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Research

Effect of Malaria Parasitaemia and Antimalarial/Antioxidant Treatment on Body Weight and Some Reproductive Hormones of Male Mice

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ABSTRACT

This study examined the effect(s) of malaria parasitemia and coartem/vitamin E co-administration on serum Testosterone and Follicle Stimulating Hormone (FSH) levels in male mice. Twenty-eight (28) adult male mice were procured, acclimatized for two (2) weeks and randomly selected into four (4) groups of seven (7) mice per group. Group 1 (Control) received standard mice diet and water *ad libitum*, while group 2 mice were infected with malaria (*Plasmodium berghei*) and left untreated. Groups 3 and 4 were inoculated with malaria (*Plasmodium berghei*); then treated with coartem and coartem+vitamin E respectively. After three (3) weeks of administration of test substance, the mice blood samples were obtained from mice (for each group) and assayed for serum FSH and Testosterone levels. Statistical comparison was then conducted (using the student t-test) against those of the control group to ascertain the effects of the changes due to coartem and/or antioxidant vitamin E co-administration to *Plasmodium berghei* infected mice. The study found a statistically significant decrease in serum testosterone levels of male mice after inoculation with *Plasmodium berghei*. This decrease was noticed more even with coartem administration but showed non-significant amelioration with coartem/vitamin E co-administration, implicative of a possible effect in fecundity levels of male mice. Levels of FSH was significantly increased in infected and untreated male mice, but following treatment with coartem and/or antioxidant vitamin E, there was a reversal to about the same levels as control. Comparative results on the body weights of mice also revealed a drastic fall due to infection with *Plasmodium berghei*. Further studies aimed at corroborating and extrapolating the results of this work are also recommended.

Keywords: Malaria; Testosterone; Parasitaemia; Follicle-stimulating hormone

INTRODUCTION

Malaria is a vector-borne infectious disease by protozoan parasites of the genus *Plasmodium* and is presently endemic in broadband around the equator, in the areas of Americas, and much of Africa, however, most (85-90)% of the fatalities occur in sub-Sahara Africa [1,2]. It is one of the most prevalent infections worldwide and occurs in the tropics, sub-tropics and some parts of the temperate regions. Malaria attacks man and other species of animals [3]. This disease inflicts a considerable adverse effect on the organs of its host. Organs affected include spleen, liver, brain, and placentae. It is known that the processing of most biochemical effectors of metabolism and reproduction occurs in the liver. It is therefore tempting to speculate that reproductive hormone levels and function can be affected during or by malaria infection [3]. Presently there are five species of the genus *Plasmodium* which can cause disease in man. They are *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, *Plasmodium knowlesi* [4]. *Plasmodium berghei* was identified in the middle of 19th century as the first *Plasmodium* of rodents such as mice, rats and hamsters. It has since been used to identify the effective therapeutic action of antimalarial compounds [5]. WHO recommends Artemisinin-Based Combination Therapies (ACTs) for the treatment of

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uncomplicated malaria. By combining two active ingredients with different mechanisms of action, ACTs are the most effective antimalarial medicines available today, but they certainly leave some adverse effects on the patients [6]. Many antimalarial drugs have been associated with male reproductive dysfunction. Chloroquine has been reported to reduce sperm motility and hence fertility by a reduction in the average number of fetuses of cohabited female rats [7]. Orisakwe et al. [8] reported that halofantrine adversely affected sperm parameters. Pyrimethamine was reported to cause the spermatogenic arrest and male infertility in a dose-dependent manner [9]. A study by Raji et al. [10] showed that an oral artemisinin derivative, artemether, caused a significant reduction in the progressive sperm motility, viability, sperm count and serum testosterone levels in a dose-dependent fashion during an acute administration of the drug in male rats. It has been reported that high doses of artesunate could produce neurotoxicities such as selective damage to brainstem centers, gait disturbances [11] and loss of spinal cord and pain response mechanisms in mice and rats [12]. Artesunate has also been shown to cause a decrease in sperm motility of guinea pigs [13,14]. Testosterone is a steroid hormone that is the most potent naturally occurring androgen that regulates the development of the male reproductive system and male secondary sex characteristics. It is produced primarily by the testes/testicles in cells called the Leydig cells [15]. In men, testosterone plays a key role in the development of the male reproductive tissues suchas the testis and prostate as well as promoting secondary sexual characteristics such as increase muscle, bone mass and the growth of the body hair [16]. It is a primary male sex hormone. Also, testosterone is essential for health and wellbeing as well as the prevention of osteoporosis [15] Follicle-Stimulating Hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone. FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes. It enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes and is critical for the initiation of spermatogenesis. The process of spermatogenesis requires testosterone [15]. Administration of vitamin E has been shown to alleviate induced toxic effects and improve testosterone levels in dioxin treated mice [17,18]. There is a paucity of studies assessing the effects of co-administration of antimalarial and antioxidant on the reproductive parameters.

AIM OF THE STUDY

This study investigated Malaria Parasitaemia and changes in Follicle Stimulating Hormone (FSH) and testosterone levels. Specifically, Study:

- Evaluated the effect of on body weight
- Observed the effects of on FSH levels
- Observed the effect of on testosterone levels
- Assessed for ameliorative effects of antioxidant vitamin E/ antimalarial coartem on Malaria Parasite infected mice

MATERIALS AND METHODS

Location of study

Study was carried out at Delta State University, Department of Human Physiology. The study was done in the animal house of the College of Health Sciences, Delta State University using mice. The study focused on the effect of Malaria parasitemia and coartem/vitamin E co-administration on follicle-stimulating hormone and testosterone levels in the male reproductive system of mice.

Study design

A total of 28 male mice were randomly divided into four groups as follows:

- Which comprised of 7 (non-malaria infected) mice
- Which consist of 7 mice infected with malaria () but not treated
- Which comprised of 7 mice infected with malaria (); then treated with coartem
- Consisting of 7 mice infected with malaria (); then treated with coartem and vitamin E (co-administration)

Animal procurement

Twenty-eight (28) healthy adult male mice of (140-200) g were procured from the breeding unit of the animal house of faculty of the basic medical sciences, Delta State University Abraka, Delta State Nigeria. The handling of the animal was carried out following ethical guild lines for investigation and approved by the local ethical committee for the care of and use of laboratory animals. The mice were housed in isolated steel cages and kept under a 12 hours light and dark cycle at room temperature.

Acclimatization

After the purchase of these animals, they were kept in the cage for two weeks and fed with water and mice chow for proper adaptation to the environment, after which they were divided into control and experiment.

Treatment of mice

Preparation for coartem solution: Coartem tablets were grounded into powder form using a mortal. Thereafter, 1000 ml of distilled water was measured with the aid of a graduated cylinder. 1 g of Coartem powder was thereafter added into the distilled water and mixed thoroughly.

Preparation for anti-oxidant (vitamin E) solution: About 0.2 ml of distilled water was measured and added to 0.2 ml of alcohol, 9.6 ml of vitamin E was measured with the aid of 1 ml syringe and added to the mixture of alcohol and water, and was stored in a conical flask/crucible. The alcohol used here was to avoid the coagulation of the vitamin E (anti-oxidant) solution.

Inoculation with *Plasmodium Berghei***:** Malaria parasites, *Plasmodium berghei* were obtained from the Nigerian Institute of Medical Research (NMR), Yaba, Lagos. Mice in the experimental group were infected by obtaining parasitized blood (3-4) drops

from the cut tail tip of the infected mice (donor). Then 0.1 ml of the collected infected blood was diluted in 0.9 ml of phosphate buffer, pH 7.2 and the mice were inoculated with 0.1 ml of the parasitized blood intraperitoneally as described by which contains about twelve thousand (12000) parasites (Figure 1 and Table 1).

Drug administration: Using a 1ml syringe, 5.7 mg/kg body weight of Coartem solution was measured and administered to the infected mice orally. This was carried out in the morning and evening for 3 days respectively. Also using 1ml syringe, 20.8 mg/kg body weight of vitamin E. solution was measured and administered to the infected mice orally. This was carried out once a day, in a period of a two (2) day interval for vitamin E treatment.

Sample collection: The mice were decapitated and blood samples were obtained from the mice though cardiac puncture into an anticoagulant (EDTA) container and shaken for about 10seconds to allow for proper mixing to avoid coagulation.

Procedure for accessing serum testosterone

Serum testosterone was estimated by Enzyme-Linked Immunosorbent Assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK) 10 µl of serum sample was added to appropriately labelled microtitre wells and 100 µl enzyme-conjugated detection antibody was also added to the wells. Also, 5 µl of rabbit anti-testosterone reagent was added in each well. The same procedure was performed for the standard as well as experimental serum samples. They were incubated at 37°C for 90 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100 µl TMB reagent was added and incubated at room temperature for 29 minutes for the color to develop. 100 µl of HCL was added to the various wells to stop further development of color. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standard (0.1, 0.5, 2, 6 and 18) ng/ml provided by the manufacturer of the kit (Figure 2 and Table 2).

Procedure for hormonal assay

Determination of follicle stimulating hormone (FSH): The FSH quantitative test is based on a solid phase enzyme-linked immunosorbent assay system that utilizes a mouse monoclonal anti-a, FSH antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-FSH in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in FSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 minutes of incubation at room temperature, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color which is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of FSH is directly proportional to the color intensity of the test sample. The desired number of coated wells in the holder was secured. 20 µl of standard, specimens, and controls were dispensed into appropriate wells. 100 µl of enzyme conjugate. The reagent was added to each well. This was mixed thoroughly for 30minutes and was further incubated at room temperature (18-25)°C for 45 minutes. The incubation mixture was removed by flicking plate contents into each well and then mixed gently for 10seconds. The optical density was read at 450nm with a microtiter plate reader within 15 minutes (Figure 3 and Table 3).

Calculation of results

Calculate the mean absorbance value (A450) for each set of reference standards, specimens, controls, and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in mlU/ml linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis. Using the mean absorbance value for each sample, determine the corresponding concentration of FSH in mlU/ml from the standard.

Ethical clearance

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals.

Statistical analysis

Statistical significance of treatment effect(s) was analyzed with the student's t-test, with values expressed as Mean ± SEM (Standard Error of Mean). All of these were automated and achieved with the Statistical Package for Social Sciences (SPSS) version [19]. Differences between means were considered at $p<0.05$.

RESULTS

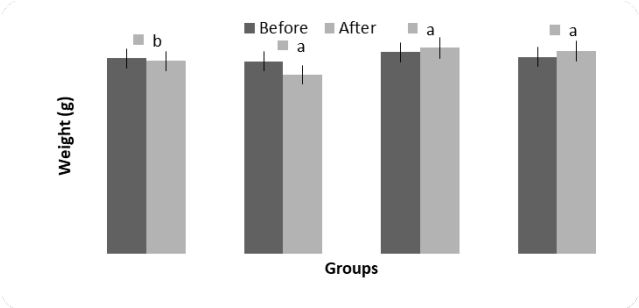

Figure 1: Changes in body weights before and after the treatment of malaria parasite-infected mice. a=statistically significant; b=statistically insignificant.

Table 1: t-test result of weights changes in malaria parasite-infected mice. $p \leq 0.05$=Statistically significant.

Groups	Before	After	t-cal	p-value	Remark
1	131.93	130.33	0.59	0.3012	Insignificant
2	129.93	120.93	0.54	0.0026	Significant
3	136	139	0.48	0.0054	Significant
4	133	137	0.56	0.0006	Significant

Figure 2: Changes in testosterone levels in malaria parasite-infected mice. *=statistically insignificant decrease (p<0.05) compared with the control group.

Table 2: t-test result of testosterone changes in malaria parasite-infected mice. p ≤ 0.05=Statistically significant decrease compared to control.

Groups	Experimental	Control	t-cal	p-value	Remark
2	3.02 ± 1.11	12.10 ± 2.01	0.21	0.0001	Significant
3	1.00 ± 0.08	12.10 ± 2.01	0.11	0.03	Significant
4	3.36 ± 1.43	12.10 ± 2.01	0.26	0.021	Significant

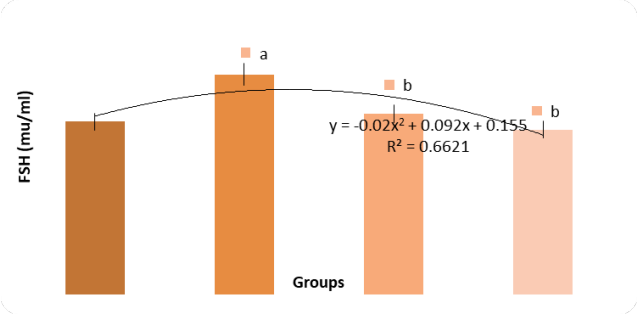

Figure 3: Changes in Follicle Stimulating Hormone (FSH) levels in malaria parasite-infected mice. a=statistically significant increase (p<0.05); b=statistically insignificant decrease (p<0.05) compared with control group.

Groups	Experimental	Control	t-cal	p-value	Remark
2	0.28 ± 0.20	0.22 ± 0.11	0.91	0.0211	Significant
3	0.23 ± 0.13	0.22 ± 0.11	0.13	0.01	Significant
4	0.21 ± 0.10	0.22 ± 0.11	0.11	0.0021	Significant

Table 3: T-test Result of FSH changes in malaria parasitemia infected mice. p ≤ 0.05= Statistically significant.

DISCUSSION

The different male reproductive hormone changes due to malaria infection and treatment with coartem and vitamin E were investigated specifically on FSH and testosterone in male mice.

The change in weight at the end of the experiment showed that malaria parasitemia was associated with a significant weight loss (120.93-129.93) g at p<0.05.Treatment with coartem was associated with a significant weight gain (139.00-136.00) g which got even better when vitamin E (antioxidant) was added to coartem (137.00-133.00) g at p<0.05. The weight loss in untreated mice is most probably due to the deleterious effects of malaria parasitemia. This is in keeping with the observation of Uraku working at Awka [20]. Nigeria but disagrees with Raji and colleagues working at Ibadan [5], Nigeria who found no significant differencein weight of infected mice after 10 days at 15%, 30% and 45% parasitemia levels. The parasitemia level was not assessed in this study but is likely to be heavier than in the study at Ibadan. The finding of recovery of weight when treated with coartem (ACT) is likely due to a reversal of the deleterious effects of malaria parasitemia in mice. This recovery is interesting especially when compared to findings by Samuel SA et al. [15,19] that administration of ACT or artesunate in unparasitized rats resulted in significant weight loss [15,16]. It appears paradoxical that untreated malaria parasitemia would be associated with weight loss, unparasitized treatment was associated with weight loss and ACT treated malaria is associated with weight gain as seen in this study. More studies are needed to evaluate the ameliorative effects of ACT/ antioxidant treatment on physiological parameters of infected persons.

This study showed a significant increase in FSH of *Plasmodium berghei* infected and untreated mice (p-value 0.0211) and this increase was reversed in mice treated with coartem/vitamin E. Considering that the main function of FSH is the induction and maintenance of spermatogenesis, it follows that Malaria appears to stimulate production of more sperm in the infected mice. This effect appears to be reversed with treatment even to the point of a non-significant reduction in FSH when coartem and vitamin E was co-administered. Akinsomisoye and Yinusa [5], found a significant rise in FSH levels with chronic administration of artesunate in unparasitized mice but Samuel et al. reported no difference in FSH levels between ACT treated unparasitized mice and controls [15]. This effect ofmalaria parasitemia on FSH and the ameliorative effects of treatment may have important extrapolation to human fertility especially in the malaria-endemic sub-Sahara Africa, where ACT use is very robust.

Our study revealed a general and significant reduction in testosterone levels of infected mice despite coartem treatment but this reduction was mildly ameliorated when Vitamin E was combined with coartem. Testosterone is responsible for libido and spermatogenesis and this shows how malaria and its treatment can affect the sex and reproductive drive in males, an effect that directly threatens the sustenance of the human species. The possible ameliorative effect of the antioxidant, Vitamin E is here highlighted. The findings in this study areto compare positively with the findings by Rajiet al.in Ibadan Nigeria who found a progressive and significant reduction in testosterone levels of parasitized mice at 15%, 30%, and 45%

parasitemia levels [5]. It, however, contrasts with Adejuwon and Adejuwon who noted a significant rise in serum levels of testosterone inparasitized and untreated mice [3]. Both Samuel et al. and Nwanjo et al. reported no difference in testosterone levels between unparasitized but ACT treated mice and controls [15,19]. However,bNwanjo and colleagues noted a significant but transient drop in serum testosterone levels when high doses of artesunate were used [19].

The effects of malaria parasitemia and its treatment on male reproductive hormones studied here highlight the possible challenges posed by this infection and its treatment to male factor fertility and the continuation of the human species especially in sub-Sahara Africa. More studies, especially with human subjects, are needed to evaluate this.

CONCLUSION

In conclusion, antimalarial treatment with Coartem and antioxidant treatment with vitamin alone orcombined, ameliorated the impact of malaria parasitemia. Hence the use of antioxidants in combinationwith antimalarial treatment may be of benefit to the reproductive system.

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