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Maternal Serum Levels of Alpha Tumour Necrotic Factor, Interleukin 10, Interleukin 6 and Interleukin 4 in Malaria Infected Pregnant Women Based on Their Gestational Age in Southeast, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OEI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EBN, EIS, AEI, AAA and CSN managed the analyses of the study. Authors AFN, UMC and CSK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Malaria has been reported as a condition caused by infestation with Plasmodium parasite species, is a major public health problem globally especially in developing countries like Nigeria. This study was carried out in Federal Medical Centre Umuahia in Abia State, Nigeria. A study was done to determine the maternal serumlevels of alpha tumour necrotic factor, interleukin 10, interleukin 6and interleukin 4 in malaria infected pregnant women based on their gestational age in Southeast, Nigeria. A total of 150 subjects between the ages of 18-45 years were recruited for the study comprising of fifty (50) subjects each of the 3 trimesters. Commercial ELISA Kit by MELSIN Medical Co Limited was used to measure all the cytokines. The results of Table 1 showed no significant difference of TNF-α (p=0.346), IL-10 (p=0.059), IL-6 (p=0.811) and IL-4 (p=0.257) of malaria infected pregnant women at first trimester and second trimester respectively. The results of Table 2 showed no significant difference of TNF-α (p=0.642), IL-10 (p=0.678), IL-6 (p=0.551) and IL-4 (p=0.280) of malaria infected pregnant women at first trimester and third trimester respectively. The results of Table 2 showed no significant difference of TNF-α (p=0.062), IL-10 (p=0.016), IL-6 (p=0.352) and IL-4 (p=0.914) of malaria infected pregnant women at first trimester and third trimester respectively. The study showed no changes in the cytokines studied among the malaria infected pregnant women based on gestational ages except when IL-10 was compared between the subjects on second trimester and third trimester. This study shows that malaria infection does not changes these cytokines in pregnant women based on gestational ages except the il-10 when compared at second trimester and third trimester but changes when compared at other trimesters.

Keywords: Alpha tumour necrotic factor; interleukin 10; interleukin 6 and interleukin 4 malaria infected pregnant women; gestational age.

1. INTRODUCTION

Malaria has been reported as a condition caused by infestation with Plasmodium parasite species, is a major public health problem globally especially in developing countries like Nigeria causing considerable morbidity and mortality especially in sub Saharan Africa where it accounts for up to 1 million death annually [1]. Dellicour et al. opined that pregnant women are vulnerable to malaria infection. Malaria during pregnancy is a substantial public health problem in endemic tropical countries, especially sub Saharan Africa [2]. Desai et al. opined that approximately 125 million pregnant women live in malaria endemic areas in sub Saharan Africa and 32 million of these pregnant women are at risk of malaria [3].

Pregnant women are at high risk of being infected with malaria owning to the ability of the parasite to adhere to trophoblastic villous epithelium and sequester in the placenta which could eventually lead to poor pregnancy outcome [4]. It shown that over 200,000 infants die annually in sub-Saharan Africa as a result of their mother becoming infected with malaria during pregnancy [5]. Malaria during pregnancy can lead to maternal and foetal adverse effects, mainly anaemia, cerebral malaria, hemorrhage and low birth weight.

Cytokines are low molecular weight regulatory proteins that are secreted by many cells of the immune system in response to a number of stimuli. They are involved in virtually all physiological responses in the body and are key players in coordinating immune responses between cells, by binding to a variety of receptors and to induce cell-specific immune responses. They are secreted by many cells of the immune system in response to a number of stimuli. During successful pregnancies, fetal trophoblasts and maternal leukocytes secrete predominantly T-helper 2 type cytokines to prevent initiation of inflammatory and cytotoxic type responses that might damage the integrity of the materno-fetal placental barrier [6]. In response to invading malaria parasites, however it has been documented that Th-1 type cytokines are produce to reverse the Th-2 type bias within the placenta [7]. Inconsistence reports on the response of some pro-inflammatory interleukins to peripheral and placental malaria have been documented [8,9]. Both pro and anti inflammatory cytokines are found at significantly increased levels in the peripheral blood and in the intervillous spaces of placentas of malaria infected woman. Productions of these cytokines is responsible for the resulting Th-1:Th-2 imbalance observed in Plasmodium falciparum infected placentas [4,10].

Severe malaria has long been associated with high circulating levels of inflammatory cytokines such as tumour necrosis factor (TNF-a), IL-1, IL-6. Studies have demonstrated a link between TNF-a, IL-6, IL-10 and the severity of the disease in human malaria [11]. Anti inflammatory cytokines has also been found to have important roles in the immune response against Plasmodium. IL-10 has an important role as an immunoregulator during *Plasmodiumfalciparum* infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells [12,13]. Additionally, IL-10 and granulocyte colony stimulating factor (G-CSF) have been found to be elevated and correlated with parasitaemia in asymptomatic pregnant women in Ghana [14], suggesting that these cytokines may act to reduce symptoms.

A study was done to determine the maternal serumlevels of alpha tumour necrotic factor, interleukin 10, interleukin 6 and interleukin 4 in malaria infected pregnant women based on their gestational age in Southeast, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Federal Medical Centre Umuahia in Abia State, Nigeria. Federal Medical Centre is the major tertiary hospital located in Umuahia, Abia State. This hospital is a referral centre which provides adequate medical care to pregnant women and the sick ones at large.

2.1.1 Subjects

A total of 150 subjects between the age of 18-45 years were recruited for the study. 0 subjects each of the 3 trimesters.

2.2 Experimental Design

A cross sectional prospective study was carried out on 3 groups.

Group 1 =50 Malaria Infected Pregnant Subjects at first trimester,
Group 2 =50 Malaria Infected Pregnant Subjects at second trimester,
Group 3 =50 Malaria Infected Pregnant Subjects at third trimester.

An oral consent was gotten from the patients after which a structured questionnaire was administered to all respondents who was also part of clinical study and the subjects were allowed to join in the study voluntarily and can withdraw at any stage of the study.

2.3 Inclusion Criteria

- Pregnant women who have no evidence of other infection, other inflammatory or chronic diseases.
- Pregnant women who presented symptoms of malaria.
- Pregnant women between the age of 18-45 years.
- Pregnant women in all trimesters

2.4 Exclusion Criteria

Those excluded from the study were:

- Pregnant women with evidence of chronic infection like HIV, tuberculosis and inflammatory disease;
- Women who did not give their informed consent;
- Pregnant women in need of emergency care or having an at-risk pregnancy such as gestational diabetes, pre-eclampsia and eclampsia.

2.5 Sample Collection

Eight milliliters (8ml) of venous blood was drawn from each participant using standard veno puncture techniques.

2.5 mls was dispensed EDTA container for malaria detection, and 5.5mls dispensed into a plain container to obtain serum. The sample in the plain test tube was allowed to clot at room temperature and centrifuged to separate the serum.

2.6 Laboratory Procedures

All reagents were commercially purchased and the manufacturer's Standard Operating Procedures (SOP) were strictly followed.

2.6.1 Malaria estimation using rapid test kit [15]

As modified by SD BIO LINE One Step Malaria antigen P.F (HRP-II) rapid kit was used.

2.6.2 Test procedure

The kit was allowed to equilibrate at room temperature. The test device was opened for and

labeled for each patient. The specimen was collected with the aid of capillary pipette provided and then transferred into the round specimen well. Four drops of assay diluents was dispensed into the diluents well. The kit was left on a flat bench for a period of 15 minutes before taking result.

2.7 Malaria Parasite Identification Using Giemsa Staining Technique [16]

2.7.1 Methodology

A drop of blood was placed on the slide to cover the diameter 15-20mm. The blood was smeared evenly on the slide to obtain a thick film and then allowed to air dry with the slide in a horizontal position. Before staining, the stock giemsa stain was diluted in 1:10 dilution using phosphate buffer at pH 7.2. The working solution of the giemsa stain was used to cover the dried thick film for 30 minutes and at the end of the staining period, water was used to gently flush the stain off the slide. The slide was rinsed briefly in gently running tap water and the under surface of the slide blotted dry to remove excess stain. It was left to air dry in a vertical position and then viewed microscopically using x40 and x100 objectives[17].

2.8 Alpha Tumour Necrosis Factor (TNF-α) Assay

Human Alpha Tumour Necrosis Factor Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0110

2.8.1 Procedure

Dilutions of standard was prepared to get a concentration of 80 pg/mL, 40png/mL, 20 pg/mL, 10 pg/mL, 5pg/mL and 0pg/mL. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample diluents was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37^{o}C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37°C. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

2.8.2 Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

2.9 Interleukin 1 (1L-6) Assay

Human Interleukin 6 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0102

2.9.1 Procedure

Dilutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample diluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37°C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37°C. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration[17].

2.9.2 Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

2.10 Interleukin-10 (1L-10) Assay

Human Interleukin 10 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-1035

2.10.1 Procedure

Dilutions of standard was prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample diluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution).

50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37°C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37°C. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

2.10.2 Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

2.11 Interleukin-4 (1L-4) Assay

Human Interleukin 4 Commercial ELISA Kit by MELSIN Medical Co Limited was used. Catalogue Number: EKHU-0065

2.11.1 Procedure

Dilutions of standard was prepared to get a concentration of 300 ng/L, 200 ng/L, 100 ng/L, 50 ng/L, and 25ng/L. 50 uL of standards were pipette into the standard wells. 10uL of test serum were pipette into the each sample well. 40uL of sample diluent was added to the sample well. Sample blank was included (to contain only chromogen solution A and B, and stop solution). 50 uL of HRP-conjugate reagent was added to all wells except blank, covered with an adhesive strip and incubated for 30minutes at 37°C. It was washed for four times. 50uL of chromogen solution A and 50uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37°C. 50uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450nm wavelength within 15 minute taking the blank well as zero concentration.

2.11.2 Calculation

A standard curve of optical density against concentration of standards was plotted and the concentration of the tests determined from there.

2.12 Statistical Analysis

All statistical analysis was performed using SPSS version 20. The results were expressed as mean plus or minus standard deviation in tabular form. Student t- test was used for comparison of differences in various groups. All test performed were two tailed and the level of significant was set at $p<0.05$.

3. DISCUSSION

The study showed no changes in the cytokines studied among the malaria infected pregnant

Table 1. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 of malaria infected pregnant women at first trimester and second trimester

Parameters	First Semester	Second Semester	t-value	p-value
TNF-α(pg/ml)	13.58±1.74	15.03±2.23	-1.023	0.346^{NS}
IL-10(pg/ml)	24.79±0.64	31.22±5.51	-2.320	0.059^{NS}
IL-6(pg/ml)	25.98±5.10	26.69±2.61	-.249	0.811^{NS}
IL-4 (pg/ml)	14.51±10.88	27.56±17.76	-1.253	0.257^{NS}

The results of Table 1 showed no significant difference of TNF (13.58±1.74 pg/ml, 15.03±2.23 pg/ml, p=0.346), IL-10 (24.79±0.64 pg/ml, 31.22±5.51 pg/ml, p=0.059), IL-6 (25.98±5.10 pg/ml, 26.69±2.61 pg/ml, p=0.811) and IL-4 (14.51±10.88 pg/ml, 27.56±17.76 pg/ml, p=0.257) of malaria infected pregnant women at first trimester and second trimester respectively

Table 2. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 of malaria infected pregnant women at first trimester and third trimester

Parameters	First Semester	Third Semester	t-value	p-value
TNF-α(pg/ml)	13.58±1.74	13.12±1.88	0.469	0.642^{NS}
IL-10(pg/ml)	24.79±0.64	25.66±4.12	-0.418	0.678^{NS}
IL-6 (pg/ml)	25.98±5.10	24.58±4.38	0.601	0.551^{NS}
IL-4 (pg/ml)	14.51±10.88	26.36±21.23	-1.095	0.280^{NS}

The results of Table 2 showed no significant difference of TNF (13.58±1.74 pg/ml, 13.12±1.88 pg/ml, p=0.642), IL-10 (24.79±0.64 pg/ml, 25.66±4.12 pg/ml, p=0.678), IL-6 (25.98±5.10 pg/ml, 24.58±4.38 pg/ml, p=0.551) and IL-4 (14.51±10.88 pg/ml, 26.36±21.23 pg/ml, p=0.280) of malaria infected pregnant women at first trimester and third trimester respectively

Table 3. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 of malaria infected pregnant women at second trimester and third trimester

Parameters	Second Semester	Third Semester	t-value	p-value
TNF-α(pg/ml)	15.03±2.23	13.12±1.88	1.912	0.062 ^{NS}
IL-10 (pg/ml)	31.22±5.51	25.66±4.12	2.510	0.016 *
IL-6(pg/ml)	26.69±2.61	24.58±4.38	0.941	0.352 ^{NS}
IL-4 (pg/ml)	27.56±17.76	26.36±21.23	0.109	0.914 ^{NS}

The results of Table 3 showed no significant difference of TNF (15.03±2.23 pg/ml, 13.12±1.88 pg/ml, p=0.062), IL-10 (31.22±5.51 pg/ml, 25.66±4.12 pg/ml, p=0.016), IL-6 (26.69±2.61 pg/ml, 24.58±4.38 pg/ml, p=0.352) and IL-4 (27.56±17.76 pg/ml, 26.36±21.23 pg/ml, p=0.914) of malaria infected pregnant women at first trimester and third trimester respectively

women based on gestational ages except when IL-10 was compared between the subjects on second trimester and third trimester. Interleukin 10 is anti-inflammatory cytokines that helps to regulate inflammatory cytokines when there are infections or other immunological changes due to some inversion of the body by any intruders. This study shows that malaria infection does not changes these cytokines in pregnant women based on gestational ages except the il-10 when compared at second trimester and third trimester but changes when compared at other trimesters. This shows that pregnant women infected with malaria should be seriously monitored at second and third trimester as the change in IL-10 predicts immunological response changes which could dangerous as there was increase in IL-10 which depicts the body of these pregnant women trying to control inflammatory cytokines. Malaria infection has long been associated with high circulating levels of inflammatory cytokines such as tumour necrosis factor (TNF-a), IL-1, IL-6. Studies have demonstrated a link between TNF-a, IL-6, IL-10 and the severity of the disease in human malaria [11]. Anti inflammatory cytokines has also been found to have important roles in the immune response against Plasmodium. IL-10 has an important role as an immunoregulator during *plasmodiumfalciparum* infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells [12,13].

4. CONCLUSION

The study showed no changes in the cytokines studied among the malaria infected pregnant women based on gestational ages except when IL-10 was compared between the subjects on second trimester and third trimester. This study shows that malaria infection does not changes these cytokines in pregnant women based on gestational ages except the il-10 when compared at second trimester and third trimester but changes when compared at other trimesters.

CONSENT

Informed consents were obtained from the participants were recruited among pregnant women booked for antenatal care in the hospital.

ETHICAL APPROVAL

As per university standard guideline, ethical approval have been collected and preserved by the authors

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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