are children less than five years and pregnant women. In these endemic countries, malaria and malnutrition coexist and forms a deadly combination.

The question of interaction between endemic malaria and nutritional status of the host is still controversial and their relationship remains difficult to establish [3,4]. Some studies show the protective effect of normal and adequate nutritional status [5-10]. On the other hand argue that malnutrition protects against exacerbation of malaria and that nutritional supplementation would increase the host susceptibility to infection [11-12]. Furthermore we know that the nutritional requirements of the host for his welfare include some trace elements [13-16]; certain of these trace elements are also essential for metabolism of parasite [17-19].

The involvement of trace elements in the protection or exacerbation of malaria has been a subject of numerous studies. The immunomodulatory properties of zinc are known [20,21]; but its interest in the treatment of malaria is variously interpreted [3,22]. Some studies show that the nutritional supplementation during treatment of malaria improves the therapy and insufficient nutritional [8,10]. Iron, copper, manganese and zinc are antioxidants or antioxidant enzyme cofactors fighting against abnormal elevation of oxidative stress both from host and the parasite [17-19,23]. Iron deficiency weakens the immune system, increases the risk of anemia and infection [18]. However, iron-deficient among individuals may confer resistance to malaria [24,25]. Thus the interest to assess these micronutrients in the blood lies in the management of intake risk and optimization of the therapy of malaria.

The aim of this work was to assess serum titers of iron, manganese and copper/zinc ratio, which is an indicator of oxidative stress, in the course *Plasmodium falciparum* malaria among ivorian patients.

2. MATERIALS AND METHODS

2.1 Study Area and Population

This study was conducted among populations of the health district of Abobo-east, between January and June 2013. Abobo is a city of Abidjan (Côte d'Ivoire). The city is spread over an area around 9.000 hectares with a population estimated about 1.5million inhabitants in the last census of the population since 1998 [26]. With a density of 167 inhabitants per hectare and benefiting of precarious socio-economic environment, Abobo belongs to endemic areas where malaria prevalence is high [27]. To ensure representative sample, three health centers have been selected based on their high rate of consultation: Banco-south Anador (a), Kennedy-Clouetcha (b) and the health facility of Abobo-Baoulé (c).

2.2 Biological Material and Eligibility Criteria

This study focused on 118 patients including 61 infected and 57 uninfected of the 297 subjects previously assessed on their dietary habits during laboratory diagnosis of malaria [28]. To be eligible, the subject should be 8 months to 45years old, have a clinical suspicion of malaria (fever, headache, polyalgie, infectious syndrome) during consultation, without known associated pathology. The patient should be fasted of at least 8 hours. The subjects who did not satisfy all these criteria were excluded from the study. The subject consent or that of exponent to the survey should be required.

2.3 Diagnosis of *P. falciparum*

The blood of subjects was collected in EDTA tubes and *P. falciparum* was detected using the thick and thin blood smear. The parasite density was determined on 200 microscopic fields and reported at 8450 standard leukocytes per µL of blood. The absence of *P. falciparum* infection was noted when 200 fields were read negative [29]. The serum samples not hæmolysed were systematically collected and aliquoted into eppendorf tubes then kept below -20ºC until assaying micronutrients.

2.4 Determination of Micronutrients and Oxidative Stress

The Atomic Absorption spectrophotometry at flame-air/acétylène (Varian AA 20 Pattern ®, France) was used for the determination of trace elements. The limit of detection was 0.001mg/L. The characteristic wavelengths were of 249, 214, 325 and 279nm respectively for iron, zinc, copper and manganese. For determining zinc, copper and manganese, serum samples were digested according to ratio 1:9 (v/v) with 6% desionized water-n-butanol during 30min at 100ºC [30,31]. The determination of iron included deprotenization of serum according to ratio 1:9 (v/v) with 5% desionized water-trichloro acetic acid [30]. A multielement standard solution of 1000ppm (Merck), diluted just before use at 1/500 with desionized water-nitric acid (0.03M), was used to prepare calibration range (0.5, 1.0, 1.5, 2.0ppm). The measurements of concentrations were performed in triplicate and adjusted against the blank (desionized water). In addition, the concentrations of copper and zinc were used to determine the level of oxidative stress by calculating the copper/zinc ratio [32].

2.5 Ethical Considerations

We certify that this study had obtained ethical clearance, and official letters were addressed to inform the health facilities selected. Informed consent was obtained for all the participants.

2.6 Statistical Analysis of Results

Data were analyzed using GraphPad Prism software. The means of concentrations (±standard error of the mean) of trace elements were calculated. The variations between the means of serum concentrations were analyzed using t-test welch. The difference was significant at $p<0.05$.

3. RESULTS

3.1 Serum Titers of Trace Elements and Malaria Infection

In total 118 serum samples, composed of 61 malaria infected serums and 57 uninfected were analyzed. The uninfected serum samples served as controls to assess the effect of infection on serum concentrations (Table 1). On all three sites: Banco-south Anador (a), Kennedy-Clouetcha (b) and Abobo-Baoulé (c), the mean of serum iron of 0.75±0.10mg/L was obtained among patients malaria infection compared to 0.94±0.09mg/L in controls. The mean of serum zinc, copper and manganese showed no significant variation between infected subjects and controls. However, the mean of concentrations of zinc and copper were lower in both studied groups, compared to reference values, while manganese titer was high. According to gender (Table 2), the serum titers of trace elements showed no significant variation ($p>0.05$) during the infection. The mean of serum iron was of 0.78±0.09mg/L in men and of 0.71±0.14mg/L in women. It was the same with the age categories (Table 3), the variations of trace elements studied were not significant.

3.2 Serum Titers of Trace Elements and Parasitæmia

The analysis of serum titers of trace elements following parasitæmia (Table 4) showed a reduction in serum iron progressively when the parasitæmia increased. When parasitæmia was high (>40000 parasites/µL of blood), the mean of iron titer (0.46±0.06mg/L) became significantly lower ($p<0.05$). While the serum concentrations of zinc, copper and manganese had not significantly varied according to parasitæmia.

3.3 Level of Oxidative Stress

The results of copper/zinc ratio (Table 5) showed that malaria would raise oxidative stress in not significant way ($p>0.05$). Among the infected group the mean value was of 1.18±0.12 against 0.93±0.09 in controls group. Whatsoever, depending of parasitæmia, gender and age categories, the variations of oxidative stress were not significant ($p>0.05$), although in children less than 5years old, this value was important (1.27±0.11).

Table 1. Distribution of means of serum titers of trace elements in infected patients and uninfected from Abobo-east health district

	Patients	Infected (n=61)	Uninfected (n=57)
	Sites	Titer±SEM	Titer±SEM
	a	0.73±0.17	0.95±0.21
Fe	b	0.66±0.20	0.84±0.19
	c	0.85±0.16	1.04±0.09
	Total	0.75±0.10	0.94±0.09
Zn	a	0.58±0.04	0.62±0.06
	b	0.51±0.08	0.60±0.06
	c	0.54±0.07	0.63±0.07
	Total	0.54±0.04	0.62±0.04
Cu	a	0.58±0.07	0.57±0.05
	b	0.65±0.09	0.58±0.06
	c	0.67±0.06	0.60±0.10
	Total	0.64±0.08	0.58±0.07
Mn	a	0.007±0.002	0.006±0.002
	b	0.005±0.003	0.007±0.004
	c	0.006±0.002	0.006±0.004
	Total	0.006±0.002	0.006±0.003

Sites: Banco-south Anador (a, n=41), Kennedy-Clouetcha (b, n=35), Abobo-Baoulé (c, n=42). SEM: standard error of the mean. Reference values of nutrients studied: Fe: 0.6-2.0mg/L, Zn: 0.7-1.2mg/L, Cu: 0.7-1.4mg/L, Mn<0.004mg/L. The variations between the titers were not statistically significant (p>0.05).

Table 2. Means of trace elements variations by gender among malaria infected patients and controls in Abobo-east health district

	Malaria infected		Control group	
	Male	Female	Male	Female
Fe	0.78±0.09	0.71±0.10	0.92±0.09	0.96±0.10
Zn	0.53±0.05	0.54±0.03	0.63±0.02	0.62±0.07
Cu	0.62±0.01	0.66±0.03	0.60±0.04	0.58±0.03
Mn	0.005±0.003	0.006±0.002	0.007±0.003	0.004±0.002

The variations between the titers were not statistically significant (p>0.05)

Table 3 Means of trace elements variations by age categories among malaria infected patients and controls in Abobo-east health district

	Malaria infected			Control group		
	Age categories (years)			Age categories (years)		
	<5	5-20	21-45	<5	5-20	21-45
Fe	0.76±0.08	0.70±0.11	0.80±0.09	0.97±0.08	0.92±0.10	0 .94±0.09
Zn	0.49±0.04	0.54±0.01	0.57±0.07	0.61±0.02	0.64±0.05	0 .63±0.04
Cu	0.62±0.13	0.67±0.04	0.64±0.04	0.58±0.04	0.61±0.10	0 .57±0.04
Mn	0.006±0.002	0.003±0.001	0.007±0.003	0.004±0.002	0.006±0.003	0.006±0.002

The variations between the titers were not statistically significant (p > 0.05)

Table 4. Means of serum titers of trace elements compared to parasite density in patients from Abobo-east health district

	Infected population (n=61)			Uninfected population (n =57)
	A P>40000 (n=12)	B P: 2000-40000 (n=31)	C P<2000 (n=18)	D
Fe	0.46 *** ±0.06	0.82±0.12	0.96±0.09	0.94±0.09
Zn	0.59±0.04	0.61±0.02	0.64±0.03	0.62±0.03
Cu	0.65 ±0.08	0.61±0.10	0.63±0.06	0.58±0.07
Mn	0.005±0.002	0.006±0.001	0.004±0.002	0.006±0.003

Parasite density (P:number of parasites/µL of blood). Significant degree: A-B (*), p=0.013; A-D (**), p=0.006; A-C (***), p<0.0001

Table 5. Cu/Zn ratio in patients from Abobo-east health district

Status	Cu/Zn (ratio±SEM)	Patients (%)
Uninfected population	0.93±0.09	48.3
Infected population	1.18±0.12	51.7
Total	1.06±0.10	100
Parasite density (/µL)		
<2000	0.98±0.24	15.2
2000-40000	1.00±0.21	27.1
>40000	1.10±0.32	10.2
Gender		
Male	1.16±0.17	21.2
Female	1.22±0.18	30.5
Age categories (years)		
<5	1.27±0.11	10.2
5-20	1.24±0.14	17.8
21-45	1.23±0.12	23.7

Reference values of oxidative stress: 1.14-1.29. The variations between the values were not statistically significant (p > 0.05)

4. DISCUSSION

It emerges from this study that *P. falciparum* infection decrease serum iron and induces a lowest iron titer at high parasitæmia. These results are in agreement with those of M'boh et al. [33]; these had found that the infection with *P. falciparum* decrease the serum iron, zinc and copper in infected during a case-control study in ivorian children of school age. The emergence of malaria is favoured by excess iron [13,15,25]. Indeed, in response to the aggression of the infectious agent, the organism induces an inflammatory reaction which adapts to the degree of aggression. The amplitude of this adaptive response varies in time according to parasitæmia and is characterized by an increase in energy loss and hypercatabolism [15]. Excessive hemoglobin digestion by the parasite for its growth, or the phagocytosis of infected erythrocytes by organism for its defense, is accompanied by iron liberation. That would increase its use by the sporozoite [34] and reduce the iron status. Afterwards, the low iron observed, would result from iron utilization by the parasite and accentuation of the host defense. The important release of iron might accentuate the transferrin saturation and could induce the production of hepcidin by the liver [35] and

release the lactoferrin of the polymorphonuclear [15]. These substances may then trap the iron and reduce its consumption by the parasite. Moreover, the titers of zinc and copper low, along with the titer high of manganese observed both in infected subjects and control group would result from causes other than infection malaria; such as impaired absorption or insufficient intake of micronutrients. That was reported by Kolia et al. [28] during the assessment of dietary habits among patients living in health district of Abobo-east. These observations are also consistent with other reports that stipulate that a nutritional deficiency weakens the defense system [20,21].

Oxidative stress is involved in the pathophysiology of malaria [32,36,37]. But our results had not confirmed that; because the variations of oxidative stress between infected malaria and controls were no significant. This absence of correlation might be explained by the sequestration of *Plasmodium* into the capillaries, which makes the parasitæmie inexact as reflection of the parasite density and thus the intensity of the disease [37]. Indeed, oxidative stress is an imbalance of pro-oxidant balance (activated oxygen species or AOS) and antioxidants in favour of pro-oxidant, potentially leading to damage [14]. Malaria provokes an overproduction of free radicals in the infected organism. These free radicals belong to activated oxygen species. These are produced by the organism permanently and having feature oxidizing properties, leading them at low concentration to act as secondary messengers in the regulation of apoptosis phenomenon and the activation of certain genes and factors involved in the immune response [16]. In contrast, overproduction of AOS will have adverse effects by inducing a series of biological reactions worse controlled that may impair antioxidant defenses (trace elements, vitamins) and cause of cellular damage. Together with several studies had demonstrated, oxygen free radicals stem from multiple sources in the organism [37,38,39]; including auto-oxidation of hemoglobin, the activation of phagocytes, the release of free iron and the self metabolism of *Plasmodium*.

5. CONCLUSION

This study shows that *P. falciparum* malaria decreases the serum iron and results in significantly lower iron at high parasitæmia. However, there was no significant variation in oxidative stress with parasitæmia. The fact that serum titers of zinc and copper in both the malaria infected subjects and the control group were lower than the reference levels raises the question of bioavailability and insufficient nutritional intake of these micronutrients, which may be a subject of further study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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