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Maternal Expressions (Serum Levels) of Alpha Tumour Necrosis Factor, Interleukin 10, Interleukin 6 and Interleukin 4 in Malaria Infected Pregnant Women Based on Parity in a Tertiary Hospital 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 OGU, AAA, AEI and CEF managed the analyses of the study. Authors AFN, CSN and KSC 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 specie, which is a great public health problem globally, particularly in developing countries like Nigeria. This study was carried out in Federal Medical Centre Umuahia in Abia State, Nigeria. The study was done to determine the maternal serum levels of alpha tumour necrosis factor, interleukin 10, interleukin 6, and interleukin 4 in malaria-infected pregnant women based on parities in Southeast, Nigeria. A total of 150 subjects between the ages of 18-45 years were recruited for the study comprising 50 subjects each of 3 parities (groups A-C). A commercial ELISA Kit was used to measure all the cytokines. Neither statistically significant differences were found for TNF-α (p=0.636), IL-10 (p=0.892), IL-6 (p=0.306) and IL-4 (p=0.222) between prime parity and second parity nor for TNF-α (p=0.356), IL-10 (p=0.896), IL-6 (p=0.304) and IL-4 (p=0.298) between prime parity and multi-parity of malaria-infected pregnant women. TNF-α (p=0.255), IL-10 (p=0.524), IL-6 (p=0.616), and IL-4 (p=0.672) between second parity and on multi-parity respectively. The study showed no changes in the cytokines studied among the malaria-infected pregnant women based on parities. It shows that the number of pregnancies in women infected with malaria has no changes in the levels of the cytokines studied.

Keywords: Maternal expressions; alpha tumour necrosis factor; interleukin 10; interleukin 6 and interleukin 4; malaria-infected pregnant women; parity.

1. INTRODUCTION

Malaria has been reported as a condition caused by infestation with Plasmodium parasite species, is a significant 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]; [2]. Dellicour *et al*. [3] reported that pregnant women are susceptible to malaria infection. Malaria during pregnancy is a major public health challenge in endemic tropical countries, like in sub-Saharan Africa. Desai *et al.* [4] opined that about 125 million pregnant women live in malaria endemic areas in sub-Saharan Africa, and 32 million of these pregnant women are at threat of malaria.

Pregnant women are at high threat of being infected with malaria owning to the capacity of the parasite to stick to trophoblastic villous epithelium and sequester in the placenta, which could eventually lead to poor pregnancy outcome [5]. It revealed that over 200,000babies die yearly in sub-Saharan Africa because of their mother becoming infected with malaria during pregnancy [6]. 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 several stimuli [7]. They are involved in virtually all physiological responses in the body and are critical players in coordinating cell-to-cell communication between immune cells; by binding to specific cell membrane receptors inducing 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 [8]. In response to invading malaria parasites, however, Th-1 type cytokines are produced to reverse the Th-2 type bias within the placenta [9]. Inconsistence reports on the response of some pro-inflammatory cytokines to peripheral and placental malaria have been reported [10]; Diouf *et al. [11]*. Both pro and anti inflammatory cytokines are found at significantly increased levels in the peripheral blood and the intervillous spaces of the placentas of malaria-infected women. The Production of these cytokines is responsible for the resulting Th-1: Th-2 imbalance observed in Plasmodium falciparum-infected placentas [12,5].

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[13]. Anti-inflammatory cytokines have also been found to have

important roles in the immune response against Plasmodium. IL-10 exerts a vital role as an immunoregulator during *Plasmodium falciparum* infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells [14], [15]. Additionally, IL-10 and granulocyte colony-stimulating factor (G-CSF) is elevated and correlated with parasitaemia in asymptomatic pregnant women in Ghana [16], suggesting that these cytokines may act to reduce symptoms.

A study was done to determine the maternal serum levels of alpha tumour necrosis factor, interleukin 10, interleukin 6, and interleukin 4 in malaria-infected pregnant women based on their parities 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.

2.1.1 Subjects

A total of 150 subjects between the ages of 18-45 years were recruited for the study comprising fifty (50) subjects for each of the 3 parities (Groups, A- C).

2.2 Experimental Design

A prospective cross-sectional study was carried out in 3 groups.

Group A =50 Malaria Infected Pregnant Subjects at prime parity,
Group B =50 Malaria Infected Pregnant Subjects at second parity,
Group C =50 Malaria Infected Pregnant Subjects a multi-parity (three or more parities).

Oral consent was obtained from the patients after which a structured questionnaire was administered to all respondents who were also part of a 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 infections, other inflammatory or chronic diseases.
- Pregnant women who presented symptoms of malaria.
- Pregnant women between the ages 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 needing emergency care or having an at-risk pregnancy such as gestational diabetes, pre-eclampsia and eclampsia.

2.5 Sample Collection

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

2.5 ml was dispensed in EDTA container for malaria detection, and 5.5 ml were 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 [17]

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

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

2.6.2 Malaria parasite identification using Giemsa Staining Technique [18]

Methodology: A drop of blood was placed on the slide to cover the diameter of15-20 mm. 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 flush the stain off the slide. The slide was rinsed briefly in gently running tap water, and the undersurface 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.

2.6.3 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

Procedure: Dilutions of standard were prepared to get a concentration of 80 pg/mL, 40 png/mL, 20 pg/mL, 10 pg/mL, 5 pg/mL and 0 pg/mL. 50 uL of standards were pipette into the standard wells. 10 uL of test serum were pipette into each sample well. 40 uL of sample dilluent 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 30 minutes at 37^{o}C. It was washed four times. 50 uL of chromogen solution A and 50 uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{o}C. 50 uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450 nm wavelength within 15 minutes taking the blank well as zero concentration.

2.6.4 Interleukin 1 (1L-6) assay

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

Procedure: Dilutions of the standard were prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20 ng/L. 50 uL of standards were pipetted into standard wells. 10 uL of test serum were pipette into 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 30 minutes at 37^{o}C. It was washed for four times. 50 uL of chromogen solution A and 50 uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{o}C. 50 uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450 nm wavelength within 15 minutes taking the blank well as zero concentration.

2.6.5 Interleukin-10 (1L-10) assay

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

Procedure: Dilutions of the standard were prepared to get a concentration of 240 ng/L, 160 ng/L, 80 ng/L, 40 ng/L, and 20 ng/L. 50 uL of standards were pipette into the standard wells. 10 uL of test serum were pipette into the each sample well. 40 uL 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 30 minutes at 37^{o}C. It was washed for four times. 50 uL of chromogen solution A and 50 uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37^{o}C. 50 uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450 nm wavelength within 15 minutes taking the blank well as zero concentration.

2.6.6 Interleukin-4 (1L-4) assay

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

Procedure: Dilutions of the standard were prepared to get a concentration of 300 ng/L, 200 ng/L, 100 ng/L, 50 ng/L, and 25 ng/L. 50 uL of standards were pipette into the standard wells. 10 uL of test serum were pipette into the each sample well. 40 uL 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 30 minutes at 37^{o}C. It was washed for four times. 50 uL of

chromogen solution A and 50 uL of chromogen solution B was added to each well. They were mixed incubated for 10 minutes at 37°C. 50 uL of stop solution was added to each well. Optical densities of the samples were read in a microtiter plate reader at 450 nm wavelength within 15 minutes taking the blank well as zero concentration.

2.7 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. The student t- test was used for comparison of differences in various groups. All tests performed were two-tailed and the level of significance significant was set at $p<0.05$.

3. RESULTS

The results showed no significant difference in the mean values of TNF-α (14.38±0.68 pg/ml, 13.73±1.86 pg/ml, p=0.636), IL-10 (26.15±0.21 pg/ml, 26.61±4.60 pg/ml, p=0.892), IL-6 (28.07±5.76 pg/ml, 25.17±3.47 pg/ml, p=0.306) and IL-4 (9.37±5.85 pg/ml, 27.98±20.10 pg/ml, p=0.222) of malaria infected pregnant women of prime parity and on second parity respectively.

The results showed no significant difference in TNF-α (14.38±0.68 pg/ml, 13.04±1.99 pg/ml, p=0.356), IL-10 (26.15±0.21 pg/ml, 25.74±4.34 pg/ml, p=0.896), IL-6 (28.07±5.76 pg/ml, 24.51±4.64 pg/ml, p=0.304) and IL-4 (9.37±5.85 pg/ml, 25.29±20.94 pg/ml, p=0.298) of malaria infected pregnant women of prime parity and on multi-parity respectively.

The results showed no significant difference in TNF-α (13.73±0.68 pg/ml, 13.04±1.99 pg/ml, p=0.255), IL-10 (26.61±4.60 pg/ml, 25.74±4.34 pg/ml, p=0.524), IL-6 (25.17±3.47 pg/ml, 24.51±4.64 pg/ml, p=0.616) and IL-4 (27.98±20.10 pg/ml, 25.29±20.94 pg/ml, p=0.672) of malaria infected pregnant women of second parity and on multi-parity respectively.

Table 1. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 serum levels of malaria-infected pregnant women of prime parity and second parity

Parameters	Prime parity (n=50)	Second parity (n=50)	t-value	p-value
TNF-α (pg/ml)	14.38±0.68	13.73±1.86	0.483	0.636^{NS}
IL-10 (pg/ml)	26.15±0.21	26.61±4.60	-0.138	0.892^{NS}
IL-6 (pg/ml)	28.07±5.76	25.17±3.47	1.058	0.306^{NS}
IL-4 (pg/ml)	9.37±5.85	27.98±20.10	-1.271	0.222^{NS}

Table 2. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 serum levels of malaria-infected pregnant women of prime parity and multi- parity

Parameters	Prime parity (n=50)	Multi-parity (n=50)	t-value	p-value
TNF-α (pg/ml)	14.38±0.68	13.04±1.99	0.937	0.356^{NS}
IL-10 (pg/ml)	26.15±0.21	25.74±4.34	0.131	0.896^{NS}
IL-6 (pg/ml)	28.07±5.76	24.51±4.64	1.044	0.304^{NS}
IL-4 (pg/ml)	9.37±5.85	25.29±20.94	-1.059	0.298^{NS}

Table 3. Mean ± standard deviation of TNF, IL-10, IL-6 and IL-4 serum levels of malaria-infected pregnant women of second parity and multi- parity

Parameters	Second parity (n=50)	Multi-parity (n=50)	t-value	p-value
TNF-α (pg/ml)	13.73±0.68	13.04±1.99	1.153	0.255^{NS}
IL-10 (pg/ml)	26.61±4.60	25.74±4.34	0.642	0.524^{NS}
IL-6 (pg/ml)	25.17±3.47	24.51±4.64	0.504	0.616^{NS}
IL-4 (pg/ml)	27.98±20.10	25.29±20.94	0.426	0.672^{NS}

4. DISCUSSION

The study showed no changes in the cytokines studied among the malaria infected pregnant women based on their parities. Cytokines are immune regulators that are vital to the response in different conditions. Pregnancy is known to exert stress on women and changes in immunological systems. The cytokines studied are inflammatory and anti-inflammatory. A balance has to be established between these cytokines. This study shows that malaria infection does not change these cytokines in pregnant women based on their parities. Malaria infection has long been associated with high circulating levels of inflammatory cytokines such as 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 [13]. Anti-inflammatory cytokines have also been found to play essential roles in the immune response against Plasmodium. IL-10 has an important role as an immunoregulator during plasmodium falciparum infection, neutralizing the effect of the other cytokines produced by Th-1 and CD8 cells[14,15]. These cytokines are known to be elevated in malaria infected pregnant women as reported in the study of Okorie *et al.* [14]. Interleukin 10 (IL-10) and IL-4 modulate TNF alpha, act exerting immunoregulatory role during pregnancy by impeding the inflammatory response. Cytokines have a significant role in the pathogenesis of malaria and their levels can be useful as diagnostic markers for malaria and for monitoring the severity of the disease [14]. In a study carried out by Obeagu and his research team, there were no changes in these 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 [7].

5. CONCLUSION

The study showed no changes in the cytokines studied among the malaria infected pregnant women based on parities. This study shows that malaria infection does not change these cytokines in pregnant women based on their parities.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company, instead rather it was funded by the personal efforts of the authors.

CONSENT

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

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Murray CJL, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghav, M, Lozano R, Lopez AD. Global malaria mortality between 1980 and 2010: A systematic analysis. Lancet. 2012;379(9814):413-431.
2. Okorie HM, Obeagu EI, Obarezi HC, Anyiam AF. Assessment of Some Inflammatory Cytokines in Malaria Infected Pregnant Women in Imo State Nigeria. International Journal of Medical Science and Dental Research. 2019;2(1):25-36.
3. Dellicour S, Tatem AJ, Guerra CA, Snow RW, Ter Kuile FO. Quantifying the Number of Pregnancies at Risk of Malaria in 2007: A Demographic Study. Plos Medicine. 2010;7(1): e1000221.
4. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, Newman RD. Epidemiology and burden of malaria in pregnancy. Lancet Infectious Disease. 2007;7(2):93-104.
5. Suguitan Jr, AL, Leke RGF, Fouda G, Zhou A, Thuita L, Metenou S, Fogako J, Megnekou R, Diane Taylor W. Changes in the Levels of Chemokines and Cytokines in the Placentas of Women with Plasmodium falciparum Malaria. The

Journal of Infectious Diseases. 2003;188(7): 1074-1082.
6. Steketee RW, Nahlen BL, Parise MN, Menendez C. The Burden of Malaria in Pregnancy in Malaria-Endemic Areas. American Journal of Tropical Medicine and Hygiene. 2001;64(1).
7. Obeagu EI, Esimai BN, Ekelozie IS, Asogwa EI, Amaeze AA, Chukwu SN, Amaeze FN, Ugwuja MC, Chukwu SK. 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. Journal of Pharmaceutical Research International. 2020;32(14):64-70.
8. Bennet WA, Lagoo-Deenadayalan S, Whitworth NS, Stopple JA, Barber WH. First trimester human chorionic villi express both immunoregulatory and inflammatory cytokines a role for interleukin 10 in regulating the cytokines network of pregnancy. American journal of reproductive immunology. 1999;41:70-78.
9. Rogerson SJ, Mkundika PC, Kanjala MK. Diagnosis of plasmodium falciparum malaria at delivery. Comparison of blood film preparation methods and of blood films with histology. Journal of Clinical Microbiology. 2013;41:1370–1373.
10. Ismaila J, Van Der Sande, Holland MJ, Sambou. Plasmodium Fal. Infection of the placenta affects new born immune responses. British Society of Immunology. 2003;133(3):414-421.
11. Diouf NF, Souleymann DM, JaneFranciols T, Plullipine D. IL12 producing monocytes and IFN8 and FNFand producing Tlymphocytes are in placenta infected by *P. falciparium*. *Journal of* Reproductive Immunology. 2007;74(1):152-162.
12. Kabyemela ER, Midial Fried, Jonathan DF, Patrick E. Fetal Responses during placental malaria modify the risk of low birth weight. American Society of Microbiology. 2008;76(4):527.
13. Akanmon BD, Kurtzhal JA, Goka BQ. Plama antibodies from malaria exposed pregnant women recognized variant surface antigens of *P. Falciparum* infected erythrocytes in a parity dependent manner and black parasites. To chondroitin sulfate. The Journal of Immunology. 2010;165(6):3301-3315.
14. Couber KN, Bolnut DG, Riley EM. The master regulator of immunity to infection. Journal of immunology. 2008;180:5771-5777.
15. Langharne F, Ndugu FN, Sponaas AM, Marsh K. Immunity to malaria. More questions then answers. *Nature* Immunology. 2008;9:725-732.
16. Wilson NO, Bythwood T, Solomon W, Jolly P, Yatich N, Anderson W. Elevated level of interleukin 10 and G-CSF associated with asymptomatic malaria in pregnant women. Infectious Disease of Obstetics and Gyneocology. 2010;317-340.
17. Iqbal SS, Mayo MW, Bruno JG, Bronk BV, Batt CA, Chambers JP. A review of molecular recognition technologies for detection of biological threat agents. Biosensors and Bioelectronics. 2000;15(11):549-578.
18. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. second edition. Cambridge University Press. Cambridge. 2005;454.

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