

Serum ferritin and other haematological measurements in apparently healthy adults with malaria parasitaemia in Lagos, Nigeria

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Summary

We studied 300 apparently healthy residents of Lagos aged 16-57 years. Their mean ferritin levels were $99.6 \pm 50.5 \mu\text{g/l}$ (men aged 20-57) and $66.5 \pm 44 \mu\text{g/l}$ (women aged 20-53) in aparasitaemic individuals. In parasitaemic subjects, mean ferritin levels were $133.1 \pm 48.3 \mu\text{g/l}$ (men aged 20-56) and $114.8 \pm 51.1 \mu\text{g/l}$ (women aged 16-50). Mean haematocrit values for aparasitaemic males were $45.7 \pm 5.6\%$ and $37.9 \pm 5\%$ for females, while mean haemoglobin levels were $153.2 \pm 1.5 \mu\text{g/l}$ and $124 \pm 3 \mu\text{g/l}$, respectively. The mean values for MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), MCHC (mean corpuscular haemoglobin concentration) were $101.7 \pm 8 \text{ fl}$, $30.6 \pm 2.2 \text{ pg}$, $335 \pm 0.4 \text{ g/l}$ and $99.8 \pm 10 \text{ fl}$, $29.1 \pm 6.5 \text{ pg}$, $335 \pm 6 \text{ g/l}$. Serum iron levels were $34.2 \pm 5 \mu\text{mol/l}$ and $29.5 \pm 7.7 \mu\text{mol/l}$. All haematological parameters measured were similar in both malaria parasitaemia positive and negative subjects, except ferritin level which was significantly higher in parasitaemic individuals ($P < 0.05$). Ferritin concentration and malaria density ($r = 0.76$ in males, $r = 0.74$ in females, $P < 0.05$) were positively correlated. Ferritin levels of subjects infected with *Plasmodium falciparum* were significantly higher than of those infected with *P. malariae* ($P < 0.05$). Hence ferritin estimation without examination for malaria parasitaemia in a malaria-endemic region such as Nigeria is not reliable. Asymptomatic malaria parasitaemia increases the ferritin level. Considering the mean ferritin level we found in normal subjects on a balanced diet, routine iron supplementation may not be necessary in the treatment of malaria-induced anaemia in Nigeria.

keywords ferritin, apparently healthy, malaria parasitaemia, adult, Lagos

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Introduction

Mild iron deficiency is frequently not detected by simple haematological measurements in community surveys because of overlap in the values of the iron deficient and normal subjects (Garby 1970). In an iron-deficient state, the iron store is depleted first before significant changes are noticed in serum and erythrocyte iron concentration (Cook *et al.* 1974). The serum ferritin level is widely accepted as an accurate indicator of body iron store (Bezawada *et al.* 1979), being the only factor that can give a semiquantitative indication of the levels of storage iron (Worwood 1997). Its measurement is thus useful in the diagnosis of iron deficiency anaemia and iron overload (Philips *et al.* 1986). Serum ferritin increases as iron accumulates in the iron store and decreases as storage iron levels drop. Increased

serum ferritin has been reported in symptomatic malaria infection (Oluboyede & Topley 1981; Fleming 1982; Das *et al.* 1997; Stoltzfus *et al.* 1997) liver disease, malignancies (Bentley & Williams 1974), haemolysis and ineffective erythropoiesis (Philips *et al.* 1986; Stoltzfus *et al.* 1997). The haematological parameters commonly used for diagnosis of iron deficiency anaemia - mean corpuscular volume, mean corpuscular haemoglobin concentration, haematocrit and haemoglobin levels - only become abnormal after iron stores have been measurably reduced.

In malaria-endemic countries of Africa, asymptomatic malaria infection is common, and it has always been a subject of debate whether this constitutes a significant disease burden that should be treated. The indicator for health risk by asymptomatic parasitaemia has always been whether such infected

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persons have lower haemoglobin concentration than uninfected persons. But since haemoglobin concentration is not the most sensitive measure of blood loss through low grade haemolysis, a false notion of the iron balance status may be obtained in such individuals. We examined the iron status of Nigerians with asymptomatic malaria parasitaemia and compared it with that of malaria parasite-free subjects. Ferritin and serum iron levels as well as haematocrit, haemoglobin, MCV, MCHC, MCH, WBC (white blood cells), and RBC (red blood cells) were measured to see whether ferritin would detect differences not revealed by the usual haematological parameters. This should throw further light on the potential hazards of asymptomatic parasitaemia.

Materials and methods

Three hundred apparently healthy adults (200 males, 100 females) aged 16-57 years residing in Lagos were studied. Informed consent was obtained from all subjects and none were clinically ill at the time of study. Those with fever, thalassaemia and genotype SS were excluded. The participants were asked about their age, history of blood transfusion, use of malaria prophylactics, and underwent physical examination to identify those who were ill. Subjects were considered healthy if they had no symptoms or signs of diseases and their temperature was normal. 10 ml of blood (5 ml in EDTA bottle and 5 ml in plain bottle) were collected from each. Haematocrit, WBC, haemoglobin, RBC, MCH, MCHC and MCV were determined using blood in EDTA bottles by Coulter counter model S. Haemoglobin electrophoresis to identify those with thalassaemia and genotype SS was done on cellulose acetate paper. The sera were stored at -20 °C and analysed in batches for ferritin using the DAKO sandwich ELISA method (Revenant 1983). The ferritin standard was supplied by National Institute for Biological

Standard and Control (Hertfordshire, UK). Serum iron was analysed by the calorimetric test method (Garcia 1979; Brivio *et al.* 1981; Tobacco *et al.* 1981). Thick blood films were Giemsa stained and examined for malaria parasites by a single microscopist using a $\times 100$ oil immersion lens and $\times 7$ eyepiece. One hundred fields were examined before a slide was declared negative (WHO 1991). Thin blood films were stained with Leishman's stain and examined for red cell morphology and malaria parasite species identification.

Statistical analysis was by student's *t* test and the level of significance taken as $P < 0.05$. FigP (Software Corporation, Durham, NC, USA) was used for the graphics.

Results

None of the 300 subjects had undergone blood transfusion, only two had donated blood for transfusion and none was on malaria prophylactics. The haematological parameters and iron status of all participants are presented in Tables 1 and 2. The mean ferritin levels of a parasitaemic subjects were $99.6 \pm 50.5 \mu\text{g l}^{-1}$ in males ($n = 113$) and $66.5 \pm 44 \mu\text{g l}^{-1}$ in females ($n = 70$). The mean ferritin levels for parasitaemic subjects were $133.1 \pm 48.3 \mu\text{g l}^{-1}$ (males) and $11.4 \pm 51.1 \mu\text{g l}^{-1}$ (females). Mean ferritin in subjects infected with *P. falciparum* and *P. malariae* was $150 \pm 50.6 \mu\text{g l}^{-1}$ and $123 \pm 53.6 \mu\text{g l}^{-1}$, respectively. The mean values for MCV, MCH, MCHC were $101.7 \pm 8.3 \text{ fl}$, $30.6 \pm 2.2 \text{ pg}$, $335 \pm 4 \text{ g l}^{-1}$ in men and $99.8 \pm 10.1 \text{ fl}$, $29.1 \pm 0.5 \text{ pg}$, $335 \pm 6 \text{ g l}^{-1}$ in women. Serum iron was $33.9 \pm 15 \mu\text{mol l}^{-1}$ for males and $28.2 \pm 12.5 \mu\text{mol l}^{-1}$ for females.

On comparison of data between malaria parasite-positive and negative subjects, only the serum ferritin level was found to be significantly higher in the malaria parasite-positive subjects (Tables 1 and 2; $P < 0.05$). The highest ferritin level was ob-

Table 1 Comparison of haematological parameters and ferritin levels between malaria parasitaemia-positive and negative males

	Malaria-positive ($n = 87$)		Malaria-negative ($n = 113$)		Difference
	Range	Mean \pm SD	Range	Mean \pm SD	
Haemoglobin (HB) (μl^{-1})	113-177	150 \pm 3	113-185	153 \pm 1.5	ns
Haematocrit (percentage)	34-53	43.6 \pm 4.1	35-56	44.3 \pm 9	ns
RBC ($\times 10^{12} \text{ l}^{-1}$)	3.5-5.8	4.9 \pm 6.4	3.5-6.1	5.1 \pm 2.5	ns
MCC (μl^{-1})	300-370	335.1 \pm 6.0	304-380	335 \pm 9.4	ns
MCH (pg)	23.9-35.8	31.2 \pm 3.0	24.7-35.2	30.6 \pm 2.2	ns
MCV (fl)	82-119	102.6 \pm 5.0	80-119	101.7 \pm 8.3	ns
MP density (μl^{-1})	30-1009	234.9 \pm 220	0	0	
Serum Ferritin ($\mu\text{g l}^{-1}$)	66.2-265.4	133.1 \pm 48.3	16.8-182.7	99.6 \pm 50.5	$P < 0.05$
Serum iron ($\mu\text{mol l}^{-1}$)	4.3-77.8	34.5 \pm 18.4	11.3-77.8	34.2 \pm 14.6	ns
Age (years)	20-57	34.3 \pm 8.8	20-56	35.5 \pm 9.3	ns

fl = femtoliter = 10^{-15} L; pg = picogram; HB = haemoglobin; MP = malaria parasite; ns = not significant

Table 2 Comparison of haematological parameters and ferritin levels between malaria parasitaemia-positive and negative females

	Malaria parasitaemia-positive		Malaria parasitaemia-positive		Difference
	20-53 (n = 30)		16-50 (n = 70)		
Age (years)	Range	Mean \pm SD	Range	Mean \pm SD	
Haemoglobin (g l ⁻¹)	89-145	123 \pm 2	90-147	124 \pm 3	ns
Haematocrit percentage	29-46	37.2 \pm 2	30-48	37.9 \pm 5	ns
RBC ($\times 10^{12}$ l ⁻¹)	2.8-4.6	4.0 \pm 3.0	2.9-5.35	4.1 \pm 0.8	ns
MCC (gl ⁻¹)	300-394.9	334 \pm 3	300-380	335 \pm 6	ns
MCH (pg)	23.6-36.2	32.1 \pm 0.5	20-35	29.1 \pm 0.5	ns
MCV (fl)	100-127	103.4 \pm 6.7	74-101	99.8 \pm 10.1	P < 0.5
Serum ferritin (μ g l ⁻¹)	49.6-265.4	114.8 \pm 51.1	4.5-115.7	66.5 \pm 44	P < 0.5
MPI density μ l ⁻¹	30-1500	262 \pm 321	0	0	
Serum iron (μ mol l ⁻¹)	33-60	29.1 \pm 10.6	5-50	27.2 \pm 0.9	ns

fl = femtoliter = 10⁻¹⁵ l; pg = picogram

served in subjects with the highest malaria parasite density. The ferritin level was significantly higher in those infected with *Plasmodium falciparum* than in subjects infected with *P. malariae* ($P < 0.05$). The figures show a linear relationship between the serum ferritin level and malaria parasite density ($r = 0.76$, $P < 0.05$; $r = 74$, $P < 0.05$; $r = 0.53$, $P < 0.05$, $n = 76$; $r = 54$, $P < 0.05$, $n = 23$; $r = 51$, $P < 0.05$, $n = 18$). Figure 1 shows a linear relationship between serum ferritin levels and malaria parasite density irrespective of *Plasmodium* species. There is no significant difference between ferritin levels of malaria parasitaemic males and females, $P = 0.086$. Figures 2, 3 and 4 show the relationship between serum ferritin levels and malaria parasite density for *Plasmodium falciparum*, *P. malariae* and mixed infection. Serum ferritin is sig-

nificantly higher in normal males than in normal females ($P < 0.05$).

Discussion

We found a significantly higher level of serum ferritin in apparently healthy subjects with malaria parasitaemia than in healthy subjects without malaria parasitaemia. There was a significant difference between aparasitaemic adult males and females but not between parasitaemic men and women. This insignificant difference is most likely related to malaria parasitaemia. The presence of malaria parasites has distorted the normal gender differences in ferritin levels. Our findings support earlier reports (Oluboyede & Topley 1981; Das *et al.* 1997;

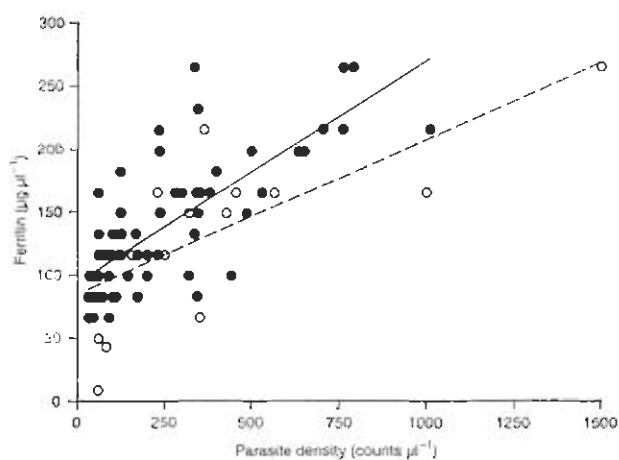


Figure 1 Regression line between serum ferritin levels and malaria parasite density among the two sexes in Lagos, Nigeria. The unbroken line indicates males, the broken line indicates females.

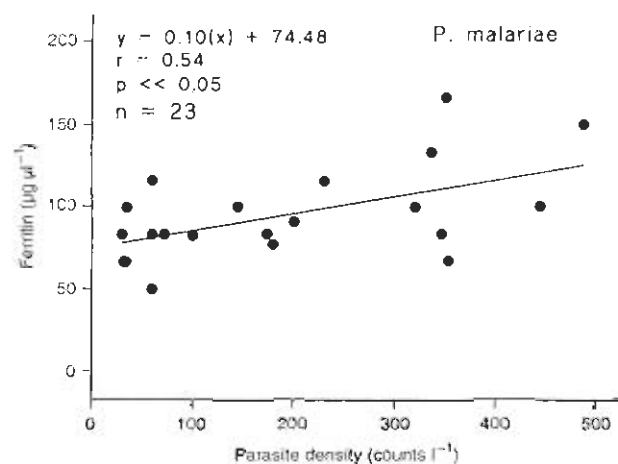


Figure 2 Regression line between serum ferritin and *P. falciparum* parasitaemia among adults in Lagos, Nigeria.

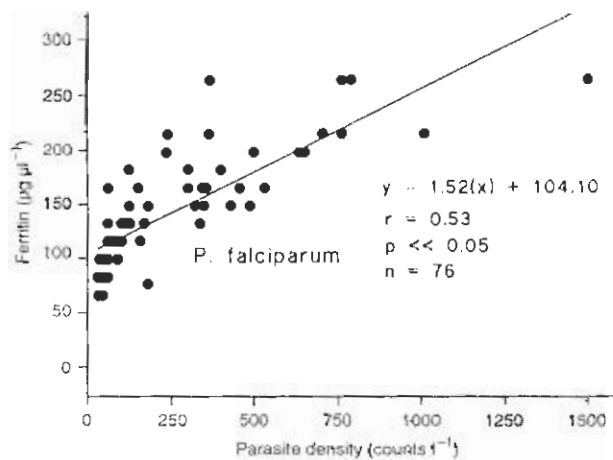
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Figure 3 Regression line between serum ferritin and *P. malariae* parasitaemia among adults in Lagos, Nigeria.

Stoltzfus *et al.* 1997) that serum ferritin is elevated in symptomatic malaria infection. Adelekan & Thurnham (1990) reported very high ferritin ranging from 170 to 1000 µg/l in children with acute malaria. Several mechanisms have been put forward to explain the elevation of serum ferritin in malaria infection. As a known acute-phase reactant protein, its concentration in the serum is known to increase during acute inflammatory reactions. High ferritin level has been reported in liver disease, haemolytic process and folic acid deficiency (Birgegard *et al.* 1979; Das *et al.* 1997). All these reported causes of high ferritin level are common in malaria infection. In our *P. falciparum* patients serum ferritin was significantly higher

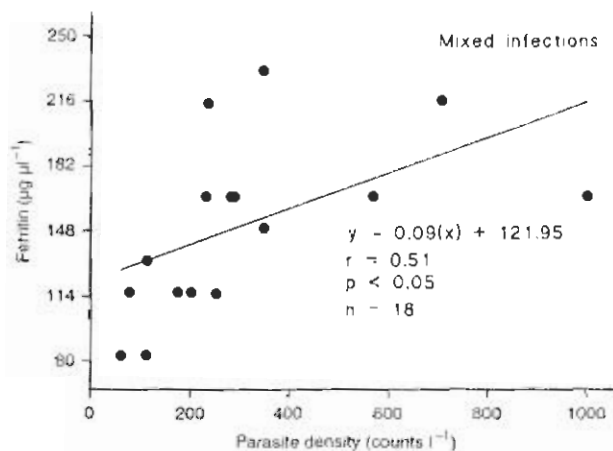


Figure 4 Regression line between serum ferritin and mixed *P. falciparum* and *P. malariae* density among adults in Lagos, Nigeria.

than in those infected with *P. malariae*. This could be explained by the fact that haemolysis is most severe with *P. falciparum* which invades red cells of all ages while *P. malariae* invades mainly the normoblasts (Strickland 1986). There was a linear correlation between the serum ferritin and the malaria parasite density. This could explain the differences in ferritin levels between *P. falciparum* and *P. malariae*, since the malaria density was higher with *P. falciparum* as shown on Figures 2 and 3. These findings may also throw more light on the actual sources of ferritin in malaria infection. There is evidence that damage to liver and spleen by the malaria parasite may contribute to elevated serum ferritin concentration during malaria infection (Oluboyede & Topley 1981).

Interestingly, a good number of subjects in this study who were asymptomatic had malaria parasitaemia. This study exposes the fallacy of measuring the serum ferritin level in a malaria-endemic area without first determining the malaria parasitaemia status. This influence of malaria infection on the serum ferritin level has prompted some workers, such as Oluboyede & Topley (1981), to suggest that direct examination of the bone marrow for iron might be preferable to serum ferritin estimation as a method of assessing iron stores in malarious areas. However, since bone marrow aspiration is a traumatic procedure, a means should be devised for the interpretation of serum ferritin results despite malaria infection. Considering our results, every serum ferritin estimation in a malaria-endemic country should be preceded by examination of the blood for malaria parasites and the result obtained corrected with the formula

Serum ferritin obtained $-(0.08 \mu\text{g} \times \text{malaria density}) = \text{Actual ferritin level } (\mu\text{g/l})$.

The significant difference between the ferritin levels of parasitaemic and aparasitaemic subjects in this study indicates that asymptomatic malaria parasitaemia constitutes a significant disease burden that should be treated.

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