

Assessment of blood iron, manganese and Cu/Zn ratio in the course of *falciparum* malaria among ivorian people (Côte d'Ivoire)

Abstract

Objectives

The question of interaction malaria-endemic and nutritional status of host is always controversial and their relationship remains difficult to establish. Furthermore, the nutritional requirements of host include some trace elements also essential for parasite. Their evaluation in blood is primordial for improve the treatment. The aim of this work was to assess serum titers of iron, zinc, copper and manganese during *falciparum* malaria among ivorian people.

Design & Methods

For this purpose, we determined serum titers of these micronutrients by atomic absorption spectrometry and the copper/zinc ratio, an indicator of oxidative stress in 61 infected and 57 controls, old 8 months to 45 years; subjects evaluated previously about their dietary habits during diagnosis by thick/thin blood smear between january-june 2013.

Results

Iron titer was of 0.75 ± 0.10 mg/L in infected against 0.94 ± 0.09 mg/L in controls; significant low iron ($p < 0.05$) of 0.46 ± 0.06 mg/L to $P > 40000$, raising the oxidative stress level to 1.10 ± 0.32 . Serum variations of zinc, copper and manganese were not significant. However, serum titers of zinc and copper in both groups studied were lower than the references; while manganese titer was high.

Conclusions

These results report that *falciparum* malaria would decrease serum iron and elevate the oxidative stress level. Then they would reveal the bioavailability problem and hypothesis of inadequate nutritional intake. For this purpose, beneficial malaria therapy should incorporate a healthy and balanced diet, that is a guarantee for optimize the management and reduce the cost of treatment.

Key words: *falciparum malaria*, Blood, Trace elements, Ivorian, Côte d'Ivoire

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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 bad observance 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 old to less five years and pregnant women. In these endemic countries, malaria and malnutrition coexist and forms a deadly combination.

The question of interaction malaria-nutritional status of 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 [5-8]. Contrariwise, other argue that malnutrition protects against exacerbation of malaria and that nutritional supplementation would increase the host susceptibility to infection [9-12]. Furthermore we know that the nutritional requirements of 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 subject to numerous studies. Immunomodulatory properties of zinc are known [20, 21]; but its interest in the treatment of malaria is variously interpreted [3, 22]. Iron, copper, manganese and zinc are antioxidants or antioxidant enzyme cofactors fighting against anormal elevation of oxidative stress both from host as in parasite [17-19, 23]. Iron deficiency weakens the immune system, increases the risk of anemia and infection [18]. However, iron-deficient among individuals may have a 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, zinc, copper and manganese in the course *Plasmodium falciparum* malaria among ivoirian people.

2. Materials and methods

2.1. Study area and population

66 This study was conducted among populations of the health district of Abobo-east,
67 between january and june 2013. Abobo is one of municipalities in the district of Abidjan; its
68 area is about 9,000 hectares with an estimated population of 1.5 million inhabitants in the last
69 census population. Its density is 167 inhabitants per hectare [26]. Abobo has a subtropical
70 climate marked by a precarious socio-economic environment, giving it the status of malaria-
71 endemic area [27]. For reasons of sample representativity, we selected three health centers:
72 Banco-south Anador (a), Kennedy-Clouetcha (b) and the health facility of Abobo-Baoulé (c).

73 2.2. *Biological material and eligibility criteria*

74 Our study included 118 (61 infected and 57 uninfected) of 297 subjects previously
75 assessed on their dietary habits during laboratory diagnosis of malaria. To be eligible, the
76 subject should be old 8 months to 45 years, have a clinical suspicion of malaria (fever,
77 headache, polyalgie, infectious syndrome) during consultation, without known associated
78 pathology; and to be fasted. Subjects not having at least one of these criteria were excluded
79 from the study. The subject consent or that of exponent to the survey should be required.

80 2.3. *Diagnosis of P. falciparum*

81 The blood of subjects was collected in EDTA tubes and *P. falciparum* was detected
82 using the thick and thin blood smear. The parasite density was determined on 200 microscopic
83 fields and reported at 8450 standard leukocytes per μL of blood. The absence of *P. falciparum*
84 infection was noted when 200 fields were read negative [28]. Serums not haemolysed were
85 systematically collected and aliquoted into eppendorf tubes then kept below $-20\text{ }^{\circ}\text{C}$ until
86 assaying micronutrients.

87 2.4. *Determination of micronutrients and oxidative stress*

88 The Atomic Absorption spectrophotometry at flame-air/acétylène (Varian AA 20
89 Pattern ®, France) was used for the determination of trace elements. The limit of detection
90 was 0.001 mg/L. The characteristic wavelengths were of 249, 214, 325 and 279 nm
91 respectively for iron, zinc, copper and manganese. For determining zinc, copper and
92 manganese, serums were digested according to ratio 1:9 (v/v) with 6% desionized water-n-
93 butanol during 30 min at $100\text{ }^{\circ}\text{C}$ [29, 30]. The determination of iron included deprotenization
94 of serum according to ratio 1:9 (v/v) with 5% desionized water-trichloro acetic acid [29]. A
95 multielement standard solution of 1000 ppm (Merck), diluted just before use at 1/500 with
96 desionized water-nitric acid (0.03 M), was used to prepare calibration range (0.5, 1.0, 1.5, 2.0

ppm). 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 [31].

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 ($\pm$ 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

The total sample of this study was 118 subjects, composed of 61 (51.7%) infected against 57 (48.3%) uninfected. These 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), we obtained a iron titer of 0.75 ± 0.10 mg/L among infected against 0.94 ± 0.09 mg/L among controls. We have no significant variations with zinc, copper and manganese. However, the concentrations of zinc and copper were lower in both studied groups, compared to reference values, while manganese titer was high.

According to sex (Table 2), serum titers of trace elements had not significantly varied ($p > 0.05$) during the infection. The serum iron was 0.78 ± 0.09 mg/L with men and 0.71 ± 0.14 mg/L with women. It was the same with the age groups (Table 2), the variations of trace elements studied were not significant.

3.2. Serum titers of trace elements and parasitaemia

The analysis of serum titers of trace elements following parasitaemia (Table 3) showed a reduction in serum iron progressively, when the parasitaemia increased. When parasitaemia was high ($P > 40000$ parasites/ μ L of blood), iron titer became significantly ($p < 0.05$) low

(0.46 ± 0.06 mg/L). While the serum concentrations of zinc, copper and manganese had not significantly varied according to parasitaemia.

3.3. Level of oxidative stress

The results of copper/zinc ratio (Table 4) showed that malaria would raise oxidative stress in not significant way ($p > 0.05$). Among the infected group the value was 1.18 ± 0.12 against 0.93 ± 0.09 with the controls. Whatsoever, depending of parasitaemia, sex and age categories, the variations of oxidative stress were not significant ($p > 0.05$), although in children less than 5 years, this value was important (1.27 ± 0.11).

4. Discussion

It emerges from this study that *P. falciparum* infection decrease serum iron and cause a low iron at high parasitaemia. These results are in agreement with those of M'boh et al. [32]; 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 parasitaemia 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 of iron liberation. That would increase its use by the sporozoite [33] 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 [34] 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 in both groups studied would result from faulty absorption of micronutrients or stem from insufficient intake. That also supports the hypothesis reported by our dietary survey (in review); that survey would show that current dietary habits in some individuals living in health district of Abobo-east did not provide adequately the micronutrients required to reinforce the immune response. These observations are consistent with other reports that stipulate that a nutritional deficiency weakens the defense system [20, 21]. This study justifies also, the advantages associated with nutritional supplementation

during treatment of malaria reported by previous studies [7, 8, 10].

Oxidative stress is involved in the pathophysiology of malaria [31, 35, 36], our results had confirmed. Indeed, we found that oxidative stress was higher in the infected population. 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]. Indeed, 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 serie 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 [36, 37, 38]; including auto-oxidation of hemoglobin, the activation of phagocytes, the release of free iron and the self metabolism of *Plasmodium*.

5. Conclusion

The assessment of trace elements and oxidative stress during this study was a priority for preventing the risk of nutritional intake and the possible consequences on the development of *Plasmodium* and exacerbation of malaria. Our work reports that malaria *P. falciparum* decrease the serum iron, and provoke significant low iron at high parasitaemia. These effects are associated with increased oxidative stress. It further showed low titers of zinc and copper and a titer higher of manganese in both groups studied. These results are therefore related to hypothesis of bioavailability problem and inadequate nutritional intake; concluding that current dietary habits do not provide sufficient and adequately micronutrients to reinforce the immune response against infection. For this purpose a improved therapy of malaria should integrate a healthy and balanced diet; this is the guarantee of optimizing the management of malaria and reducing the cost of treatment.

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Table 1
Distribution of serum titers of trace elements in people
infected malaria or uninfected from health district of
Abobo-east to january-june 2013

		Infected (n = 61)	Uninfected (n = 57)
<i>Sites</i>		Titer ± SEM	Titer ± SEM
Fe	a	0.73 ± 0.17	0.95 ± 0.21
	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: 0.6-2.0 mg/L, Zn: 0.7-1.2 mg/L, Cu: 0.7-1.4 mg/L, Mn < 0.004 mg/L. The variations between the titers were not statistically significant (p > 0.05).

Table 2
Serum trace elements variations according to sex and age groups among malaria patients and controls, from health district of Abobo-east to january-june 2013

		Malaria patients		Uninfected	
		Titers $\pm$ SEM		Titers $\pm$ SEM	
<i>Sex</i>					
Fe	Male	0.78	$\pm$ 0.09	0.92	$\pm$ 0.09
	Female	0.71	$\pm$ 0.10	0.96	$\pm$ 0.10
Zn	Male	0.53	$\pm$ 0.05	0.63	$\pm$ 0.02
	Female	0.54	$\pm$ 0.03	0.62	$\pm$ 0.07
Cu	Male	0.62	$\pm$ 0.01	0.60	$\pm$ 0.04
	Female	0.66	$\pm$ 0.03	0.58	$\pm$ 0.03
Mn	Male	0.005	$\pm$ 0.003	0.007	$\pm$ 0.003
	Female	0.006	$\pm$ 0.002	0.004	$\pm$ 0.002
<i>Age groups</i>					
Fe	< 5 years	0.76	$\pm$ 0.08	0.97	$\pm$ 0.08
	5 to 20 years	0.70	$\pm$ 0.11	0.92	$\pm$ 0.10
	21 to 45 years	0.80	$\pm$ 0.09	0.94	$\pm$ 0.09
Zn	< 5 years	0.49	$\pm$ 0.04	0.61	$\pm$ 0.02
	5 to 20 years	0.54	$\pm$ 0.01	0.64	$\pm$ 0.05
	21 to 45 years	0.57	$\pm$ 0.07	0.63	$\pm$ 0.04
Cu	< 5 years	0.62	$\pm$ 0.13	0.58	$\pm$ 0.04
	5 to 20 years	0.67	$\pm$ 0.04	0.61	$\pm$ 0.10
	21 to 45 years	0.64	$\pm$ 0.04	0.57	$\pm$ 0.04
Mn	< 5 years	0.006	$\pm$ 0.002	0.004	$\pm$ 0.002
	5 to 20 years	0.003	$\pm$ 0.001	0.006	$\pm$ 0.003
	21 to 45 years	0.007	$\pm$ 0.003	0.006	$\pm$ 0.002

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

Table 3
Serum titers of trace elements following parasitaemia in people from health district of Abobo-east to january-june 2013

Infection district of 19000 East to January - June 2019				
	Infected population (n = 61)			Uninfected population (n = 57)
	A	B	C	
	P > 40000 (n = 12)	P: 2000-40000 (n = 31)	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

Parasitaemia (P: number of parasites/ μ L of blood). Significant degree: A-B (*), $p = 0.013$; A-D (**), $p = 0.006$; A-C (***), $p < 0.0001$

Table 4
Cu/Zn ratio of malaria patients in the health district
of Abobo-east to january-june 2013

<i>Status</i>	Cu/Zn ratio $\pm$ SEM	n (%)
Uninfected population	0.93 $\pm$ 0.09	48.3
Infected population	1.18 $\pm$ 0.12	51.7
Total	1.06 $\pm$ 0.10	100
Parasitaemia		
P > 40000	1.10 $\pm$ 0.32	10.2
P: 2000-40000	1.00 $\pm$ 0.21	27.1
P < 2000	0.98 $\pm$ 0.24	15.2
<i>Sex</i>		
Male	1.16 $\pm$ 0.17	21.2
Female	1.22 $\pm$ 0.18	30.5
<i>Age groups</i>		
< 5 years	1.27 $\pm$ 0.11	10.2
5 à 20 years	1.24 $\pm$ 0.14	17.8
21 à 45 years	1.23 $\pm$ 0.12	23.7

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