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Iron Status of Malaria Patients in Douala - Cameroon

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Abstract: In Africa, anaemia associated with malaria infection is a major cause of childhood morbidity and mortality. Problem of severe anemia linked to malaria is increasing as antimalarial drugs resistant parasites are widespread throughout Africa. In Cameroon, malaria turns out to be the major disease with the higher number of annual deaths, especially among children under fives and pregnant women. To assess the iron status among malarial patients in Douala where malaria is endemic, 163 malarial subjects (aged 0 to 60 years) and 98 uninfected volunteer subjects (aged between 0 - 65 years) were screened for this study. Iron status was evaluated using three biochemical (Serum Iron: SI, Total Iron Binding Capacity: TIBC and Transferrin Saturation: TS) and five haematological (Haemoglobin: HGB, Haematocrit: HTC, Mean Cell Volume: MCV, Mean Cell Haemoglobin: MCH and Mean Corpuscular Haemoglobin Concentration: MCHC) parameters. It was observed that 41.7% and 63.20% of malaria patients were serum iron and haemoglobin deficient respectively. Moreover, the rates of SI, TS, HGB, HTC, MCV and MCH were significantly lower in malarial than controls ($P<0.01$). However, the TIBC rates were significantly higher among the malarial in comparison with the uninfected subjects ($P<0.01$). This rate decreased with age while HGB, HTC and MCV percentages increased with age. Parasitic density is higher in patients aged between 0 - 3 years than those between 4 - 25 years and between 26 - 60 years of age. We noticed significant ($P<0.01$) increase of SI with moderate parasitemia. Significant correlations ($P<0.001$) were observed among malarial. Malaria negatively affects iron status, but we need further research on iron metabolism for the better comprehension of the mechanism by which *Plasmodium falciparum* interact with iron status.

Key words: Iron, malaria, parasitaemia, plasmodium falciparum, ferritin, transferrin

Introduction

Iron is an essential micronutrient necessary for the transportation of respiratory gases via haemoglobin in the red blood cells. Iron also intervenes in the constitution of enzymatic systems such as catalases, peroxydases and cytochromes that play an essential role in cellular respiratory mechanisms, in mitochondrial respiratory channel (Heberg and Galan, 1991). Iron has three levels of distribution in human body: "Functional" iron in haemoglobin, tissues and various haeminic enzymes; "Store" iron as ferritin and haemosiderin and "Circulating" iron bound to transferrin in the plasma.

Aneamia is a major and pressing problem around the world. Recent WHO statistics indicate a worldwide prevalence of about 30% with higher figures in developing countries. Many causes of anaemia have been identified: Nutritional deficiency due to lack of bioavailable dietary iron or vitamin folate, parasitic infections such as hookworm or malaria.

Iron deficiency anaemia is the main nutritional deficiency in the world. According to the WHO estimations, more than 700 millions people are affected by iron deficiency anaemia (INACG, 1986). The vulnerable groups are: Children, women of reproductive age and pregnant women (Crawley, 2004). Iron status of human is affected by the quality of the diet, the physiological and pathological status. Many infections among others: Schistosomiasis, ankylostomiasis and malaria (Olsen *et al*., 2000) are able to modify iron status. These modifications can cause iron deficiency or iron excess. The consequences of iron deficiency anaemia ranging from effects on energy metabolism and immune function to effects on cognitive and motor development (Walter, 1993).

Malaria is a disease caused by protozoa of the genus *Plasmodium*; it is a serious health problem in tropical and subtropical areas. World wide, more than 400 millions people are affected by malaria, with about 200 millions in Sub - saharan Africa. In Cameroon, malaria is a public health priority (Breman *et al*., 2004).

It would be important to investigate the effect of malaria on the iron status and to establish the relation between intravascular haemolysis and iron circulation, since red blood cells are the target for infection by *P. falciparum*.

Materials and Methods

This was a prospective study, carried out in Laquintinie Hospital of Douala - Cameroon; a littoral town situated along the river Wouri, in a transitional zone between the forest and savannah. The climate is the hot humid

equatorial climate which favors the development of anopheles mosquitoes, a vector for the transmission of malaria parasite. 163 volunteers' of malaria patients (78 males and 85 females) aged 0 to 60 years and 98 controls (42 males and 56 females) aged 0 to 65 years were enrol after informed consent.

Thick and thin blood films prepared from a finger prick with Giemsa stained and examined by light microscopy under oil - immersion objective, at 100X magnification. Parasitaemic (asexual or sexual) in thick films was estimated by counting asexual or sexual parasites relative to 1000 leukocytes. From this figure, the parasite density was calculated assuming a leukocytes count of 8000 $\mu 1^{-1}$ of blood.

Further, blood (1 - 2 ml) was collected by venipuncture from the arm and stored in sample tubes (one EDTA tube and one dry tube) and properly labelled. The tubes were then stored and transported to the laboratory where the dry tube was centrifuged at 4500 rpm for 5 min.

The serum obtained from it was used for biochemical analysis: Serum iron (SI), Total iron binding capacity (TIBC), Transferin saturation (TS). The EDTA tubes was used for Haematological analysis: Haemoglobin (HGB), Haematocrit (HTC), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using an Automatic Counter of blood cells (ABX). The study was approved by the Ethical Committee of Clinical Study (ECCS) at the University of Douala.

Statistical analysis was done using Pearsons's correlation coefficient. Student's t - test and the analysis of variances (ANOVA) with Statistica and SPSS 6.1 software.

Results

An overview of Table 1 shows that: age and parasitaemia varies from 0 to 60 years and from 1 to 12% respectively, whilst controls, aged between 0 to 65 years, were distinguished by the presence of no positive thin or thick blood smear during malaria diagnosis. The analysis of biochemical and haematological parameters using Student t-test shows significant decrease (at 1%) of SI, TS, HGB, HTC, MCV and MCH in malaria patients; but the TIBC is significantly high in malaria patients. As far as MCHC is concerned, no significant variation was observed between malaria patients and controls.

Frequencies distributions of parameters studied (SI, TS, HGB and HTC) of 163 malaria patients and 98 controls are presented in Table 2. In the population of patients, 41.71% and 63.19% have low values in SI and HGB respectively. More than half of these patients were between 0 - 3 years of age with 20.85% and 32.50% of deficiencies for SI and HGB respectively. Whereas in the controls population, 5.10% and 23.46 % of subject were respectively deficient on SI and HGB.

Table 3 presents the results by parasitaemia intervals. We notice that patients with smooth parasitaemia (< 1%) have an SI mean of 53.59±2.69µg/dl. The SI mean (81.68±6.16 µg/dl) is significantly high ($P<0.01$) with moderate parasitaemia (1 - 5%); But we notice a significant decrease ($P<0.01$) in SI (57.35±8.53 µg/dl) with high parasitaemia (>5%). Concerning the TIBC, the mean is proportional to parasitaemia ($P=0.01$). But HGB, HTC and MCV values decreased with high parasites densities.

In Table 4, the results by age intervals in malaria patients are presented. The TIBC is significantly higher in patients aged between 0 - 3 years than in those between 4 - 25 and 26 - 60 years ($P<0.01$). However, we noticed a significant variation at 1% of HGB, HTC and MCV between age intervals; the mean in infants are then less high than those in adults.

The analysis of results by sex at 5% shows that no significant difference between males and females concerning the parameters of our interest in our patients. In Table 5 we noticed the high number of correlations between parameters analysed.

Discussion

After a general analysis of the results, we have observed that SI, TIBC, TS, HGB, HTC and MCV, are highly affected by malaria ($P<0.01$), whereas MHCH does not shows significant variations at 5%. These differences observed between the two groups of individuals (malaria patients and controls) could be essentially due to clinical manifestations of malaria: Fever, pulse acceleration, sweating and shivering (Lariviére *et al.*, 1987); physiological phenomena, that upset iron metabolism. Moreover, during an infection such as malaria, minerals are redistributed from circulation to tissues and cause a reduction of minerals in the circulation (Keusch, 1998). All these explain why SI, TS, HGB, TC and MCV are significantly lows in malaria patients than in controls. The increase of TIBC proves that stored iron is exhausted (INACG, 1986). This rise also shows liberation of iron in the plasma, an increase in transferin synthesis and of transferin activity.

The rise of serum iron with moderate parasitaemia is due to iron from haemolysis, because we have also noticed a reduction of HGB. Moreover, during certain diseases, SI increases after haemolysis (Olsen *et al.*, 2000). HGB is a constituent of red blood cells and the destruction of red blood cells lead to the release of iron into the plasma. Therefore, the rise of SI is not for a long time because significant reduction of HGB brings significant decrease of SI. Further, clinical manifestations of malaria and the non respect of RDA (Recommended Dietary Allowance) can affect iron bioavailability and it metabolism (Caulfield *et al.*, 2004). HGB, HTC, MCV are lows in the age interval of 0 - 3 years compared to those of 4-25 and 26-60 years (Table 4). These differences could be explained by the parasitaemia, higher in the 0 - 3 years old patients than

Table 1: Means and ranges of results in malaria patients and controls

	Groups			
Parameters	Malaria patient (n=163)	Controls (n=98)	F	P
Age (years)	16.25±1.27[a]	22.05±1.99	12.4267	<0.01**
	0-60[b]	0 - 65		
Parasiteamia (%)	2.96±0.23	-	49.5049	/
	1-12			
[SI] (µg/dl)	59.73±2.49	83.68±3.50	32.3222	<0.01**
	20.09-196.45	35.05-199.27		
[TIBC] (µg/dl)	305.49±9.24	257.51±7.98	12.7415	<0.01**
	85.24-773.28	120.12-482.43		
TS(%)	20.93±0.73	32.89±0.83	109.2634	<0.01**
	5.02-39.73	19.16-64.58		
[HGB] (g/dl)	10.07±0.13	12.00±0.13	89.4905	<0.01**
	5.2-13.90	10.00-15.10		
HTC (%)	30.25±0.43	35.42±0.37	66.2080	<0.01**
	16.50-44.50	26.20-44.10		
MCV (μm^3)	76.52±0.48	83.40±0.467	90.6304	<0.01**
	54.00-90.00	78.00-94.00		
MCH (pg)	25.86±0.24	27.79±0.24	28.4648	<0.01**
	14.40-32.00	22.5-34.60		
MCHC (g/dl)	32.97±0.14	33.18±0.20	0.7039	0.40 NS
	26.60-38.20	29.70-37.50		

[a]:Mean±SE, [b]: ranges, SI: Serum Iron, TIBC : Total Iron Binding Capacity, HGB: Haemoglobin, HTC: Haematocrit, TS: Transferrin Saturation, MCV: Mean Cell Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Cell Haemoglobin,
**:Significant at 1%, NS: Not Significant. N: Number of subjectL.

Table 2: Frequencies distribution of parameters analyzed according to age levels

	Patients				Controls
Parameters	0-3 years n=70	4-25 years n=59	26-60 n=34	Total n=163	n=98
SI<45µg/dl or TIBC<16% (low)	34[a] (20.85%)[b]	21 (12.88%)	13 (7.97%)	68 (41.71%)	5 (5.10%)
SI ≥ 45 and ≤ 160 µg/dl or TIBC ≥ 16 and ≤ 35%:(normal)	32 (19.63%)	36 (22.08%)	19 (11.65%)	87 (53.37%)	78 (79.59%)
SI>160µg/dl or TIBC>35%: (high)	4 (2.45%)	2 (1.22%)	2 (1.22%)	8 (4.90%)	15 (15.30%)
HGB<11g/dl or HTC<33% (low)	53 (32.50%)	32 (19.63%)	18 (11.04%)	103 (63.19%)	23 (23.46%)
HGB ≥ 11 and ≤ 15g/dl or HTC ≥ 33 and ≤ 44% (normal)	17 (10.12%)	27 (16.59%)	16 (9.81%)	60 (36.80%)	73 (74.48%)
HGB>15g/dl or HTC>44% (high)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (1.02%)

[a]: number of subjects. [b]: frequencies

Table 3: Results obtained by ranges of parasitaemia

	Ranges of parasitaemia			
Parameters	Smooth: <1% N=104	Moderated: 1-5% N=32	High: >5% N=27	P (ANOVA)
Age (years)	18.37±1.69[a]	8.80±2.35	3.90±1.06	<0.01**
	0-60[b]	0 - 60	0-22	
Parasiteamia (%)	0.426±0.022	2.659±0.256	7.929±0.436	<0.01**
	0.1-0.9	1-5	5-12	
[SI] (µg/dl)	53.59±2.69	81.68±6.16	57.35±8.53	<0.01**
	20.58-125.29	38.76-160	20.09-196.45	
[TIBC] (µg/dl)	291.70±11.71	298.55±17.10	366.82±23.05	0.01*
	88.26-773.28	104.33-500.20	80.24-696.27	
TS(%)	19.83±0.76	28.01±1.63	16.76±2.09	<0.01**
	7.86-39.39	10.91-39.73	5.02-36.04	
[HGB] (g/dl)	10.28±0.16	10.59±0.21	8.61±0.33	<0.01**
	5.20-13.90	8.20-12.30	5.50-11.90	
HTC (%)	31.02±0.55	31.19±0.67	26.16±1.04	<0.01**
	17.30-44.50	25.00-38.20	16.50-36.50	
MCV (μm^3)	77.31±0.63	75.46±0.89	74.74±0.88	0.08 NS
	54.00-90.00	60.00-83.00	65.00-86.00	
MCH (pg)	26.17±0.33	25.51±0.44	25.07±0.42	0.19 NS
	14.40-32.00	19.20-29.90	22.10-30.40	
MCHC (g/dl)	32.82±0.20	33.17±0.22	33.32±0.31	0.38 NS
	26.60-38.20	30.50-35.30	29.70-36.20	

NS: Not significant *:Significant at 5% **:Significant at 1%, [a]:mean ± SE. [b]Ranges.

Table 4: Results by age intervals in malaria patients

	Age intervals (Years)			
Parameters	0-3 [N =70]	4-25 [N = 59]	26-60 [N =34]	P (ANOVA)
Parasiteamia (%)	3.365±0.432[a]	1.483±0.293	0.602±0.108	<0.01**
	0.1-12[b]	0.1-9.2	0.1-3.5	
[SI] (µg/dl)	61.18±4.11	58.58±4.17	58.73±4.54	0.88NS
	20.09-196.45	20.69-159.02	20.58-125.29	
[TIBC] (µg/dl)	335.38±12.45	294.26±16.13	262.79±20.74	<0.01**
	128.79-696.27	80.24-773.28	90.00-502.62	
TS(%)	19.29±1.18	21.40±1.22	23.47±1.30	0.09NS
	5.02-39.73	7.50-39.16	9.21-39.39	
[HGB] (g/dl)	9.47±0.21	10.42±0.15	10.68±0.37	<0.01**
	5.20-12.60	6.80-12.60	5.20-13.90	
HTC (%)	28.21±0.60	31.49±0.56	32.29±1.20	<0.01**
	16.50-40.20	23.50-42.00	17.30-44.50	
MCV (µm^3)	73.95±0.60	77.30±0.74	80.47±1.15	<0.01**
	58.00-84.00	55.00-90.00	54.00-90.00	

NS: Not significant *: Significant at 5% **: Significant at 1%. [a]: mean±SE [b]: Ranges.

Table 5: Correlations observed among subjects

Para meters	FS	CTF	CST	HGB	HTC	VGM	TGMH	CCMH	Para
A-Infected subjects									
CTF	0.4172"***[b]	/							
CST	0.6310***	-0.3776***	/						
HGB	0.3442***	-0.1456[NS]	0.4536***	/					
HTC	0.2717***	-0.1067[NS]	0.3774***	0.8673***	/				
VGM	0.2033**	-0.1504[NS]	0.3053***	0.5139***	0.4856***	/			
TGMH	0.1046[NS]	-0.2009**	0.2447**	0.4767***	0.3447***	0.7916***	/		
CCMH	-0.0404[NS]	-0.1939*	0.0895[NS]	0.1142[NS]	-0.1055[NS]	0.1943*	0.4847***	/	
Para	0.0949[NS]	0.2454**	-0.0984[NS]	-0.3759***	-0.3711***	-0.1550*	-0.0960[NS]	-0.1551*	/
Age	-0.0238[NS]	-0.2568***	0.2010**	0.2520***	0.2915***	0.4099***	0.2756***	-0.1486[NS]	-0.3231***
B-Uninfected subjects									
CTF	0.7937"***	/							
CST	0.5523***	0.0170[NS]	/						
HGB	0.3018**	0.1626[NS]	0.3906***	/					
HTC	0.4557***	0.2462*	0.4401***	0.7635***	/				
VGM	0.2913**	0.1547[NS]	0.2900**	0.4267***	0.4460***	/			
TGMH	0.0958[NS]	0.0618[NS]	0.0545[NS]	0.3104**	0.1083[NS]	0.4885***	/		
CCMH	-0.1078[NS]	0.0435[NS]	-0.0160[NS]	0.2616**	-0.0807[NS]	0.2638**	0.6194***	/	
Age	0.1605[NS]	0.1179[NS]	0.0821[NS]	-0.1776[NS]	0.0304[NS]	0.1607[NS]	0.1316[NS]	0.0137[NS]	

[a] = Coefficient de corrélation de PEARSON, [b]= Valeur de P. NS = Non significatif, * = Significatif au seuil de5%, ** = Significatif au seuil de 1%. *** = Significatif au seuil de 1% 0.

in others. Parasitic densities of malaria in the tropical and equatorial zones are higher in infants, but decrease with age (Crawley, 2004), because of acquired immunity and consolidation of immune system in adults whereas this system seems weak in infants. Among infected subjects, positive correlations (P<0.001; r = 0.63; P<0.001, r = 0.86) was observed between SI and TS, HGB and HTC respectively. But a negative correlation between age and parasitemia (P<0.001, r<-0.3) was also observed.

We have also noticed that 63.19% of malaria patients have low levels of haemoglobin, a trend toward anaemia. That is in line with WHO data which consider HGB as a true maker of iron deficiency anaemia and that infant are more affected than adults (Mungala *et al.*, 2004).

Our results suggest that malaria negatively affects the iron status in human. This is in accordance with the result obtained by Oppenheimer *et al.* (1986a, 1986b). In fact they have reported a carefully controlled clinical trial of the effects on morbidity of Fe supplementation in a chronically Fe-deficient population. Because of the degree of Fe deficiency they hypothesized that Fe would have a beneficial effect on infection morbidity; on the contrary they found that the children who were given Fe had an increased morbidity; in particular, they had a higher prevalence of malaria and respiratory infection with undefined organisms. Some authors (Murray *et al.*, 1975, 1978, 1980, Keusch and Farthing, 1986) have provided evidence from studies in East Africa that there is an increased risk of malaria and tuberculosis when Fe supplements are given to humans. These reports have been supported by research on animals (Keusch and Farthing, 1986).

Thus, several studies provide strong evidence that Fe deficiency can protect against particular infections and Fe supplementation may increase the risk of these infections. However further investigations taking into

account iron metabolism in *P. falciparum* are necessary. Because, much of the confusion and argument that surrounds the relationship between Fe stains and infection stems from a failure to appreciate the heterogeneity of infections, the different strategies adopted by organisms, the relative importance of the various host defences, the different ways in which the host is compromised and the consequences of changes in Fe metabolism on both host and parasite need to be investigated.

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References

Breman, J.G., M.S. Alilio and A. Millo, 2004. Conquering the intolerable burden of malaria: What's new, what's needed: A summary; Am. J. Trop. Med. Hyg., 71: 1-15.

Caulfield, L.E., S.A. Richard and R.E. Black, 2004. Under nutrition as an underlying cause of malaria morbidity and mortanity in children less than five years old; Am. J. Trop. Med. Hyg., 71: 53-63.

Crawley, J., 2004. Reducing the burden of Anemia in infants and youngs children in malaria - endemic countries of Africa; from evidence to action; Am. J. Trop. Med. Hyg., 71: 25-34.

Heberg, S. and P. Galan, 1991. Apports conseillés en minéraux (Fer) - Apports nutritionnels conseillés pour la population française; 2nd Edn. Lavoisier, Paris, pp: 39-41.

INACG, 1986. Lutte contre la carence en fer: Etude de cas réalisé au Chili, The Nutrition Foundation Inc. Washington DC, pp: 16-22.

Keusch, G.T., 1998. Infection: Nutritional interaction; Encyclopaedia Hum. Nutr., 2: 1117-1121.

Keusch, G.T. and M.J.G. Farthing, 1986. Nutrition and infection. Ann. Rev. Nutr., 6: 131-154.

Lariviére, M., B. Beauvais, F. Derouin and F. Traoré, 1987. Parasitologie médicale; *Ellipses*, pp: 18-28.

Mungala - Odera V., R.W. Snow and C.R.J.C. Newton, 2004. The burden of the neurocognitive impairment associated with P. falciparum malaria in sub-saharan Africa. Am. J. Trop. Med. Hyg., 71: 64-70.

Murray, M.J., A.B. Murray, M.B. Murray and C.J. Murray, 1978. The adverse effect of iron repletion on the course of certain infections. Br. Med. J., pp: 113-115.

Murray, M.J., A.B. Murray and C.J. Murray, 1980. The salutary effect of milk on amoebiasis and its reversal by Iron. Br. Med. J., 1: 1351-1352.

Murray, M.J., A.B. Murray, C.J. Murray and M.B. Murray, 1975. Refeeding - malaria and hyperferraemia. *Lancet*, 1: 653-654.

Olsen, A., J. Nawiri and H. Friis, 2000. The impact of iron supplementation on re infection with intestinal helminths and schistosoma mansoni in Western Kenya. Am. J. Trop. Med. Hyg., 71: 493-499.

Oppenheimer, S.J., F.D. Gibson, S.B. Mac Fariane, J.8. Moody, C. Harrison, A. Spencer and O. Bunan, 1986a. Iron supplementation increases prevalence and effects of malaria: report on clinical studies in Papua New Guinea. Transactions of the Royal Soc. Trop. Med. Hyg., 80: 603-612.

Oppenheimer, S.J., S.B.J. Mac Fariane, J.B. Moody, O. Bunari and R.G. Hendrickse, 1986b. Effect of iron prophylaxis on morbidity due to infectious disease: report on clinical studies in Papua New Guinea. Transactions of the Royal Soc. Trop. Med. Hyg., 80: 596-602.

Walter, T., 1993. Impact of iron deficiency on cognition in infancy and childhood. Eur. J. Clin. Nutr., 51: 658-664.

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