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Full Length Research Paper

Effect of falciparum malaria on some plasma proteins in males: With special reference to the levels of testosterone and cortisol

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Sex-associated hormones were evidenced to modulate immune responses and consequently directly influence the outcome of infection. Testosterone is known to influence both protein metabolism and the level of cortisol which is the hormone of stress. This work was conducted to explore the influence of the degree of parasitemia in *Plasmodium falciparum* malaria on the male sex hormone testosterone, plasma proteins and the stress hormone cortisol in male patients. The study targeted male subjects whose ages ranged between 20 and 40 years old. The subjects were divided into three groups: lightly-infected patients (Infected with *P. falciparum* density 1 - 10 asexual form of the parasite per 100 fields), heavily-infected patients (Infected with *P. falciparum* density 11 - 99 asexual form of the parasite per 100 fields) and a group of malaria-free individuals who were used as a control group. Blood samples were taken from the median cephalic vein to investigate for malaria parasite, plasma proteins and hormones. The effect of the degree of parasitemia was considered for all parameters studied. The study revealed that low parasitemia in malaria-infected patients resulted in significantly ($p < 0.05$) higher level of plasma total proteins and it was found to be due to a significant ($P < 0.05$) increase in the total globulins fraction which reached 5.85 ± 1.03 g/dl compared to 3.44 ± 0.4 g/dl in the control group. The opposite was true for the heavily- infected group as it reported significantly ($P < 0.05$) lower total plasma proteins value which was found to be due to a significant ($p < 0.05$) reduction of the total globulins fraction reported only 2.96 ± 0.20 g/dl. The albumin fraction maintained levels similar to that of the control group in both infected groups. The levels of the hormones tested also showed significant changes, manifested as significantly ($p < 0.05$) lower values in both groups of patients compared to the control group for the testosterone hormone, with significant ($p < 0.05$) difference between the two groups. Thus, high parasitemia resulted in the least testosterone level in the heavily-infected group of 2.64 ± 0.28 ng/ml, compared to 6.03 ± 0.86 ng/ml in the control group. In contrast, the stress hormone, the cortisol, showed the highest level in the heavily- infected patients of 191.03 ± 18.17 ng/ml, with significant ($p < 0.05$) difference compared to 166.28 ± 10.63 ng/ml in the control group.

Key words: Malaria, testosterone, cortisol, parasitemia.

INTRODUCTION

Malaria is one of the most widespread transmissible diseases distributed throughout the world. Although the cause of malaria was identified more than hundred years ago, it still remains one of the leading causes of morbidity and mortality in the tropics. The worldwide incidence of malaria is estimated to be 300 - 500 million clinical cases and 1.5 – 2.7 million deaths (mostly children) annually, representing 2 - 3% of the overall global disease burden. Malaria is a serious problem e.g. in Sudan. It accounts for 32% of all cases and an estimated 7 – 7.8 million cases of the disease occur annually with a 20% mortality rate (Robert et al., 2004). Several studies have shown the relation between malaria-induced stress and the level of proteins. Abdelgadir (2002) reported that no significant

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difference was observed between males and females in total proteins and total globulins, but the albumin showed significantly higher values in male patients compared to females with the same pattern being observed in normal individuals. Adebisi et al . (1998) reported significant decrease in plasma total proteins in malaria patients compared to the normal individuals.

Numerous epidemiological and clinical studies have noted differences in the incidence and severity of parasitic diseases between males and females. Although in some instances this may be due to gender-associated differences in behavior, there is overwhelming evidence that sex hormones can also modulate immune responses and as a consequence, directly influence the outcome of parasitic infection (Benten et al., 1997). However, Bello et al. (2005) evaluated the pattern of infection in *P. falciparum* malaria cases at nested sentinel points in northern Nigeria and he reported that there were significantly more female patients than male.

In the present study, the male sex hormone testosterone and cortisol were assayed during *P. falciparum* infection in males and the levels of total proteins, albumin and total globulins were also measured. This is to investigate the effect of malaria as a stressing disease on the level of testosterone which is known to influence both the protein metabolism (Fahey, 1998) and cortisol which is the stress hormone (Resumo, 1998). Determining how all this is influenced by the degree of parasitemia was done by studying malaria patients as lightly-infected (one cross) and heavily- infected (two crosses), their results compared to values from malaria-free subjects of the same age.

MATERIALS AND METHODS

Forty-five men (age range 20 - 40 years) were employed in this study. Thirty of them were malaria patients, 15 were lightly-infected and other 15 were heavily-infected with *P. falciparum*. The immuno-chromatographic (ICT) malaria Pf test described by Garcia and Marlborough (1996) was used for rapid diagnosis of *P. falciparum* malaria. The other fifteen were free from infection and included as a control group. All individuals were divided into three groups: Group A as the control group (malaria-free individuals); Group B (one-cross patient) infected with *P. falciparum* density 1 - 10 asexual form of the parasite per 100 fields and Group C (two-cross patients) infected with *P. falciparum* density 11 - 99 asexual form of the parasite per 100 fields.

Blood samples for the laboratory test were collected in the morning after subjects signed a consent form and 5 ml of blood from the medium cephalic vein was collected into heparin bottles. Plasma was obtained by centrifugation at 4˚C (x 800 g, 15 min) and kept frozen at – 20˚C until used for the quantification of hormones, total proteins and albumin and the difference between total protein and albumin was calculated as total immunoglobulin.

Microscopic examination

Thick and thin films were prepared and examined microscopically for malaria parasite identification. Universal precautions were followed during preparation of the smears. The thick smears blood films were left to dry and the samples here were not fixed with methanol. This allowed the red blood cells to be hemolysed and leukocytes and any malarial parasites presented were the only detectable elements. The presence and relative parasite count of *P. falciparum* in each blood sample was determined from Giemsa stained thin and thick films after staining for 30 min. The identification of the species of human parasites in the blood films was carried out according to (WHO, 1980). A slide was scored as negative when 100 high power fields (at 1000x magnification) had been examined for about 30 min without seeing any parasites. Then blood films were stained by Giemsa stain and left to dry and examined microscopically at 10×100 magnification by oil immerse objective. Parasites count was estimated according to the plus system described by Dayachi et al. (1991) . The number of asexual forms of parasite (rings, trophozoites and schizonts) was counted against 100 fields as follows, one cross as 1 - 10 and two cross as 11 - 99 per 100 thick fields, respectively.

Biochemical measurements

Kits used for biochemical measures of total protein, albumin, testosterone and cortisol were obtained from Linear Chemicals Laboratory in Spain. Total protein was determined by the method described by Henary et al. (1974) using Biuret reagent kit. Albumin was determined by the method described by Doumas and Waston (1971) using Bromocresol green kit. Testosterone was determined by using radioimmunoassay (RIA) according to the method described by Soini and Kojola (1983) . Cortisol was measured by RIA according to the method described by Prasad (1979).

Statistical analyses

Data were entered into an access database that was then imported into SAS for statistical analysis with SAS/STAT software. Differences in parasitemia categories between male cases were assessed using the Fisher' s exact ^{2}. Differences in variable levels between cases and the control group were assessed using Wilcoxon rank sum exact test, the non- parametric counterpart of the independent *t* test. For the repeated measures analyses, mixed analyses of variance (ANOVA As) was used. Power analyses were calculated for all tests that revealed significant results ($p < 0.05$).

RESULTS

P. falciparum malaria infection and the degree of parasitemia in male individuals, showed clear changes in all parameters measured compared to malaria free subjects. The mean value of total protein, in all infected individuals as a group and the group of one-cross patients, was significantly ($p < 0.05$) higher than in the patients with two crosses and the control group as shown in Table 1 and Figure 1, while albumin levels, presented in Table 1 showed similar levels in the two groups of patients and the control group, with slightly lower levels in the groups of patients specially those of one-cross parasitemia.

Table 1 and Figure 1 present the effect of malaria infection on total globulins. The results showed significantly ($p < 0.05$) higher level in the group of all infected patients and in the one-cross parasitemia patients than the patients of two-cross parasitemia and control group. In two-cross patients the mean values showed significantly ($p < 0.05$) lower level than in the control group.

Table 1. The effect of Plasmodium falciparum malaria on plasma total proteins, albumin, total globulins, testosterone and cortisol in male individuals (N = 15).

Subjects	Total proteins g/dL	Albumin g/dl	Total globulins g/dL	Testosterone ng/mL	Cortisol ng/ml
The control group	7.97 ± 0.11[a]	4.53 ± 0.07[a]	3.44± 0.4[a]	6.03 ± 0.86[a]	166.28 ± 10.63[a]
One-cross patients	10.28 ± 0.24[b]	4.43 ± 0.09[a]	5.85 ± 1.03[b]	3.93 ± 0.57[b]	184.77 ± 14.70[ab]
Two-cross patients	7.42 ± 0.23[c]	4.45 ± 0.12[a]	2.96 ± 0.20 [c]	2.64 ± 0.28[c]	191.03 ± 18.17[b]

Means within columns followed by different letters are significantly different at ($p < 0.05$). N: Number of individual.
One cross = 1 - 10 asexual form of parasites per 100 leukocytes. Per 100 fields
Two crosses = 11 - 100 asexual form of parasites per 100 leukocytes. 11 - 99 asexual form of parasite per 100 fields
Means above columns followed by different letters are significantly different at ($p < 0.05$)

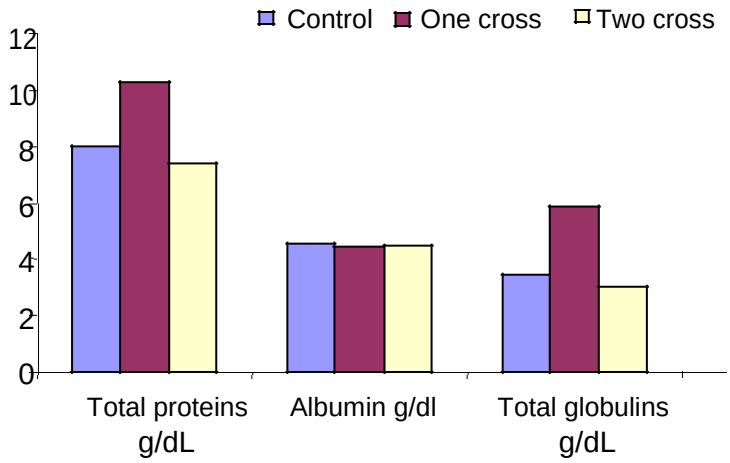

Figure 1. Effect of degree of parasitemia on plasma total proteins, albumin and total globulins in male (Means ± S.E).

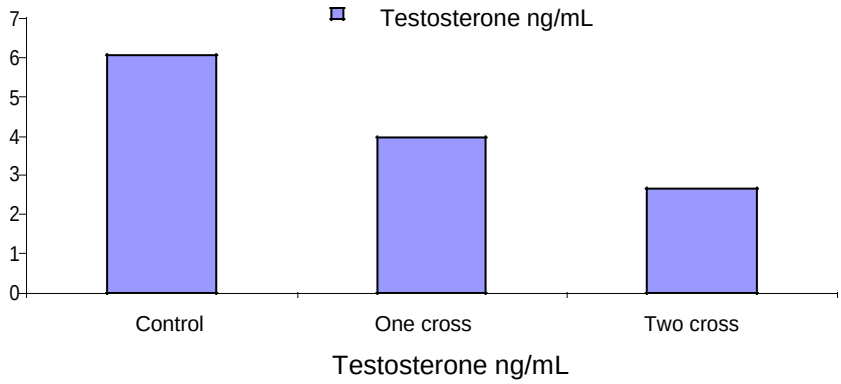

Figure 2. Effect of degree of parasitemia on testosterone in males (Means ± S.E).

The effect of presence and density of infection on the hormones is also presented in Table 1 and on the testosterone and cortisol in Figures 2 and 3 respectively. Testosterone mean value showed significantly ($p < 0.05$) lower level in the mean value of all infected individuals and each of the two groups of patients compared to the control group. The mean value in the patients with two crosses was significantly ($p < 0.05$) lower compared to

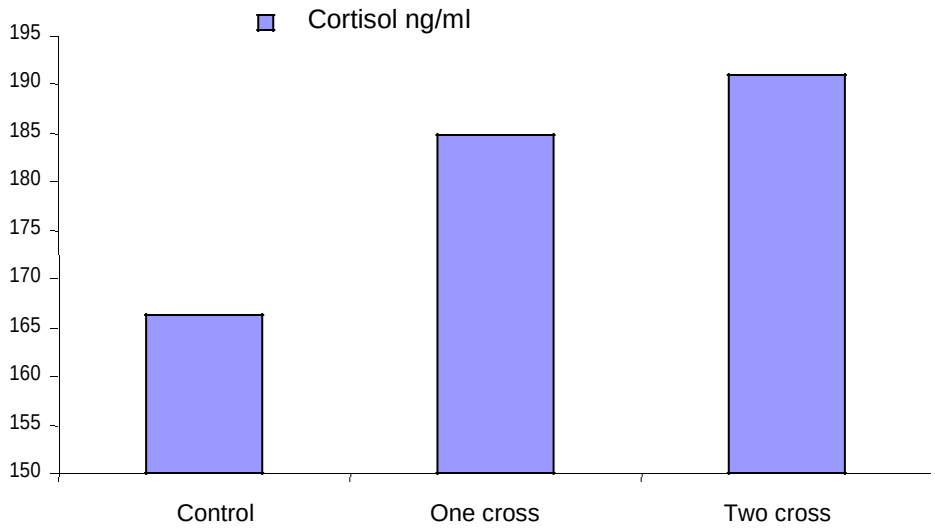

Figure 3. Effect of degree of parasitemia on cortisol in males (Means ± S.E).

the patients with one cross. In contrast, the cortisol showed higher values in the two groups of patients compared to the control group. Also significantly ($p < 0.05$) higher level of cortisol was observed in the two-cross patients compared to the control group, while the level of this hormone was just higher in the one-cross patients than that of the control group.

DISCUSSION

Effect of *P. falciparum* malaria infection on plasma proteins

Several studies showed that the level of total proteins in plasma decrease after the infection with *P. falciparum* malaria. Adebisi et al. (1998), Abdelgadir (2002) and Adeosun et al. (2007) reported significant decrease of plasma total proteins in malaria patients compared to non-infected individuals. They explained their results by the fact that the concentration of plasma proteins determines the colloid osmotic pressure of plasma and this is influenced by the nutritional status, hepatic and renal function. Chang and Herzog (1976) mentioned that malaria has an effect on all these functions and that it results in decreased plasma total proteins. It is also well known that hepatic protein biosynthesis shifts during inflammation from albumin synthesis to the synthesis of proteins involved in the acute inflammatory response such as C-reactive protein, coagulation factors, fibrinogen and complement components (Mac and Whaley, 2001).

In the present study the plasma total proteins in all infected patients as one group and the two-cross patients group agree with these finding and were found to have significantly ($p < 0.05$) low levels compared to the control group subjects and the one-cross patients. But the level of total proteins was found to be significantly ($p < 0.05$) high in one-cross patients compared to the control group. These findings showed that the values of total proteins can be influenced by the degree of parasitemia. However, It is well known that the first attack of the infection results in severe clinical symptoms e.g. diarrhea and vomiting (Dayachi, 1991). This might result sometimes in hemo concentration and elevated plasma proteins. In the present study the estimation of total globulin fraction showed significantly ($p < 0.05$) higher level in one-cross patients compared to the normal individuals, while the albumin was of similar levels in the patients and the control group. The very low levels of total proteins in the two-cross patients agree with the results of Chang and Herzog (1976) who mentioned that patients of high parasitemia develop liver disease, chronic kidney failure, malnutrition, or decrease in the immunoglobulins fractions. Abdelgadir (2002) found that the total protein values in children patients with one-cross parasitemia were within the same levels of the patients with two-cross parasitemia, so it was suggested that since all patients studied were children and supposed not to be suffered high frequency malaria infection (as in adult individuals) and the synthesis of the protein in the liver is not yet affected. The level of plasma albumin is very important. Matsuda et al. (1986) reported that a patient with lower levels of albumin has an unfavorable outcome, due to the fact that the decreased protein level may be a reflection of the severity of systemic illness rather than nutritional deficiency. In the present study, no changes were found in albumin levels of all groups. Lower albumin levels were observed in the two groups of patients compared to the control group individuals (Table 1) though the difference was not significant. This may be because the pronounced decline in plasma albumin (hypoalbuminemia) is usually known to follow prolonged malnutrition due to inadequate

dietary intake of protein, impaired digestion of protein, chronic loss of protein or inability to synthesize albumin in chronic liver disease (Change and Herzog, 1976). This may also indicate that hepatic condition in all patients in the present study was not yet seriously affected. In a previous work, Golden (1982) found that in patients infected with malaria, serum albumin levels dropped by 15%. This was explained to be due to the fact that inflammation leads to rapid decrease in albumin levels. The findings in the present study suggest that the levels of albumin were not affected by the degree of parasitemia, or all subjects employed in this work did not suffer repeated infection with malaria.

Previous studies showed that total globulins fraction levels increase in plasma after the infection with *P. falciparum* malaria. Lunn et al. (1966) reported a rise in the - globulins level at the initial period of the disease which was correlated with the increasing of malaria antibodies. He also reported that stable levels of hyper gamma globulinaemia are observed in populations of endemic malarious areas. Many authors reported that cytokines and other proinflammatory mediators initiate both cellular and hormonal changes that induce skeletal muscle proteolysis, all of which are components of the metabolic responses observed following injury and infection (Cannon et al., 1990; Hill et al., 1996; Ling et al., 1997; Mitch and Goldberg, 1996; Michie et al., 1988). In the present study the plasma total globulin in all infected patients as one group and in the one-cross patients agree with these findings and were found to be signifi-cantly ($p < 0.05$) higher compared to the control group individuals. But the level was found to be significantly ($p < 0.05$) lower in the two-cross patients compared to the control group. These findings suggested that the level of total globulins is influenced by the degree of parasitemia. Very high levels of total globulins in patients are known to be due to liver disease, acute or chronic infection (Bams and Miranda, 1985). It is also known that in the first attack of the infection the immune system stimulates the lymphocytes to produce specific antibodies (Roitt, 1993). Infection with malaria in the present study increased the level of total globulins in the group of low infection. Whereas, the total globulins level in the patients of heavy parasitemia in the present work showed values signifi-cantly ($p < 0.05$) lower than in the non-infected group. This finding is similar to the results obtained by Cohen et al. (1974) and Benten et al. (1992) who reported that total globulin levels decreased in heavy malaria cases which can be explained as immunity- suppression that is caused by acute *P. falciparum* malaria parasite infection.

Effect of *P. falciparum* malaria infection on plasma testosterone

Several studies showed a characteristic hormonal response pattern when the body is subjected to chronic or acute stress (Bessey et al., 1984; Bessey and Lowe, 1993; Watters et al., 1986). Hilary (2002) reported that the hypothalamic-pituitary-adrenal (HPA) axis which is an important hormonal system is acted upon by the testos-terone and this inhibits the release of the stress hormone cortisol. He suggested that this is the reason why males react differently to stress than females. In the present study testosterone levels showed significantly ($p < 0.05$) lower values in the two- cross patients compared to the patients with one-cross parasitemia (Table 1). These findings suggested that testosterone levels decrease significantly ($p < 0.05$) with the increase in the degree of parasitemia. Stephen (1999) reported that approximately 50% of the circulating testosterone is tightly bound to sex hormone binding globulin (SHBG) produced by the liver and high percentage bound to albumin, so that increased or decreased levels of SHBG or albumin influenced testosterone level. He also reported that the most accurate indicator of hypogonadism is the concentration of unbound testosterone. However, the very low levels of testosterone in malaria patients in the present study may be due to impaired biosynthesis of SHBG in the liver and this might increased the unbound testosterone (Matsuda et al., 1986; Stephen, 1999). Zhi et al. (2000) explained the possible modulation by male sex hormone of Th1/Th2 function in protection against *Plasmodium chabaudi* infection in mice. He suggested a possible counterbalan-cing between the immune and the endocrine systems in the response of a host to malarial infection. Benten et al. (1991) reported that testosterone has been shown to inhibit the ability of a host to overcome *P. chabaudi* infection, they also reported that although the mechanisms of testosterone-mediated inhibition are not clear, malaria- specific T and non-T cell suppression were observed with an increase in the number of CD8+ cells and are all thought to be involved in this immune sup-pression. It has also been shown that certain cytokines modulate male sex hormone production. Orava (1989) demonstrated that productions of interferons (IFNs) inhibit testosterone production *in vitro* in porcine leydig cells. Meikle et al. (1992) showed that IFN - , interleukin-2 and tumor necrosis factor- when used as therapy to treat chronic viral hepatitis, serum testosterone levels decreased. All these results clearly suggest that not only does male hormone inhibit immune responses, but also that immune responses can in turn modulate hormone production, resulting in the formation of an endocrine immune circuit. However, it is well known that malaria stimulate the immune system (Roitt, 1993) which may explain the low levels of testosterone in the present study which is in agreement with all these findings. On the other hand, the effect of testosterone on protein biosyn-thesis may be mediated by stimulation of intramuscular insulin like growth factor-1 (IGF-1) system (Fahey, 1998) and it is well known that cortisol inhibits (IGF-1) expression by stimulating the release of somatostatin (Resumo, 1998). In the present study, increased levels of cortisol and decreased levels of testosterone may de-monstrate the low levels of total proteins in heavy infection

patients.

Benten et al. (1992) stated that the influence of gender itself on the severity of malaria in human remains a matter of contention, the impact is clearly evident in certain rodent models of disease. Benten et al. (1997) found that following infection with *P. chabaudi* , female C57BL/10 mice self-cure, whereas male mice do not. The susceptibility of male mice is dependent on testosterone, as castration prior to infection makes male mice resistant, whereas testosterone administration to females impairs their ability to self-cure. Cernetich et al. (2006) examined the hormonal and immunological mechanisms that mediate sex differences in susceptibility to malaria infection, intact and gonadectomized (gdx) C57BL/6 mice were inoculated with *P. chabaudi* AS-infected erythrocytes and the responses to infection were monitored. In addition to reduced mortality, intact females recovered from infection-induced weight loss and anemia faster than intact males.

Effect of *P. falciparum* malaria on plasma cortisol

Resumo (1998) reported that when persons experience stress, our bodies release cortisol. He also reported that levels of cortisol in serum sample collected from malaria patients were significantly higher than those of normal subjects. He concluded that corticosteroid may interfere with initial response of *P. falciparum*-infected patients to treatment. In the present study also cortisol showed higher levels in the whole infected patients as one group and the two groups of patients compared to the control group. The highest cortisol level in his study was observed in the group of two-cross parasitemia and was significantly ($p < 0.05$) higher compared to the control group. These findings suggested that cortisol levels were increased with the increase of the degree of parasitemia and reach significant ($p < 0.05$) levels in heavy infection. Hilary (2002) reported that infection with *P. falciparum* malaria increases the secretion of pro-inflammatory hormones and mediators which induce resistance to cortisol, such as tumor necrosis factors (TNFs) and anti-microbial agents, also the infection reduce the synthesis of cortisol receptors that increase plasma cortisol level. It is also well known that the degree of parasitemia has effects on the immune system (Deans and Scohen, 1983). Very high levels of cortisol found in patients of two-cross parasitemia in the present study are in agreement with all these findings (Table 1).

Conclusions

This study showed the clear effect of falciparum malaria infection and degree of parasitemia on the levels of testosterone and cortisol. Though the number of the studied subjects was very little, still the differences between groups were very significant. The results showed that the higher the parasitemia the lower the testosterone levels and the higher the cortisol levels. This indicates that the degree of infection by the disease and the produced cytokines burden greatly on testosterone production. This can influence its effect on the HPA axis hormonal system and result in higher cortisol levels in malaria patients, increasing with the increase of parasitemia. Though findings concerned plasma proteins cannot easily explained, both hormones studied are known to influence protein metabolism and immunity. The influence of gender itself on the severity of malaria in humans remains a matter of contention and is an area of research that is likely to grow. Therefore, careful consideration should be given to these factors when designing and operating programs to study falciparum malaria infection.

Authors' contributions

Nabiela M. ElBagir conceived, designed and coordinated the study. M. A. Abdagalil obtained and prepared data and contributed to policy and manuscript preparation. All authors read and approved the final manuscript.

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