Effect of Falciparum Malaria on Plasma Proteins in Males: With special reference to the levels of testosterone and cortisol hormones

By :

Muawia Abdalla Abdalgalil
B.Sc. of Chemistry 1998
University of Khartoum

Athesis Submitted for Partial Fulfillment
For Requirements of Degree of Master of Science in Biochemistry

Supervisor :
Dr. Nabiela Musa Elbagir

Department of Biochemistry
Faculty of Veterinary Medicine
University of Khartoum

June 2003

بسم الله الرحمن الرحيم

قال تعالي

(وقل اعملوا فسيري الله عملكم ورسوله والمؤمنين)

صدق الله العظيم

Dedication

To my parents, my brothers and sisters, friends, relatives and to the department of Biochemistry – Faculty of Veterinary Medicine – University of Khartoum.

Contents

CHAPTER TWO : MATERIALS AND METHODS

CHAPTER THREE: RESULTS

CHAPTER FOUR : DISCUSSION

Acknowledgement

I am greatly indebted to my supervisor Dr. Nabiela Musa Elbagir the head department of Biochemistry – Faculty of Veterinary Medicine – University of Khartoum, for her valuable advice, guidance, continuous encouragement and patience throughout the process of my study.

Great thanks are also extended to my dear friend Ibrahim Matar for his support throughout the period of this work.

I would like to thank Mr. Eltayieb Abbas Babiker for his help also the thank is extended to all staff in the department of biochemistry for their help and support .

Also I would like to express my thanks to all these persons who made my study so successful and without whom it would have not been possible to overcome many obstacles that were encountered.

LIST OF FIGURES

ABSTRACT

This work was conducted in the Biochemistry Department, Faculty of Veterinary Medicine, University of Khartoum to study the effect of Plasmodium falciparum malaria on some plasma proteins, the male sex hormone testosterone, and the stress hormone, cortisol.

The study targeted male subjects their age ranged between 20-40 years old. Fourty five subjects employed in this work. The subjects were divided into three group, 15 one cross patients (1-10 a sexual form of parasite per 100 fields), 15 two cross patients (11-100 a sexual form of parasite per 100 fields), and the other fifteen were uninfected individuals and were included as control.

The blood samples were taken from the median cephalic vein for investigation of malaria parasite, plasma proteins and hormones.

Testosterone and cortisol were measured using Radioimmunoassay method (RIA). The effect of degree of parasitemia was considered for all parameters studied.

The result obtained showed that, the total proteins and total globulins were significantly ($P< 0.05$) higher in one cross patients compared to the control, and showed significantly ($P< 0.05$) lower value in two cross patients compared to the control.

Whereas the albumin showed slightly lower values in patients compared to control.

In all malaria patients studied the mean values of testosterone were significantly ($P< 0.05$) lower than control. In one cross patients, cortisol showed a rather higher level in patients compared to control, while two cross patients showed significantly ($P< 0.05$) higher value compared to the control.

When only infected individuals were compared, patients with two cross showed significantly ($P< 0.05$) lower values of total proteins, and total globulins compared to one cross patients, this also observed in the level of testosterone, while the albumin and cortisol showed no significant different between the one cross and two cross patients.

ملخص الأطروحة

أجريـت هـذه الدراسـة بقسـم الكيمياء الحيوية – كلية الطب البيطري بجامعة الخرطوم لمعرفة اثر الملاريـا علـي بعـض بروتينات البلازما في مجموعة من الشباب وتأثير بعض الهرمونات علي مستوي هذه البروتينات.

استهدفت الدراسة 45 شابا تتراوح أعمارهم بين 20 – 40 سنة. 30 مصاباً بالملاريا تم تقسيمهم إلى مجموعتين بالتساوي بناءً علي درجة الإصابة بطفيل الملاريا و15 من الأصحاء لضبط النتائج.

جمعـت عيـنات الدم من وريد اليد لإجراء الفحص المجهري للملاريا أولا ثم قياس مستوي البروتين الكلي والزلال والفلوبيولين الكلي وهرموني التستوستيرون والكورتيزول وقد أظهرت النتائج الآتي :

1- ارتفاع تركيز البروتين الكلي والفلوبيولين الكلي معنوياً عند مرضي الملاريا.
2- عدم تأثر مستوي الزلال معنوياً بمرض الملاريا بالرغم من نقصه عند المرضى
3- ارتفاع مستوي هرمون الكورتيزول عند مرضي الملاريا.
4- انخفاض مستوي هرمون التستوستيرون معنوياً عند مرضي الملاريا.
5- ازدياد البروتين الكلي والفلوبيولين الكلي معنوياً عند زيادة درجة الإصابة.
6- لم يتأثر الزلال عند زيادة درجة الإصابة.
7- ازداد هرمون الكورتيزول عند زيادة درجة الإصابة ازدياداً ملحوظا.
8- نقص هرمون التستوستيرون معنوياً عند زيادة درجة الإصابة عند المرضي.

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 cause 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 (Nature, 1996).

Malaria is a serious problem in Sudan. It acounts 32% of all cases, in an estimation of 7 – 7.8 million cases of the disease occurs annually, and 20% of the mortality cases (Federal Ministry of Health – Sudan, 2001).

Several studies showed the relation between malaria stress and the level of proteins. Abdelgadir, (2002) reported that, no significant deference observed between males and females in total proteins and total globulins, but the albumin showed significantly higher values in male patients compared to females and this was also observed in the normal individuals. Adebisi, et al., (1998) reported significant decrease in plasma total proteins in malaria patients compared to the normal individuals.

In the present study, an attempt was made to measure the levels of the male sex hormone testosterone and the cortisol during P. falciparum

infection in (20-40 years old men), and to measure the levels of total proteins, albumin and total globulins to investigate the effect of malaria as stress on the level of testosterone which is known to influence protein metabolism (Fahey, 1998) and on the cortisol which is the hormone of the stress (Resumo, 1998). Malaria patients were studied as slight infected (one cross) and heavy infected (two cross), their results compared to values from normal subjects of the same age as control.

CHAPTER ONE
LITERATURE REVIEW

1.1. Etiology of malaria

Malaria is caused by four species of protozoa of the plasmodium genera (P. vivax, P. falciparum, P. ovale, and P. malariae). With the except of P. malaria, which was repoted to affect some higher primate, humans are the only known hosts of the plasmodium parasite. Anopheles mosquitoes are the only mosquitoes capable of transmitting malaria, but of the 380 Anopheles species, only 64 actually carry the parasites (Nayar, *et al*., 1996).

1.2. Symptoms

The time between the mosquito bite and the appearance of symptoms varies, depending on the strain of parasite involved. The incubation of period is usually between 8 and 12 days for falciparum malaria, but it can be as long as a month for other species. Symptoms from some strains of P. vivax may not appear until 8-10 months after the mosquito bite occurred.

The primary symptom of malaria is the "malaria ague" (chills and fever). In most cases, the fever has three stages, beginning with uncontrollable shivering for an hour or two,followed by a rapid spike in temperature ,which lasts three will quickly bring down the fever.Other symptoms may includ[illegible] headache, or nausea and vomiting.As the sweating subside, the pateint typically feels exhausted and falls asleep. In many cases, this cycle of chills, fever, and sweating occurs every other day, or every third day, and may last for between a week and a

month if not treated. Those with the chronic form of malaria may have arelapse as long as 50 years after the initial infection.

Falciparum malaria is far more severe than other types of malaria because the parasite attacks all types of red blood cells, not just the young or old cells, as do other types. It causes the red blood cells to become very "sticky". A patient with this type of malaria can die within hours of the first symptoms. The fever is prolonged. So many red blood cells are destroyed and block the blood vessels in vital organ (especially the kidneys), and the spleen becomes enlarged. There may be brain damage, leading to coma and convulsion. The kidneys and liver may fail (Kristof, and Nicholas, 1997).

1.3. Life cycle of plasmodium parasite

The human malaria parasite has a complex life cycle that requires both a human host (carrier) and an insect host. In the Anopheles mosquito, the Plasmodium parasite reproduces sexually (by combing sex cells). In people, the parasite reproduces a sexually (by cell division), first in liver cells and then, repeatedly, in red blood cells.

When an infected fe 4 :s a human, sporozoites from the mosquitoes gut enter tl pon entering, the sporozoites travels to the liver, perhaps by chemotaxis (Wernsdorfer, 1980). They infect hepatocytes and begin to divide (some may become dormant in the liver and are known as hypnozoites). As the hepatocyte lyses, merozoites

are released. This portion of the cycle is known as exoerythrocytic schizogony (division outside erythrocytes).

The merozoites enter red blood cells and mature and divide. The fully developed asexual stage (called a schizont) breaks into individual merozoites, which again invade erythrocytes. This portion is known as erythrocytic schizogony (division inside erythrocytes). Upon invading an erythrocyte, the merozoite can repeat the above process or develop into a gametocyte. When gametocytes mature and unite, the result is azygote that matures into an ookinete, a motile cell.At this point in the process, another mosquito must feed upon the infected human.Ookinetes that are taken up into the mosquitoes gut mature into oocysts. Oocysts divide and eventually release sporozoites, and the process begins a new cycle (Hallowes, 1999).

1.4. Pathogenesis

Three factors affect the pathology of malaria:

1) Parasitemia (degree of parasite in the blood).
2) Destruction of the erythrocytes.
3) Defense response of the immune system.

5

The first event parasiti rocytes. The parasite enters the red blood cells and become part of vacule within the cell cytoplasm. The malaria acquire their characteristic hemozosin pigment here most likely a byproduct of hemoglobin (Wernsdorfer, 1980). .

The next event is hemolysis. Erythrocytes destroyed by both the plasmodia and by own immune system. It is this stage that is to cause the pathology behind the paroxysms that are very closely correlated with the levels of the merozoites in the blood.

The third stage is the body's response to the parasites. This stage is poorly understood.The immune system eventually consumes the parasites and the paroxysm comes under control. Physiological adaptations occur over time of individuals with repeated infections exposure to higher levels of parasitemia without symptoms (Aikawa, *et al*., 1980).

1.5. Complications of malaria

1.5.1. Anemia and reticulocytosis

The primary cause for morphological changes in malaria is the destruction of erythrocytes. Their destruction causes a hemolytic anemia as well as anemia of chronic disease.

The body responds by accelerating erythrocyte production and patients with malaria may exhibit chronic reticulocytosis.

The mechanism of anemia are as follows: hemolysis of parasitized RBCs due to rupture of the schizonts, decline in the production of erythrocytes due to depression of erythr 6 ased phagocytosis of the RBCs due to the change in sodiur and hemolysis of parasitized as well as unparasitized red cells through immunological process (Aikawa,1980).

1.5.2. Splenic enlargement

The first organ to show morphological changes is the spleen. Changes in the spleen can be seen as early as two weeks after infection, but the size of the spleen is not a good indicator of infection time. The spleen actively participates in the destruction of red blood cells and hematopoiesis; malaria also results in the hyperplasia of splenic lymphocytes and macrophages. So with stimulation of both splenic activities, splenomegaly is common. Individuals with malaria are at risk for splenic rupture with very small amounts of trauma (Aikawa, 1980).

1.5.3. Liver enlargement

Hepatomegaly in malaria seems to be associated with the level of nutrition, but can occur in well-nourished individuals. Kupfer cells are hypertrophied and contain parasite (besides the initial destruction of a few parenchymal cells) (Nicholas, 1999).

Endothelial cells in the sinusoids transform into Kupfer cells and rapidly divide to increase the number of macrophages (Aikawa, 1980).

P.falciparum parasitized red blood cells adhere to the sinusoid endothelium, causing slowed blood flow through the liver, resulting in portal hypertension, isch [7] ıtually focal necrosis. Fatty infiltration can be found throughout the liver, especially around the contrilobular vein. As disease goes on, phagocytes become rounded instead to elongated and can be found floating free in the sinusoid. The liver eventually turns black from the deposition of malaria pigment (hemozosin).

1.5.4 Capillary occlusion and cerebral malaria

Cerebral malaria is most dangerous form of falciparum malaria. It is an indirect complication arising from capillary occlusion. Agglutinated parasitized erythrocytes mass together forming a plug. All malarias cause some occlusion of capillaries, but only P. faliciparum malaria causes the erythrocyte mass and its cause is not understood.The occlusions can occur in any system, but brain occlusion are the most dangerous (Laudicina, 1998).

1.5.5 Renal pathology

Renal changes vary with the species of plasmodium, but generally speaking, malaria can cause nephrotic syndrome and glomerulonephritis. Both are associated with changes in the basement membrane, which begins to thicken and become less elastic (Aikawa, 1980).

1.5.6 Pulmonary changes

The most important pulmonary change is sudden, massive pulmonary edema, most likely caused by increased hydrostatic pressure as blood flow through the lungs decrease .the symptoms may begin gradually or suddenly with fever 8 xpectation and vague pain.In malaria there are several mechanisms that lead to acute pulmonary edema.The patein develops tachopena, with respiratory frequency of up to 40 breaths per minute,there is reduction in the atterial oxygen, and sighns of central nervous system dysfunction (Dunn and Palmer, 1998).

1.6 Alterations in protein metabolism due to the stress of injury and infection.

Robert (1999) reported that during the severe injury or infection an overall metabolic response occurs that results in aloss in lean body mass. However, each tissue has specific response that may be unique, and net protein synthesis may even be increased in some tissues, thus protein synthesis is accelerated in the liver for the production of cute phase proteins, the immune system, and wound repair requires rapid protein synthesis.

The catabolic response largely occurs in the Skeletal muscle. Over a short period of time , the muscle has one adequate reserve of protein to maintain function despite accelerated catabolism, (Bams and Miranda, 1985). The net synthesis or catabolism of muscle protein depend on the balance between rate of protein synthesis and breakdown. The precursors for protein synthesis are derived from either protein breakdown or from transmembrane transport from the plasma.The amino acids given in nutrition can only be incorporated into protein after being transported into the muscle cells from the 9 processes of protein synthesis, breakdown, and transmembrane amino acids transport are linked, and it is necessary to evaluate the respone to stress by quantifying these three related processes (Biolo, *et al.* 1995).

The negative protein balance caused by severe injury results from a large increase in the rate of protein breakdown. Although synthesis is also increased, the increase is insufficient to offset the increased rate of breakdown. The increase in muscle protein breakdown is coupled with an

increase in the outward transport of amino acid, which is consistent with the role of muscle to provide amino acid precursors for synthesis elsewhere in the body. The negative amino acid balance persist across the muscle even for a person in the fed state. Further more , increasing the amount of protein intake has no effect on the rate of muscle protein synthesis . (Patterson, *et al*. 1997).

1.7 Regulation of the translocation of protein

Various factors have been associated with the increased skeletal muscle proteolysis and translocation of nitrogen from the carcass to visceral organ. Anorexia commonly accompanies infection, and the diminished food intake causes hormonal changes , such as a fall in insulin and insulin- like growth factor-1, which decrease skeletal muscle protein synthesis.Other factors that control intermediate metabolism are known to be associated with the altered protein metabolism that occurs during injury and infection. A chara [10] response pattern has been observed during catablism. (Bessey, *et al*. 1984) . insulin levels are low, after which the blood levels generally rise to normal or supranormal levels. However, the counterregulatery hormone glucagon, glucocorticoids, and catecholamines are all elevated and they generally remain so, throughout the period of catabolism (Watters, *et al*. 1986). The hormonal environment plays a major role in determining body protein balance. Hormonal changes during critical illness may explain most of the negative nitrogen balance

observed, especially if food intake is provided by enternal or parental support.Alteration in the hormonal environment are triggered by yet another set of signals, those of the cytokines and other pro-inflammatory mediators. Cytokines are glycoproteins synthesized by inflammatory cells and elsewhere in the body in respone to inflamation and other noxious stimuli.Although these mediators primarily signal other cells in the local environment, they may be produced in a abundance and can, on occasion, be detected in the blood stream (Cannon, *et al.* 1990) . Those cytokines central to the pro-inflammatory response are interleukin-1 (IL-1) tumor necrosis factor (TNF), and IL-8 (apotent chemoattractant). These and other pro-inflammatory mediaters (e.g., complement, fatty acid metabolites, vascular endothelial factors) initiate both cellular (Hill, *et al* . 1996) and hormonal changes (Michie, *et al* . 1988). That induce skeletal muscle proteolysis (Mitch and Goldberg, 1996).

These pro- flammortary is a result of cytokines singaling to the central nervous system to initiate a pituitary-adrenal cortical response. Glucocorticoid elaboration is an important mechanism that attenuates cytokine effects and thus modulates catabolism (Santos, *et al*. 1993). Other anti- inflammatory cytokines are elaborated (IL-4, IL –10, and IL-13), and these substance dampen or attenuate the inflammotary response and enhance tissue viability and promote repaire.

1.8 Plasma proteins

The blood consist of solid elements, the red and white cells and the platelets suspended in a liquid medium, the plasma. The major function of blood are: Respiration, transport of nutrients, hormones and metabolic waste for excretion, regulation of water electrolytes, metabolites, nutrients, proteins and hormones. The concentration of total protein in human plasma is approximately (7.0-7.5g/dl) and comprises the major part of the solids of plasma. Plasma proteins are complex mixture that includes simple protein and conjugated proteins. The most common method for analyzing plasma proteins is electrophoresis.

The concentration of the proteins in plasma is important in determining the distribution of fluid between the blood and tissues. (Murry, 2000).

12

1.8.1 Albumin

Albumin is a protein manufactured by the liver, which performs many functions including maintaining the " Osmotic Pressure" that causes fluid to remain within the blood stream instead of leaking out tissue.

Another important function of albumin is acting as general carrier in plasma because of it is ability to bind to various ligands including bilirubin, free fatty acids, ions, metals, hormones and avariety of drugs.

Liver disease, kidney disease , and malnutrition are the major cause of low albumin. A diseased liver produces insufficient albumin. Diseased kidneys some time lose large amounts of albumin into the urine faster than the liver can produce it (this is termed nephrotic syndrome). In malnutrition there is no enough protein in the patients diet for the liver to make new albumin.The normal value of albumin depends on the laboratory running the test. Most labs consider roughly 3-5 to 5 grams per deciliter to be normal.In a healthy person with normal nutrition, the liver will simply manufacture more and the level will normalize. If albumin gets very low swelling can occur in the ankles (edema) and fluid can begin to accumulate in the abdomen (ascites) and in the lungs (pulmonary edema) (Matsuda, *et al.* 1986).

13

1.8.2 effect of malaria

Malaria is characterized by moderate decrease in total protein of blood serum at the expense of reduce levels of albumin and increased concentration of globulins with corresponding diminution of the albumin-globulin coefficient. Albumin is most abundant protein in the plasma. Although it is thaught that decreased levels are indicative of protein deficiency.(Matsuda, *et al.* 1986).

In many studies of severe protein restriction one does not see a drop in albumin untill many months and then only with diets that severely restrict protein (<3% of energy). In times of the protein restriction the catabolism of albumin decreases to maintain a stable serum level. It is true that dramatic decreases in serum albumin are often seen in very ill patients. This is due to the fact that inflammation will lead to rapid drops in albumin levels. Both decreased hepatic synethis of albumin and increased catabolism results in this response. In patients infected with malaria, serum albumin levels droped by 15% acutely. In addition, deficiency of other nutrients can effect albumin levels. Zinc deficiency will lead to decrease albumin synthesis. In ill patients, albumin will shift of extravascular space. In patient with toxic shock syndrome, serum albumin will often fall below 2g/dl.(Golden, 1982)

Furthermore, decreased level of albumin is more indicative of systemic inflammatory states rather than nutritional situation. Many studises have found that patient with lower level of albumin has an unfavorable outcome. These results may be due to the dec[illegible] ing a reflection of the severity systemi[illegible] itional deficiency.

1.8.3 Immunoglobulins.

Immunoglobulins (Igs) are glycoprotein molecules which produced by plasma cells in response to an immunogen and which function as antibodies. The blood cells which are responsible for the producation of the immune system in general are the lymphocytes.

There are two types of lymphocytes:

1. B-lymphocytes (B-cells).

These are mainly derived from bone marrow in higher animals, they were produce from the stem cells and will migrate with the lymphoid tissue and later will be colonized to mature B-lymphocytes.

2. T-Lymphocytes (T-cells)

These are also produced in the bone marrow but migrate early in liver to the thymus and will be colonized there. All immunoglobulin molecules consist of two identical light chains and two identical heavy chains, held together as atetramer by disulfide bonds forming (Y) shape. Each chain is divided in specific regions or domains, this according to their structure and function.

> Immunoglobulins bind specifically to one or a few closey related antigens. I[15]lin actually binds to specific antigenic d binding by antibodies is the primary function of antibodies and can result in protection of the host. Immunoglobulins also have effector functions that binding to various cell types, and activate the cells to perform some function. (Roitt, 1993).

1.8.4 Immunological perspective of life cycle of malaria parasite

During a mosquito bite, sporozoites are inoculated into the blood stream where they remain for only few minute, being targets for the circum sporozoite protein (CSP) antibodies. Inside hepatocytes, sporozoites develop into schizonts which later relaese merozoites into the blood stream. Liver schizonts express stage specific antigens, as well as antigen that are common to sporozoites and blood stage of the parasite .

$CD8^+$ T lymphocytes specific for several antigens, have been described as the most powerful immunological mechanism that inhibits the development of the liver stages of the malaria parasite.CD4+ lymphocytes also inhibit the development of this form of the parasite. In circulation, the merozoites also become a target for antibodies. Antibodies against surface antigens can inhibit erythrocyte invasion in vitro, and

mediated opsonization by blood monocytes. Merozoites invade red blood cells and transform into trophozoites, subsequently into schizonts, and finally are released as merozoites. The intra-erythrocytic stage of malaria parasites are targets for the CD4+ T- cell mediated immunity and antibodies, as some parasitic antigens occur on the surface of infected erythrocytes. Inside erthrocytes, some invading haploid merozoites develop into male and female gametocytes, and following ingestion, gametocytes undergo gametogenesis in the mosquito midgut. Gametocytes express stage specific surface antigens which are targeted by antibodies that impede parasite development inside the mosquito, therefore, blocking the cycle of transmission. This type of immunity does not protect the host against malaria but may diminish malaria transmissiom.

Diploid zygotes, formed after fertilization, differentiate into motile ookinetes, traverse the midgut epithelium, lodge on the haemocoele side, and develop into oocysts. Sporozoites produced in the oocysts then migrate to the salivary gland of the mosquito to continue the infection cycle (Amanpreet,*et al*. 2002) .

1.9 Testosterone hormone

Testosterone is the most important androgen produced by the testes, plays crucial role in the health of male.During fetal development, testosterone and its metabolite dihydrotestosterone (DHT) are needed for normal differentiation of male internal and external genitalia.

During puberty testosterone is required for the development of male secondary sexual characteristics, stimulation of sexual behavior and function, and initiation of sperm production. In adult males testosterone maintains mus [17] fat distribution, bone mass, erythropoiesis, male hair pattern, libido and potency, and spermatogensis.

Circulating testosterone level have diurnal variation in a normal young men, usually reaching a mean maximum level of 25nmol/l (710 ng/dl) at approximately 8 A.M and declining to mean minimum level of 15 nmol/l (426ng/dl) at approximately 10 P.M. This circadian variation in testosterone level appears to be a result of temporal modulation of hormone secretion by testes rather than of diural change in testosterone clearance, although the precise mechanism is unknown. (Stephen., 1999).

1.9.1 The effect of testosterone on muscle protein synthesis

Testosterone is anabolic-androgenic steroid hormone work by stimulation of receptor molecules in muscle cells, which activate specific genes to produce proteins. It also affect the activation rate of enzyme system involved in protein metabolism, thus enhancing protein synthesis and inhibiting protein degradation (called an anti-catabolic effect) (Fahey, 1998).

1.9.2 Anti-catabolic effect of testosterone

Testosterone may block the effect of hormones such as cortisol involved in tissue breakdown during and after exercise. Testosterone may prevent tissue from breaking down following intensive work ,this would lead to speed recovery. Cortisol and related hormones, secreted by the adrenal cortex also has receptors sites within skeletal muscle cell. Cortisol cause protein breakdown 18 xercise to enhance the use of protein for fuel and supress inflammation that accompanies tissue injury.

Testosterone may block the binding of cortisol to its receptor site, which would prevent muscle breakdown and enhances recovery.

The effect of the testosterone on skeletal muscle protein synthesis may be mediated by stimulation of the intramuscular insulin like- growth factor-1 system (IGF-1) (Fahey, 1998).

1.10 The stress hormone: Cortisol

Resumo, (1998) reported that, when we experience stress, our bodies release cortisol. One of our steroid hormone, cortisol is called stress hormone and functions by helping our bodies mobilize energy to manage stress. Cortisol is the primary glucocorticoid, it is a natural hormone of he adrenal gland. Cortisol is necessary to maintain important processes in times of prolong stress. Most of its effects are not directly responsible for the initiation of metabolic or circulotary processes, but necessary for their full response. Cortisol can exert its effects on peripheral tissues, once in circulation it is typically bound to aspecific glucocorticoid- binding alpha2-globluin called transcortin.About 75% of cortisol is bound to transcortin, 15% to 20% bound less tighly to albumin and 5% of circulating cortisol is unbound . this important factor to make into consideration when measuring cortisol levels.

The major catabolic effects of cortisol involve its facilitating the conversion of protein in muscle and connective tissue into glucose and glycogen (cortisol may increase liver glycogen). Gluconeogenesis involves both the increased degradation of protein already formed and the decreased synthesis of new protein. Cortisol can also decreased the utilization of glucose by cells. Acortisol excess can also lead to decreased in insulin sensitivity. Cortisol also reduces the utilization of amino acids for protein formation in muscle cells. Acortisol excess can lead to approgressive loss of protein, muscle weakness and atrophy and loss of bone mass through increased calcium excretion and less calcium absorption (Davies, *et al* 1985).

Any type of stre 19 body signals the nervous system to rely this to the hypnothalamus. The hyphothalamus then responds by initiating the stress-hormone cascade starting with CRF (corticotrophin

releasing factor) followed by ACTH (adreno corotico- tropic hormone) release and finally glucocorticoid production. Who also reported that increased cortisol levels also increased protein breakdown by 5% to 20% Even mild elevation in serum cortisol can increase plasma glucose level and protein catabolism within a few hours in healthy individuals.Cortisol can inhibit growth-hormone levels by stimulating the release of somatostatin (growth-hormone antagonist) .

It may also reduce IGF-1 expression (IGF –1 is one of the most anabolic agents in the body and is the substance that is responsible foe most of growth hormones positive effects because GH converts into IGF –1 in the liver (Charles, 1999)..

CHAPTER TWO
MATERIALS AND METHODS

2.1. Subjects

Forty five men their age ranged between 20-40 years old were included in this study. Thirty of them are malarial patients, infected with plasmodium faliciparum. The other fifteen were uninfected and were included as control group.

2.2. Protocol of study and preparation of samples

Included individuals were divided into three groups:

1. Group A control (uninfected individuals).
2. Group B (one cross patients).Infected with *P.falciparum* density 1-10 asexual form of the parasite (rings, trophozoites and schizonts) per 100 fields.
3. Group C (two cross patients).Infected with *P.falciparum* density 11-99 a sexual form of the parasite (rings, trophophozoites and schizonts) per 100 fields.

Blood samples for the laboratory test were collected in the morning.

After included individuals agree, five ml blood from the medium cephalic vein was collected from them into heparin bottles. Plasma was obtained by centrifugation at 4 C (800 X g, 15 min) and kept frozen at -20^{o}C until used. Plasma was used for the quantification of hormones, total

proteins and albumin, and the different between total protein and albumin was calculated as total immunoglobulins.

2.3. Microscopical examination

Thick and thin films were prepared and examined microscopically for the presence of the parasite.

Preparation of smear:

Universal precautions were used while preparing the smears for malarial parasites such as gloves; only disposable lancets; wash hands; handle and dispose the sharp instruments and other materials contaminated with blood carefully to avoid injury.

The smear was prepared in six steps as follows:

1) The fifth finger of the left hand was holden and wiped its tip with spirit; and dried.
2) The finger was pricked with disposable needle; allowed the blood to ooze out.
3) A clean glass slide was taken. 3 drops of blood 1 cm from the edge of the slide were taken; another drop of blood 1 cm from the first drop of blood was also taken.
4) Another clean slide with smooth edges was used as spreader.
5) Thick and thin smear made allowed to dry.
6) Blood film was marked on the thin smear with a lead pencil.

Thick smears:

1) Blood films were left to dry and the sample here is not fixed with methanol. (This allows the red blood cells to be hemolyzed leukocytes and any malarial parasites present will be the only detectable elements).

2) Further blood films were staining by Giemsa stain and left to dry and examined microscopically at 10×100 magnification.

3) The slide blood films were examined by oil immersiobjective.

.

Parasite count:

Parasites count was estimated according to the plus system described by Dayachi, *et al*. (1991).

The numbers of asexual form of parasite (rings, trophozoites, and schizonts) were counted against 100 fields as follows:

+ = 1-10 per 100 thick fields.

++ = 11-99 per 100 thick fields.

+++ = 1-10 per thick field .

++++=11-99 per thick field.

2.4. Biochemical measurements

Kits used in biochemical measures of total protein, albumin, testosterone and cortisol, were obtained from linear chemicals laboratory in Spain.

2.4.1. Total protein

Total protein was determined by using spectrophotometer according to the method described by Henary, *et al*. (1974) using Biuret reagent kits.

Reagents:

1- Biuret reagent:

a) Potassium - sodium - Tartrate - 15 mmol/L

b) Sodium iodide. 100 mmol/L

c) Potassium iodide - 15 mmol/L

d) Copper sulphate - 5 mmol/L

2- Standard protein 7 g/dl:

Principle:

Proteins together with a basic copper-sulphate solution containing tartrate (Biuret Reagent) form a violet blue colour complex which proportional to the amount of the total protein present in samples.

Procedure:

In three different tubes:

1- Standard: 100 ml sample in 5 ml biuret reagent.

2- Blank: 5 ml biuret reagent.

3- Sample: 100 ml sample in 5 ml biuret reagent.

Then the tubes were mixed well and incubated for 20 minutes at 37° then they were kept at room temperature for 5 minutes, the absorbance of unknown and standard were measured against blank reagent at 540 nm.

Calculation:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard conc. (g/dl)} = \text{Total protein (g/dl)}$$

2.4.2. Albumin

Albumin was determined by the method described by Doumas and Waston, (1971) using Bromcresol green kits.

Principle:

Serum albumin in the presence of bromcresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue.

Reagents:

1- Bromcresol green pH 4.2. 50 mmol/L (BCG)

2- Standard-Human serum albumin.

Procedure:

In three different tubes:

1- Blank reagent: distilled water 0.2 ml in 4 ml BCG.

2- Standard: 0.2 ml standard in 4 ml BCG reagent.

3- Sample: 0.2 ml in 4 ml BCG reagent.

Then the tubes were mixed well and allowed to stand at room temperature for 10 minutes. The absorbance of sample and standard were measured against the blank at 630 nm (600-650).

Calculations:

Absorbance sample X conc. standard (g/dl)= Albumin (g/dl)
Absorbance standard

2.4.3. Testosterone

Testosterone was determined by using radioimmunoassay (RIA) according to the method described by Soini and Kojola (1983).

Principle of the assay:

The radioimmunoassay method depends on competition between europium-labeled testosterone and sample testosterone for polyclonal anti-testosterone antibodies. Standard, control and samples containing testosterone inhibit the binding of the europium-labeled testosterone to the antibody molecules.

The blocking agent in the testosterone assay buffer facilitates the release of testosterone from binding proteins in the sample. A second antibody, directed against rabbit IgG, is coated to the solid phase, and binds the IgG-testosterone complex, giving convenient separation of antibody-bound and free antigen.

Reagents:

1) Testosterone standards (the powder contain sodium azide > 1%).

2) Testosterone-Entracer (~0.5 μg/ml). Tris-HcL buffered (pH 7.5) salt solution with bovine serum albumin.

3) Testosterone Anti-serum (~ 2 μg/ml).

4) Enhancement solution with Triton X - 100[6], acetic acid and chelators.

5) Anti-rabbit IgG Microtitration strips. 8 X 12 wells coated with anti-rabbit IgG (goat).

Procedure:

1) The assay racks were labeled.

2) Pipette 100µl of each standard, control and patients plasma.

3) 200µl of [^{125}I]-testosterone tracer were added to each tube and vortex mix briefly.

4) 100µl testosterone antiserum was added to the appropriate tubes.

5) 200µl of separating agent were added to each tube.

6) Incubated at 37 C for 3 hours**.**
7) The tubes were centrifugated for 20 minutes at 1500xg
8) Determined the radioactivity of the precipitate remaining in the tubes in λ - counter.

Calculation of results:

Results were calculated using Ling-log plotting:

1- Express the counts (B) for each of the standards and unknowns as a percentage of the mean counts of the zero standards (B_o).

$$B_{/Bo}\% = \frac{\text{B of standard or unknown}}{B_o} \text{ X } 100\%$$

2- Plot the percentage values obtained for the testosterone concentration on Ling-log graph paper and constructs a standard curve.

3- Read the testosterone concentration directly from the curve for each of the unknown samples.

2.4.4. Cortisol

Cortisol was measured by (RIA) Radioimmunoassay according to the method described by Prasad (1979).

Principle:

The radioimmunoassay method depends on the competition between ^{125}I labeled cortisol (F) and cortisol contained in standards or in specimens to be assayed. After incubation, the amount of 125-labeled F (^{125}I-F) bound to the antibody is inversely related to the amount of cortisol present in sample. By measuring the proportion of ^{125}I -F bound in the presence of reference standards containing various known amounts of cortisol, the concentration of present in unknown samples can be interpolated.

Reagents:

1) Standards (F)

2) Red solution (^{125}I-F 20 ml)

3) Blue solution (F antibody 10 ml)

4) M S DA solution (20 ml).

Procedure:

1- F standard: 0.5 ml-distilled water was added to each samples.

2- The tubes were centrifuged at 2000 X g for 20 min.

3- The supernatant was discarded, and then counted in γ-counter.

Calculation of results:

Results were calculated using Ling-log plotting:

$$F_{/Fo}\% = \frac{\text{F of standard or unknown}}{F_o} \times 100\%$$

2.5 Statistical analysis

Statistical analysis was performed using Statistical Analysis System (SAS) which analyzed the mean values of all parameters with each other and compared the significant different between the patients and control using T-test Design (Nie, 1983).

CHAPTER THREE
RESULTS

3.1. Effect of degree of parasitaemia in plasma proteins, testosterone and cortisol in falciparum malaria infected males

The effect of falciparum malaria on plasma total proteins, albumin, total globulins, testosterone and cortisol in male individuals, is presented in table (1).

3.1.1 Total proteins

The effect of infection and degree of parasitaemia on total proteins is presented in table (1) fig. (1). The mean values of total protein differed significantly ($P<0.05$) higher level in the infected individuals and one cross patients compared to the patients with two cross and control. In two cross patients the mean value showed significantly ($P<0.05$) lower values compared to the control.

3.1.2 Albumin

The effect of infection and degree of parasitaemia in albumin is presented in table (1) fig. (2). The mean values of albumin showed a similar level in the two groups of patients and the control, with slightly lower levels in the groups of patients specially those of one cross parasitaemia.

3.1.3 Total globulins

The effect of infection and degree of parasitaemia on total globulins is presented in table (1)fig.(4). The mean values of total globulins showed significantly ($P<0.05$) higher level in the infected and one cross patients compared to the patients with two cross and control. In two cross patients the mean values showed significantly ($P<0.05$) lower compared to the control.

3.1.4 Testosterone

The effect of presence and density of infection on testosterone is presented in table (1) fig. (4). Testosterone mean values showed significantly ($P<0.05$) lower level in the infected and the two groups of patients compared to the control. The mean value in the patients with two cross is significantly ($P<0.05$) lower compared to the patients with one cross, while showed a similar level when compared to infected individuals.

3.1.5 Cortisol

The effect of presence and density of infection in cortisol level is presented in table (1) fig.(5).Cortisol mean values showed higher levels in the two patients groups. It was significantly ($P<0.05$) higher in the two cross patients compared to the control. But the level was a rather higher in patients of one cross than that of the control.

CHAPTER FOUR

DISCUSSION

4.1. Effect of falciparum malaria on some plasma proteins in males

4.1.1. Plasma total proteins

The level of plasma total proteins is one of the factors, which is known to be affected by falciparum malaria. Several studies showed that the level of total protein in plasma decrease after the infection with falciparum malaria. Adebisi, *et al*. (1998) and Abdelgadir, (2002) reported significant decrease in plasma total proteins in malaria patients compared to the normal individuals. They explained their results by the fact that the concentration of plasma proteins determines the colloids osmotic pressure of plasma and this is influenced by the nutritional status, hepatic and renal function. Also (Chang and Herzog, 1976) mentioned that malaria has an effect on all these functions,and it results in decreased plasma total proteins. In the present study the plasma total protein in the infected and the two cross patients (11-100 asexual form of parasites per 100 fields) agree with these finding and were found to have significantly ($P<0.05$) low levels compared to the control subjects and one cross patients (1-10 asexual form of parasites per 100 fields). But the level of total proteins found to be significantly ($P<0.05$) high in one cross patients compared to the control.

These finding showed that, the values of total proteins can be influenced by the degree of parasitaemia.

However, high level of total proteins in patients may be due to nutritional status, chronic disease, chronic infection or alcoholism (Bams and Miranda, 1985).

It is also well known that, the first attack of the infection result in severe clinical symptoms e.g. diahrria and vomiting (Dayachi, 1991) this might result in haemoconcentration and elevated plasma proteins, moreover, in the present study the estimation of total globulins fraction showed significantly ($P< 0.05$) higher levels compared to the normal individuals, while the albumin was of similar levels in one cross patients and controls.

The very low levels of total proteins in the infected and the two cross patients agree with (Chang and Herzog, 1976) who mentioned that patients of high parasitemia develop liver disease, chronic kidney failure, malnutrition, or decrease in the immunoglobulins fractions which is usually a companies malaria.

Abdelgadir, (2002) reported that the values in children patients with one cross are within the same levels of the patients with two cross, so it was suggested that, the total protein levels was not influenced by the degree of parasitaemia and explained her results as that, all patients studies were children, and supposed not to be suffered malaria infection as high frequency as in adult individuals and the synthesis of the protein in the liver is not yet affected.

4.1.2. Albumin

Multiple studies have found that, a patient with lower levels of albumin has an unfavorable outcome. This may be due to the fact that the decreased protein level being a reflection of the severity of systemic illness rather than nutritional deficiency (Matsuda, *et al*., 1986).

Golden, (1982) reported that, in patients infected with malaria, serum albumin levels dropped by 15% this was explained to be due to the fact that inflammation lead to rapid drops in albumin levels. Also it is 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 compounds, fibrinogen and complement components (Mac Sween and Whaley, 2001).

In the present study the albumin fraction showed lower levels in the two groups of patients, compared to the control individuals(Fig 2) (Table 1) though the difference was not significant, this may be because the pronounced decline in plasma albumin (hypoalbuminemia) 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 indicate that, hepatic condition in all patients in the present study was not yet seriously affected.

The findings in the present work showed similar albumin levels in both patient groups this might suggest that, the levels of albumin were not

affected by the degree of parasitaemia, or may be all subjects employed in this work were not suffered repeated infection with malaria and are in good situation,since they look healthy and have good capacity for work.

4.1.3. Total globulins

Several studies showed that the levels of total globulins in plasma increase after the infection with falciparum malaria. Lunn, *et al*. (1966) reported a rise in the γ-globulins level at the initial period of the disease which was correlated with the increasing of malaria antibodies. He also reported that, the stable levels in hypergammaglobulinaemia observed in the population of endomic malarious areas.

Cannon, *et al*. (1990) reported that, cytokines and other pro-inflammatory mediators initiates both cellular (Hill, *et al*., 1996) and hormonal changes (Michie, *et al*. 1988) that induce skeletal muscle proteolysis (Mitch and Goldberg, 1996), all of which are components of the metabolic responses observed following injury and infection (Ling *et al*., 1997).

In the present study the plasma total globulins in the infected and one cross patients agree with these findings and were found to be significantly ($P< 0.05$) higher compared to the patients with two cross and control individuals. But the level was found to be significantly ($P< 0.05$) low in the two cross patients compared the controls.

These findings suggested that, the level of total globulins is influenced by the degree of parasitaemia. 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 stimulate 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, this result was also obtained by Cohen, *et al*., (1974).Benten, *et al*., (1993) reported that total globulin levels decreased in heavy malaria cases which can be explained as immunity-suppression which caused by acute falciparum malaria parasite infection. This can be suggest the very low levels of total globulins in patients of high parasitaemia in the presence study. Anemia patients infected with malaria are known to manifest leucopenia with relative monocytosis (Mac Sween and Whaley, 2001).

However, Grimble (1993) reported that, low levels of total globulins may be due to malnutirtion or inherited acquired hypo-gammaglobulinaemia.

4.2. Effect of falciparum malaria 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) and (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 testosterone and this inhibits the release of the stress hormone the steroid cortisol. He suggested that, this is reason which explains why males react differently to stress than females.

In the present study testosterone levels showed significantly ($P< 0.05$) lower values in the two groups of patients comported to the normal individuals, and showed significantly ($P< 0.05$) lower values in two cross patients compared to the patients with one cross and shoed a similar level in the two group of patients and the infected individuals, while differed significantly ($p< 0.05$) lower in the infected compared to the control. (Table 1),(Fig 4).

These findings suggested that testosterone levels decrease significantly ($P< 0.05$) with the increase in the degree of parasitaemia.

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 patients in the present study may be due to impaired biosynthesis of albumin and (SHBG)

in the liver which is affected in malaria infection (Matsuda, *et al*., 1986) that may increased unbound testosterone which is the cause of hypogonadism (Stephen, 1999). Zhi, *et al*. (2000) explained the possible modulation by male sex hormone of Th_1/Th_2 function in protection against plasmodium chabaudi infection in mice, and he suggested a possible counterbalancing 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, he also reported that, although the mechanisms of testosterone-mediated inhibition are not clear, malaria-specific T and non-T cell suppression and an increase in the number of $CD8^+$ cells (Benten *et al*., 1991) are all thought to be involved in this immune suppression.

It has recently been shown that certain cytokines modulate male sex hormone production, Orava (1989) demonstrated that productions of (IFNs) inhibit testosterone production in vitro in porcine leydig cells and Meikle ,*et al*. (1992) showed that IFN-γ inter leukin-2, and tumor necrosis factor-α 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 inturn

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 agree with all these findings.

On the other hand the effect of testosterone on protein biosynethesis may be mediated by stimulation of intramuscular insulin like-growth factor-1 (IGF-1) system (Fahey, 1998) and it is well known that cortisol inhibit (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 demonstrate the low levels of total proteins in heavy infection patients.

4.3 Effect of falciparum malaria on plasma cortisol

Resumo,(1998) reported that ,when we experience stress, our bodies release cortisol ,he also reported that ,levels of cortisol in serum sample collected from patients were significantly higher than those of normal subjects .High levels of cortisol led us to postulated 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 infected and the two groups of patients compared to the control. Also the level of

the infected and the two cross patients bit higher compared with one cross patients, and significantly ($p<0.05$) higher compared to the control.

These findings suggest that cortisol levels were increase with the increase of the degree of parasitemia and reach significant ($p<0.05$) levels in heavy infection.The little increase in the level of cortisol in one cross patients may be because patients were having good diet ,their bodies do not overtrain ,relaxed and got sufficient hours of sleep per night ,all these factors are know to be responsible of keeping the normal range levels of cortisol according to Resumo,(1998).

Hilary, (2002) reported that infection with *P.falciparum* malaria increases the secretion of pro-inflammatory hormones and mediators which induce resistance to cortisol, tumor necrosis factors (TNFs) , anti-microbial agents, also it reduce synthesis of cortisol receptors that increase plasma cortisol level . It is also well known that the degree of parasitaemia has an effects on the immune system (Deans and Scohen, 1983). Very high levels of cortisol in two cross patients in the present study is agree with all these findings (Fig 5).

Conclusion

Several studies discussed the relationship between P. falciparum infection and plasma proteins. Adebisi, *et al*., (1998) reported that, plasma total protein, albumin and total globulin are significantly higher in control subjects when compared to falciparum malaria patients.

The present work is conducted to examine the degree of parasitemia of plasmodium falciparum malaria on some plasma proteins, testosterone and cortisol hormones in males.

Fourty-five individuals their age ranged between 20-40 years old were employed. 30 of them were malarial patients divided into two groups, 15 one cross patients and 15 two cross patients. The other 15were healthy and used as control group.

The results obtained in the low infected group is as follows:they showed significant increase in total proteins and total globulins, while albumin fraction was only a little bit lower compared to the control. Testosterone was significantly decreased, while cortisol showed a little bit higher compared to the control subjects.

Whereas in the heavy infected group the total protein and total globulins showed significant decrease compared to the control and low infection group, while albumin showed similar levels to the two cross groups of patients. Testosterone showed significant ($P< 0.05$) decrease compared to control and one cross while cortisol showed significant

increase compared to the control group and just higher value than one cross patient.

These finding showed that, the increase in the total protein was found to be mainly due to the increase in total globulins.

The progressive decreased in testosterone levels and increased cortisol levels is therefore likely to be mediated as suggested by (Resumo,1998) due to the continuous stimulation from the hypothalamic-pituitary-adrenal (HPA) axis that controls the secretion of cortisol. Thus it is suggested that in cortisol levels are affected by the level of testosterone. Also he reported that, the release of cytokines act as an acute stimulating factor for the HPA axis.

It would therefor be proposed that, there is strong interrelationships between both testosterone and cortisol levels and may be cytokines, which indicates that they seem to be influenced by the production of antibodies from P-falciparum infected patients that increased the level of total globulins in the present work.

REFERENCES

Abdelgadir, N.E., Nadia (2002). Effect of plasmodium falciparum malaria on some plasma proteins and immuoglobulins. M.Sc. research. Dept. of Biochemistry. Vet. Medicine, U. of K. Khartoum.

Adebisi, S.A., Soladoye, A.Q., Adekoya D., and Odunkanmi O.A. (1998). Serum protein fractions of Nigerian with plasmedium infection: ILRON Experience. Pp. 82-84.

Aikawa, M., Suzuki, M., and Gutierriez, Y. (1980). Pathology of malaria. In. J. P. Kreier (Ed.) Malaria, Vol. (2): pp 1-46.

Amanpreet, K., Arvind, G. Kinhikar, and Prati Pol (2002). Malaria vaccine development. National Institute of Pharmaceutical Education and Research, Sector 67, pp. 160-162.

Bams, J.L., and Miranda, D.R. (1985). Outcome and costs of intensive care. Intensive (are. Med. 11: 34-241.

Barreca, T., Piccioto, A.; Frances Chini, R.; Varagona, G.;Garibaldi, A.; Valle, F., Cataldi, and Rolandi, E. (1993). Sex hormone and sex hormone-binding globulin in males with chronic viral hepatitis during recminant INF-x. J. of INF Research 13, 209-211.

Benten, W.P.; Bettenhaeuser, U.; Wunderlich, F. and Massmann, H. (1991). Testosterone induced abrogation of self-healing of P. chabandi malaria. Infection and Immunity 59, 4486-4490.

Bessey ,P.Q. and Lowe, K.A.(1993).Early hormonal changes effect the catabolic response to trauma. Ann.Surg. 218:476-489.

Bessey, P.Q., Watters, J.M., Aoki, T.T., and Wilmore ,D.W. (1984). Combined hormonal infustion stimulate the metabolic response to injury. Ann. Surg. 200: 264-281.

Biolo, G., Fleming, R.Y.D.; Maggi, S.P.; and Wolfe, R.R. (1995). Transmemberane transport and intracellular kinetics of amino acids in human skeletal muscle. Am. J. Physiol. 268 (31): 75-84.

Cannon, J.G.; Tomokins, R.G.; Gelfond, J.A. Michic H.R., Stanford, G.G. Vander Meer, J.W.; Endres, S. Lonnemann, G. J.; Corsetti, B. Chemow, Wilmore ,D.W., Wolff,S.M.; Burke, J.F. and Dinarello, C.a. (1990). Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever, J. Infect. Dis. 161: 79-84.

Chang, F.C. and Herzog. B. (1976). Burn morbidity: A follow-up study of physical and psychological disability. Ann. Surg. 183: 34-37.

Charles Weber, (1999). The purpose of cortisol and corticosterone. Med Hypothesis (1) . 70-83.

Cohen, S., Butcher, G.A. (1974). Mechanism of immunity to malaria. Bull. Wld. Hlth. Org.

Davies, E. Keyon, C. J. ; and Fraser, R. (1985). The role of the calcium ions in the mechanism. Of ACTH stimulation of cortisol synthesis. steroids 45: 557.

Dayachi, F.,; Kabongo, L.,and Ngoie, K. (1991). Decreased mortality from malaria in children with symptomatic HIV infection. Int. Cont. AIDS, 2: 164.

Deans, J.A., and Scohen (1983). Immunology of malaria. Ann. Rev. Microbiol. 37, pp. 25-49.

Doumas, B., and Waston, W. (1971). Clin chim Acta 13, 87. Publisher spinreact, S.A. Spain (2001).

Dunn, I.J., and Palmer (1998). Newly emerging disease in a world out of balance. Seminars in Roentgneology 33 (1), 79-80.

Fahey, T.D. (1998). Anabolic-androgenic steroids: mechanism of action and effects on performance. Encyclopedia of Sports Medicine and Science. Internet Society: http: 11 sportsci.org.

Golden M. (1982). Transport proteins as indices of protein status. Am. J. clin. Nutyr. 35: 1159-1165.

Grimble, R.F. (1993). The maintenance of antioxidant defenses during inflammation. Pp. 347-366 in Metabolic support of the critically ill patient. New York: Springer-Verlag.

Hallowes, P. (1999). Malaria as are emerging disease. Epidemiologic Reviews. 18 (1), 77-89.

Henary, R.J., Cannon, D.C., and Winkelam (1974). Anal. Chem. 92. 1491. Publisher USA edition Harper and Rwo.

Hilary Thomson (2002). Sex hormones' link to stress. UBC reports. Vol. 48. No. (5).

Hill, A.G., L. Jacobson, J. Gonzalez, J. Rounds, J.a. Majzoub, and D.W. Wilmore (1996). Chronic central nervous system exposure to interleukin - 1 β causes catabolism in the rat. Am. J. Physiol. 271: 1142-1148.

Kristof, and Nicholas. "Malaria makes a come back, deadlier than ever" The New York Times (Jan. 8, 1997).

Laudicina, R.J. (1998). Hematologic changes associated with infection. Clin. Lab. 11 (4), 239-254.

Ling, P.R., J.H., Schwatz, and B.R. Bistrain. (1997). Mechanism of host wasting induced by administration of cytokines in rats. Am. J. Physiol. 272: 333-339.

Lunn, J.S., Chin, W. Contacos, P.G., Coatney, G.R. (1966). Changes in antibody titres and serum protein fractions guring the course of

prolonged infection with vavax or falciparum malaria. Amer. J.Trop. Med. Hyg. 15, 1, 13.

Mac Sween R.N.M and Whaley .K (2001).Muirs text book of pathology .Thirteen edition Arnold-Hodderline Group.London.Copublished in USA by Oxford University press ,Inc,New York.

Matsuda, Y., Ogushi. .; F., Ogaw . K; and Katunuma, N.(1986). Structure and properties of albumin. J. Biochem, 100, 375-379.

Meikle, A.W., Gardoso, Dacosta, N. and Samlowski, W.E. (1992). Direct and indirect effects of murine IL-2, -INF, and TNF on testosterone synthesis. J. of Andrology, 13, 437-443.

Michie, H.R., K.R. Manogue, D.R. Springs, A. Revhaug, S.O. Dwyer, C.A. Dinarello, A. Cerami, S.M. Wolff, and D.W. Wilmore (1988). Detetion of circulating tumor necrosis factor after endotoxin administration. N. Engl. J. Med. 318: 1481-1486.

Mitch, W.E., and A.L. Goldberg (1996). Mechanism of muscle wasting. N. Engl. J. Med. 335: 1897-1905.

Murry, R.K. (2000). Blood plasma and clotting in Harper's Biochemistry 2[1st] edition, murr, R.K. Cranner, Dikmayes, P.A. and Rodwell, V.W. Lage medical publication USA.

Nature (1996). Report of nature. London. 386: pp. 535-536.

Nayar, J.K., C.D. Morris and R.H. Baker (1996). Human malaria. Emerging infectious disease 2: 343-357.

Nicholas Regush (1999). The germs are coming. ABC News viegas. J. www.httii:more.abcnews.go.com.

Nie NH,(1983).SPSSX user guide.New York:Mc Graw-Hill

Orava, M. (1989). Comparison of the inhibitory effects of interferoms on testosterone production in porcine leyding cell culture. J. of Interferon Research, 9, 135-141.

Patterson, B.W., T. Nguyen, E. Pierre, D.N. Herndon, R.R. Wolfe (1997). Urea and protein metabolism in burned children:

Prasad, J.A. (1979). Method of hormone radioimmunoassay. Department of isotope, China Institute of Atomic Energy. Beijing, 102413.

Resumo, D.T. (1998). Evaluation of both cortisol and dehidroepiondrosterone levels in patients with non-complicated malaria due to plasmodium falciparum Soci. Bra. Med. Trop. 13 (2): 243-244.

Robert, R. Wolfe (1999). The role of protein and amino acids in sustaining and enhancing performance. Pp. 279-284. Washington, D.C. National Academy Press.

Roitt, K. (1993). Immunoglobulins structure and function. Immunology, 5th Ed., pp. 6-7.

Santos, A.a., M.R. Scheltinga, E. Lynch, E./F./ Brown, P. Lawton, E. Chambers, J. Browning, and C.a. Dinarello (1993). Receptor antagonist is not attenuated by glucocorticoids after endotoxemia. Arch. Surg. 128 (2): 138-143.

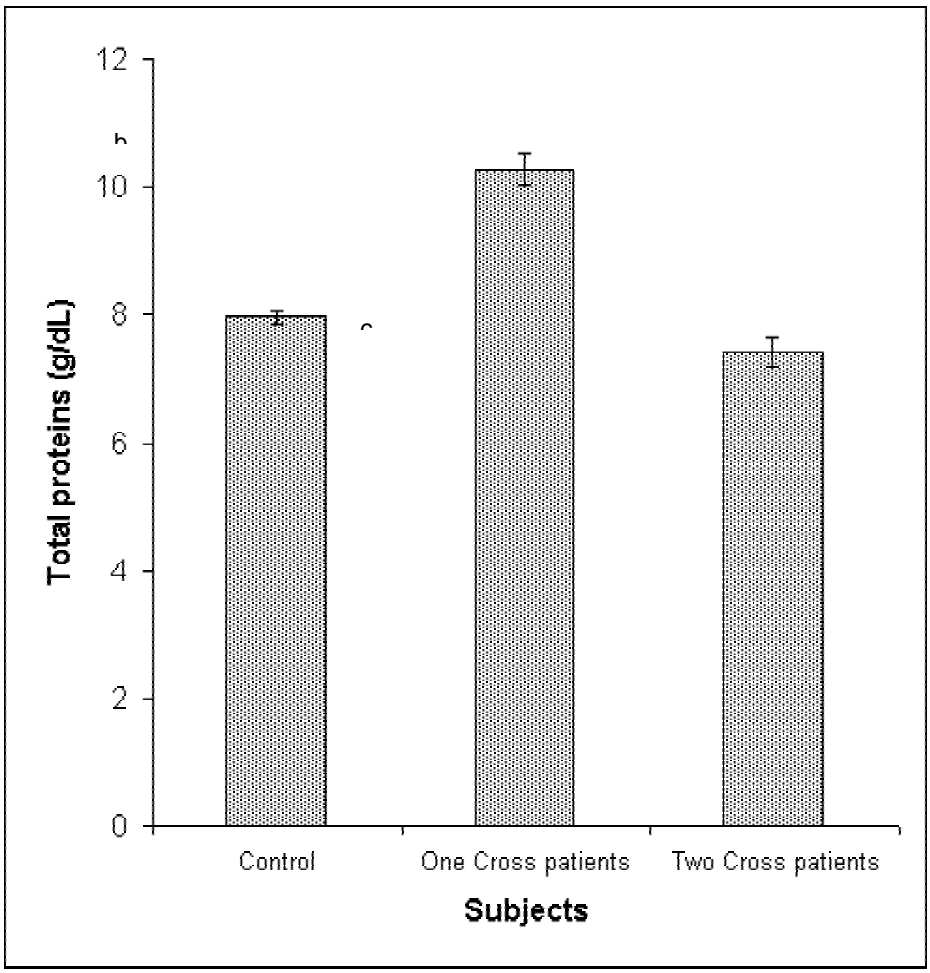
Soini, E. and Kojala, H. (1983). Time-resolved flourometer for lanthanide chelates - Clin. Chem. 29, 65-68.

Stephen J. Winters (1999). Current st5atus of lestosterone replacement therapy in men. Arch Fam Med. 8: 257-263.

Watters.J.M.,P.Q.Bessey,C.A.Dinarello,S.M.Wolf,andD.W.Wilmore(1986) Both inflammatory and endocrine mediators stimulate host responses to sepsis. Arch. Surg. 121: `7 9-190.

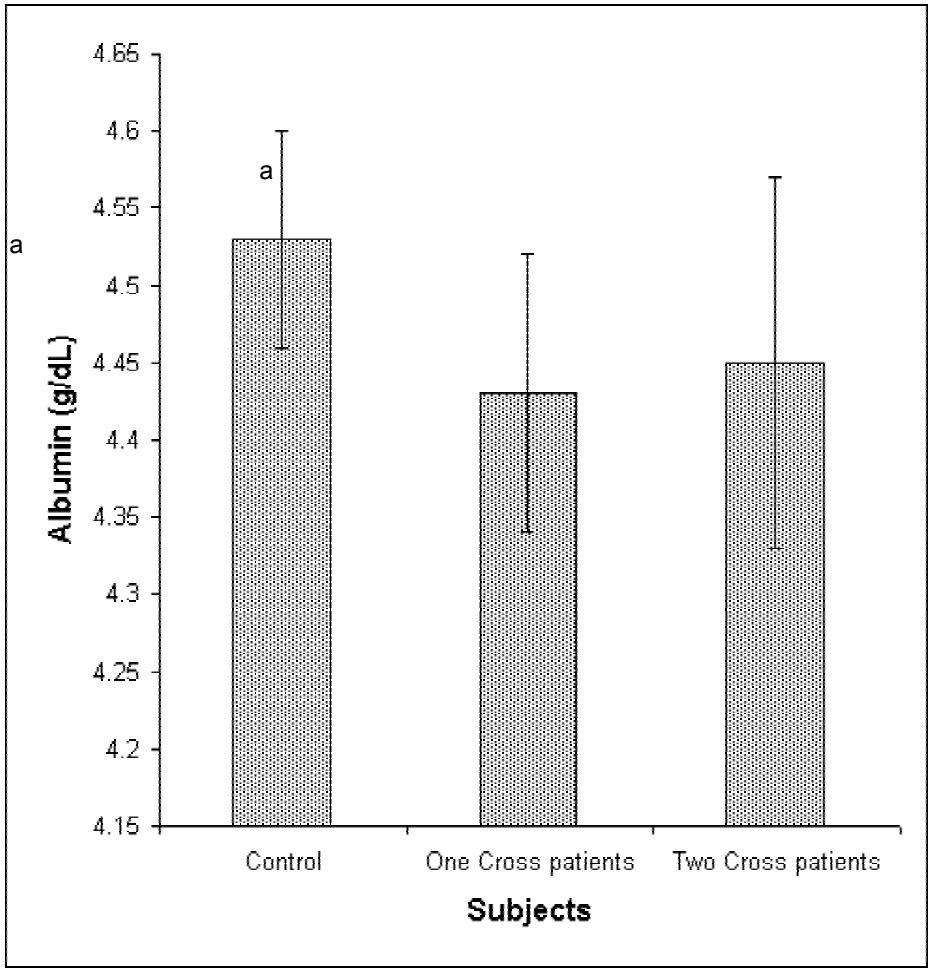
Wernsdorfer, W.H. (1980). The importance of malaria in the world. In J.P. Kreir (Ed.) Malaria, Vol. (1): pp. 1-95.

Zhi-Hui Zhang, Lin Chen, Susumu Saito, Osami, Kanagawa and Fujiro Sendo. 2000. Possile modulation by male sex hormone of Th1/Th2 function in protection against plasmodium chabaudi . As infection in mice. Experi. Parasitol. 96, 121-129.



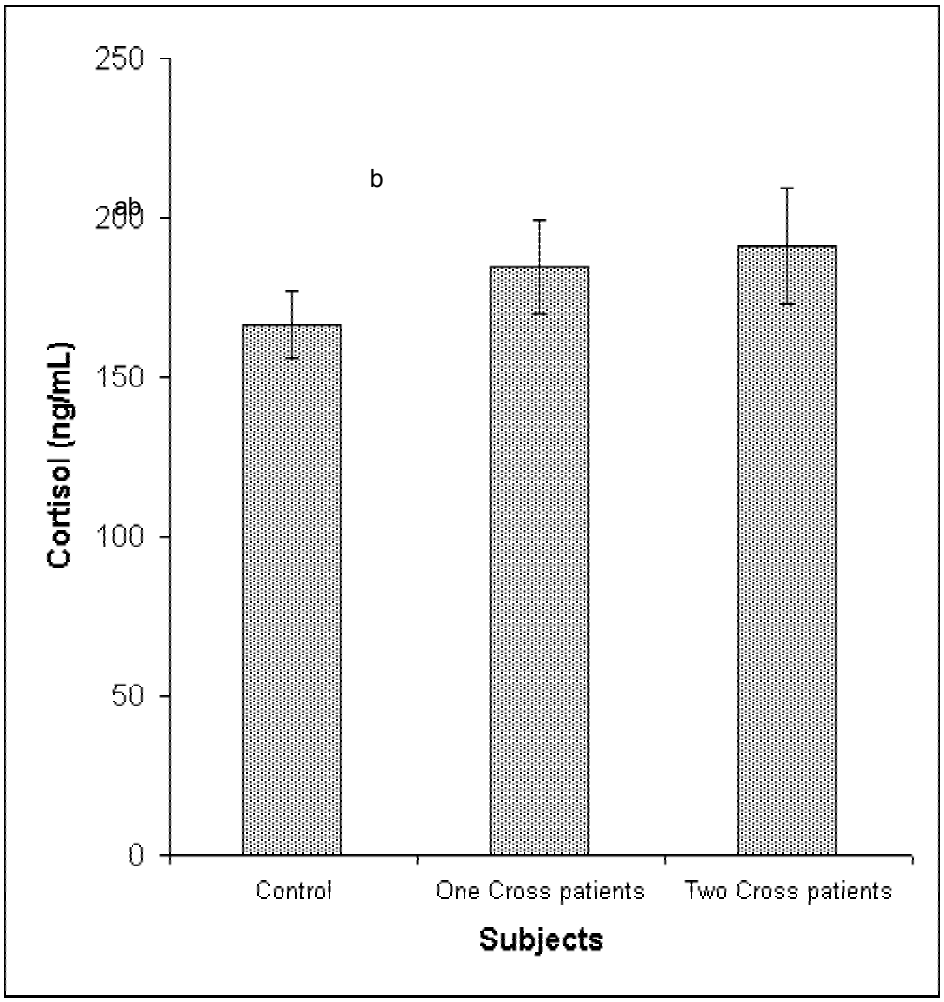
e of parasitaemia on plasma total proteins in males (Means±S.E)
ual form of parasites per 100 leukocytes. per 100 fields
sexual form of parasites per 100 leukocytes. -88 fields
followed by different letters are significantly different at ($p<0.05$)

a



e of parasitaemia in plasma albumin proteins in males (Means±S.E)
xual form of parasites per 100 leukocytes per 100 fields
sexual form of parasites per 100 leukocytes. -88 fields
followed by different letters are significantly different at ($p<0.05$)

a



e of parasitaemia on cortisol in males (Means±S.E)
exual form of parasites per 100 leukocytes. per 100 fields
sexual form of parasites per 100 leukocytes. 11-99 asexual form of parasites per 100 fields
s followed by different letters are significantly different at ($p<0.05$)