

Malaria Parasitaemia and Changes in Follicle Stimulating Hormone and Luteinizing Hormone Levels of Adult Mice

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Abstract: To understand the treatment of malaria, it is important to understand the effect of the administration of specific anti-malarials on various physiological variables. Current study investigated the effect (s) of the anti-malarial, coartem and co-administration of the antioxidant, Vitamin E on the levels of Luteinising and Follicle Stimulating Hormones [LH and FSH] of malaria parasite infected mice. Twenty-eight (28) adult mice were obtained, acclimatized for two (2) weeks and randomly selected into four (4) groups of seven (7) rats 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 LH levels. Statistical comparison was then conducted (using the student t-test) against those of control group to ascertain the effects of the changes due to coartem and/or antioxidant vitamin E co-administration to *plasmodium berghei* infected mice. Study found a statistically significant increase in serum LH levels of male mice after inoculation with *plasmodium berghei*. This decrease was noticed more even with coartem and antioxidant vitamin E co-administration, implicative of a possible effect in fecundity levels of infected male mice. The level of FSH was significantly increased in infected mice, but however showed a reversal following treatment with coartem and/or antioxidant vitamin E. The effects on reproductive hormones seen here may mirror the effects in man. More studies are needed to elucidate that.

Keywords: Coartem, Leutinizing Hormone, Follicle Stimulating Hormone

1. Introduction

Malaria is the most prevalent preventable tropical disease in the world today. It has affected human for over fifty thousand years, and may have been a human pathogen for the entire history of our species. In the tropical and subtropical regions, during the first half of the twentieth century, malaria affected every walk of life so much that its effects on developing economy been studied [1, 2] It is estimated to

have affected about 3.4 million people in 92 countries of the world in 2019. There were about 219 million cases of malaria in 2017 causing 43500 deaths. [3]

Malaria is endemic in part of Asia, Africa, central and South America, Oceania, and certain Caribbean islands. It is endemic in Nigeria and it takes a heavy toll on our pregnant women and children. Nigeria accounted for 25% of cases of malaria worldwide in 2018 and accounted for 24% of global malaria deaths in 2018. [3]

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 and luteinizing hormone (LH) work together in the reproductive system.

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. [4, 5]

Luteinizing hormone (LH) is released from the pituitary gland, and is controlled by pulses of gonadotropin-releasing hormone (GnRH). It stimulates Leydig cell production of testosterone. It acts synergistically with FSH. LH acts upon the Leydig cells of the testis and is regulated by gonadotropin-releasing hormone (GnRH). The Leydig cells produce testosterone (T) under the control of LH, which regulates the expression of the enzyme 17 β -hydroxysteroid dehydrogenase that is used to convert androstenedione, the hormone produced by the testes, to testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis. [5, 6]

Malaria causes severe disease. Severe malaria complications include cerebral malaria, miscarriages, febrile seizures, hypoglycaemia and others which can all lead to mortality. [7]

WHO recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by the *P. falciparum* parasite. 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 effect on the patients. [7, 8]

Hormonal changes due to malaria has been studied and malaria chemotherapy has been associated with adverse effects on reproductive function. [9, 10] Antifertility effects of artemisinin in male rats has been reported. [11] We therefore undertook to study the effects of malaria parasitaemia and antimalaria chemotherapy on FSH and LH in male mice.

Aim of Study

The study aimed at investigating Malaria Parasitaemia and Changes in Follicle Stimulating Hormone and Luteinizing Hormone Levels. Specifically, the study;

- i. Ascertained the changes in body weights in Malaria parasite infected mice.
- ii. Determined the serum FSH and LH level of male mice infected with malaria parasitaemia.
- iii. Determined the changes in serum FSH and LH levels of malaria infected, and treated mice

2. Materials and Methods

2.1. Location of Study

Study was conducted in the animal house of the college of

Health sciences, Department of Human Physiology, Delta State University, Abraka, Delta State.

2.2. Study Design

The study focussed on assessing the effect of Malaria parasitaemia on the follicle stimulating hormone and luteinizing hormone levels in male albino mice. Twenty-eight (28) mice were procured, acclimatized for two weeks, and then, divided into four (4) groups of seven (7) animals each as follows;

Groups 1 (control): Which received standard chow and water *ad libitum*.

Groups 2: Infected with malaria (*Plasmodium berghei*) and given only water and chow.

Groups 3: Infected with malaria (*Plasmodium berghei*) and treated with coartem solution only.

Groups 4: Infected with malaria (*Plasmodium berghei*) and treated with coartem and vitamin E.

2.3. Animal Procurement

The study was carried out on 28 healthy adult male mice, which were procured from the breeding unit of animal house of Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State Nigeria. The handling of the animals was carried out in accordance with ethical guild lines for investigation and approved by local ethical committee for the care of and use of laboratory animals. The mice were house in isolated steel cages and kept under a 12 hours light and dark cycle at room temperature.

2.4. Acclimatization

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

3. Treatment of Mice

3.1. Preparation for Coartem Solution

Coartem tablets were ground into powder form using a mortar. Thereafter, 1000ml of distilled water was measured with the aid of a graduated cylinder and 1g of coartem powder was thereafter added into the distilled water and mixed thoroughly.

3.2. Preparation for Anti-oxidant (Vitamin E) Solution

About 0.2ml of distilled water was measured and added to 0.2ml of alcohol, 9.6ml of vitamin E was measured with the aid of 10ml syringe and added to the mixture of alcohol and water, and was a stored in a conical flask/crucible. The alcohol used here was to avoid the coagulation of the vitamin E (anti-oxidant) solution.

3.3. Drug Administration

Using a 1ml syringe, 5.7mg/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, 20.8mg/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.

3.4. Sample Collection

At the end of the treatment, the mice were anesthetized in a desiccator containing cotton wool soaked with chloroform. After they had attained deep anesthesia, they were brought out of the dessicator and laparotomy was carried out (by making V-shape incision in the abdominal region with the aid of a surgical scissors) and the visceral organs were exposed, blood samples were obtained from the mice by cardiac puncture from the hepatic portal vein and hormonal assay was done immediately.

4. Procedure for Hormonal Assay

4.1. Determined of Follicle Stimulating Hormone (FSH)

Method:

Principle:

The FSH quantitative test is based on a solid phase enzyme-linked immunosorbent assay system utilizes a mouse monoclonal anti- α , FSH antibody for solid phase (microtite 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 45minutes 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 20minutes, resulting in the development of a blue color which is changed to yellow and measured spectrophotometrically at 450nm. The concentration of FSH is directly proportional to the color intensity of the test sample.

Procedure:

The desired number of coated wells in the holder was secured. 50ul of standard, specimens, and controls were dispensed into appropriate wells. 100ul of enzyme conjugate Reagent was added into each well. This was mixed thoroughly for 30minutes and was further incubated at room temperature (18-25c for 45minutes. The incubation mixture was removed by flicking plate contents into each well into the waste container. 100ul of TBM reagent was dispensed into each well and mixed gently for 10seconds. The optical density was read at 450nm with a microtiter plate reader within 15 minute.

Calculation of results;

The mean absorbance value (A450) for each set of reference standards, specimens, controls and patient samples were calculated. A standard curve was constructed by

plotting the mean absorbance obtained from each reference standard against its concentration in mIU/ml linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X axis. The mean absorbance value for each sample, determine the corresponding concentration of FSH in mIU/ml from the standard curve.

4.2. Determination of Luteinizing Hormone (LH)

Method:

Principle:

In this test, the targeted antigen LH in the sample is immobilized through binding between the streptavidin coated onto the walls of the microplate and the biotin molecule that is part of the biotinylated monoclonal antibody added in the enzyme conjugate, together with another specific enzyme-conjugated antibody, the target antigen and biotinylated antibody forms a sandwich complex on the walls of the microplate after a period of incubation. After a wash step that removes any unbound molecules, a substrate is added to react with the enzyme conjugated on the cold anti-body, a blue color develop during the incubation period. the intensity of the color is directly correlated to the concentration of the target antigen, LH in the serum sample. After the addition of the stop solution, the concentration of LH can be quantified using this correlation.

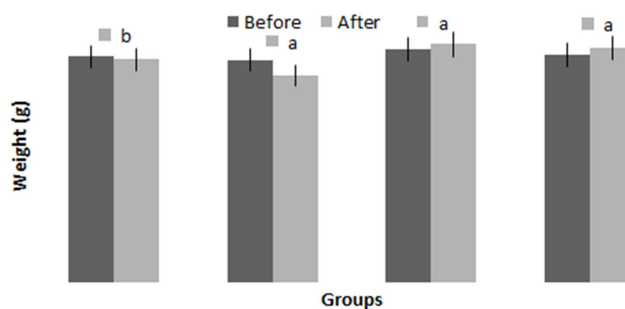
Ethical Clearance

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 (1985).

Statistical Analysis

Statistical significance of treatment effect (s) was analyzed with the students't-test, with values expressed as Mean \pm SEM (Standard Error of Mean). All of these were automated and achieved with the statistical package for social sciences (SPSS) version 20. Differences between means were considered at $p < 0.05$.

5. Results



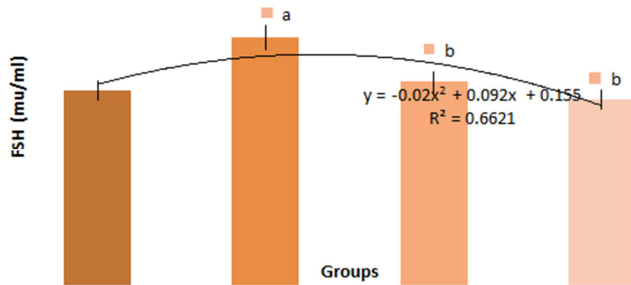
a=statistically significant, b=statistically insignificant.

Figure 1. Changes in Body Weights before and after the experiment.

Table 1. T-test Result of Weights Changes in Malaria Parasite Infected Mice.

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	136.00	139.00	0.48	0.0054	Significant
4	133.00	137.00	0.56	0.0006	Significant

$P \leq 0.05$ =statistically significant.

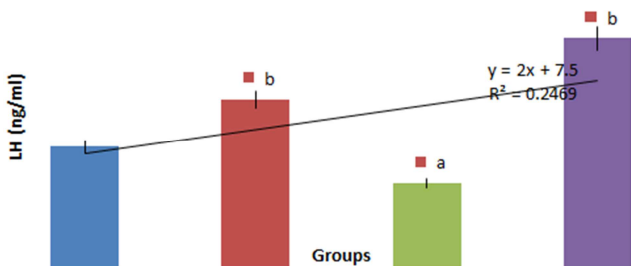
**Figure 2.** Changes in Follicle Stimulating Hormone (FSH) Levels in Malaria Parasite Infested Mice.

a=statistically significant increase ($p < 0.05$), b=statistically insignificant decrease ($p < 0.05$) compared with control group.

Table 2. T-test Result of FSH Changes in Malaria Parasitaemia Infested Mice.

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	0.0100	insignificant
4	0.21 ± 0.10	0.22 ± 0.11	0.11	0.0021	insignificant

$P \leq 0.05$ =statistically significant.

**Figure 3.** Changes in Luteinising Hormone Levels in Malaria Parasitaemia Infested Mice.

a=statistically significant decrease ($p < 0.05$), b=significant increase compared with control group.

Table 3. T-test Result of Luteinising Hormone Changes in Malaria Parasitaemia Infested Mice.

Groups	Experimental	Control	t-cal	p-value	Remark
2	15.00 ± 1.21	10.00 ± 1.11	0.13	0.0360	Significant
3	7.00 ± 0.30	10.00 ± 1.11	0.22	0.7020	Insignificant
4	19.00 ± 1.62	10.00 ± 1.11	0.14	0.0114	Significant

$P \leq 0.05$ =statistically significant increase compared to control.

6. Discussion

The study assessed the effect of malaria parasitaemia on FSH and luteinizing hormone levels. The different

reproductive hormone changes due to malaria infection and treatment with coartem and vitamin E were investigated in this study, especially on FSH and LH levels of male mice.

The results from this study showed a significant weight reduction ($p < 0.05$) in infected mice by the end of the experimental period (129.93g -120.93g). This weight loss of about 9 grams on the average is much higher than what was observed with the control group (1.6g). This is in agreement of other studies which found progressive and significant weight loss in plasmodium berghei infected mice¹². The weight losing effect of malaria parasite infection of the mice was apparently reversed with treatment of the malaria with coartem. The coartem treatment group and the coartem plus vitamin E treatment group gained weight significantly (3g and 4g respectively). This observation suggests a nutrition improving effect of treatment of malaria with effective medication.

The major change in follicular stimulating hormone (FSH) was seen in the infected group. This significant increase in FSH is remarkable especially since these are male mice. The FSH is important for the meiotic division in spermatogenesis¹³. This suggests a rise in sperm production occasioned by malaria in mice. This finding is not in agreement with a study at Ibadan, Nigeria which found no significant difference in FSH levels in adult wistar rats treated with ACTs (artemether / lumefantrine and artesunate / amodiaquine). [13] In this study, there was no significant difference in FSH levels between controls and mice treated with either coartem or coartem plus vitamin E. This suggests a reversal of the spike seen in infected mice thereby raising the possibility that the increase in FSH levels seen in infected mice may be reactionary rather than physiological.

The most riveting observation of this study is the changes observed in the levels of luteinizing hormone in the experimental mice. Malaria parasite infection and treatment of malaria all led to increase in luteinizing hormone levels. However, the increase observed in the coartem treatment group was not significant, the infected group and the co-administration group showed significant increases. Luteinizing hormone is very important for the secretion of testosterone¹³. This means that the testosterone levels of all infected mice was elevated irrespective of whether they were undergoing treatment or not. This finding is corroborated by Adejuwon and Adejuwon and Ojezele and colleagues^{14, 15}. It is however contrary to the finding by Raji *et al.*,¹⁰ who reported a reduction in testosterone levels of parasitized mice.

7. Conclusion

This study has yet again provided evidence that malaria parasitaemia does have effects on the reproductive function of male rats and this may be so in humans. More studies are required to elucidate and characterize these effects so as to continue to protect the continuity of the human species.

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