Bioscene

Bioscene
Volume- 21 Number- 03
ISSN: 1539-2422 (P) 2055-1583 (O)
www.explorebioscene.com

Enhanced Peroxidation and Cytokine Dysregulation in HIV Seropositive Pregnant Women with Malaria Co-Infection in Nauth, Nnewi, Nigeria

Nkiruka Rose Ukibe[1*], Ofia Anya Kalu[2], Chinedum Charles Onyenekwe[1], Ikechukwu Uzoma Chukwuagwu[1], Ezinne Gloria Ukibe[2], Blessing Chinyere Ukibe[2], Victory Ezennia Ukibe[3]

[1]Department of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University, Awka, P. M. B, Anambra State, Nigeria.
[2]Department of Medicine, Faculty of Medicine, College of Health Sciences, Nnamdi Azikiwe University, Awka, P. M. B, Anambra State, Nigeria.
[3]Department of Radiography and Radiological Sciences, College of Health Sciences, Nnamdi Azikiwe University, Awka, P. M. B, Anambra State, Nigeria.

Abstract

Background: the burden of HIV and malaria co-infection has contributed to adverse health complications, especially in pregnant mothers residing in endemic regions. Malaria infection is an important neglected tropical disease and has remain a major public health challenge in sub-Saharan Africa including Nigeria. This study is a case-control study aimed at evaluating the impact of oxidative stress and some cytokine imbalance on HIV seropositive pregnant women with/without malaria infection attending antenatal clinic at Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, Nigeria. The study involved 90age-matched pregnant women grouped into HIV seropositive pregnant women (n=30), HIV seropositive pregnant women with malaria (n=30), HIV seronegative pregnant women with malaria (n=30), and 30 non-pregnant women without HIV or malaria as control. **Methods**: Blood sample was collected from each participant for determination of Malondialdehyde (MDA), Superoxide Dismutase (SOD), Selenium (Sel), Zinc (Zn), Tumor necrosis factor alpha (TNF-α),interferon-gamma (IFN-γ) using colorimetric, atomic absorption spectrophotometry and enzymatic method respectively. **Results**: IFN-γ and TNF-α were significantly increased in HIV seropositive with malaria compared with HIV seronegative pregnant women without malaria and control participants (p=0.001). IFN-γ was significantly increased in HIV seronegative pregnant women with malaria compared with HIV seropositive pregnant women (p=0.001). MDA was significantly increased in HIV seropositive pregnant women with and without malaria co-infection while serum levels of zinc, selenium, and SOD were decreased in HIV seropositive pregnant women with malaria compared with HIV seronegative pregnant women with malaria and control group (p≤0.05 respectively). **Conclusion**: The study

showed increased oxidative stress with a significant degree of inflammation and reduced immunity in HIV seropositive women with malaria co-infection. This suggests evidence of disease progression and severity which may have been worsened by the co-infection.

Keywords: HIV, Oxidative stress, cytokine, pregnant women, malaria

Introduction

Background

The burden of Human Immunodeficiency Virus (HIV) and Plasmodium falciparum malaria co-infection have remained a public health importance, more especially in developing countries, Nigeria inclusive [1, 2]. Both infections have synergistically become more vulnerable in sub-Saharan Africa and the effect has increasingly affected pregnant mothers, resulting in overwhelming complications and adverse pregnancy outcomes [3]. The recent WHO report has shown evidence of increasing trends in new infections globally [4] with an estimated 39.0 million people living with HIV at the end of 2022, and about 630,000deathsfrom HIV-related causes [4]. There is a report of a 27.3% combined prevalence of malaria in HIV-positive adults and a malaria prevalence of about 32.3% in HIV-positive pregnant women [5]. However, Nigeria has recently recorded between 31–61% prevalence among HIV-positive pregnant women and between 10 and 36% in non-HIV-infected pregnant women [2].

During normal pregnancy, the body undergoes physiological adjustments, one of them being the modulation of adaptive, pro-inflammatory immune responses to ensure fetal survival [6].However, human pregnancy constitutes a metabolic, oxidative, and immune challenge for the mother as well as the unborn [7].

Cytokines are of interest since they are the fundamental messengers of adaptive immunity and are likely to be involved in pregnancy, HIV, and Malaria infection [8] and oxidant and antioxidant alterations have also been a long-standing problem in sub-Saharan Africa with severe public health implications in pregnant women [9, 10].Though the rate of mother-to-child transmission of HIV infection is on reduction, more information is still needed on the mechanism of mother-to-child transmission of malaria and the oxidative stress implication of this co-infection on pregnant mothers.

Materials and Methods

Study Design

This study is designed to assess the level of oxidative damage and cytokine imbalance in HIV seropositive pregnant women with malaria co-infection in NAUTH Nnewi Nigeria using Malondialdehyde (MDA), Superoxide Dismutase (SOD),

Selenium (Sel), Zinc (Zn), Tumor necrosis factor alpha (TNF-α), interferon-gamma (IFN-γ). The study was conducted on 90 randomly selected HIV seropositive pregnant women grouped into (30) HIV seropositive pregnant women with malaria co-infection, (30) HIV seropositive pregnant women without malaria infection, (30)pregnant women with malaria infection, and (30)non-pregnant women without malaria nor HIV infection which served control.

All the HIV seropositive pregnant women were on antiretroviral therapy. All subjects were asymptomatic and screened for HIV seropositivity and malaria infection.

Screening for HIV antibodies was done by Nigeria's national algorithm. Pregnancy testing was done by human chorionic gonadotropin (HCG) one-step pregnancy test strip. Peripheral malaria was determined from maternal venous blood by the Rapid Detection Technique (RDT) (2SD). Giemsa stain of thin and thick blood smears were assayed by microscopy to confirm malaria results.

A well-structured questionnaire was administered to each participant to obtain the history of their pregnancy and other biodata. The participants were aged between 18 and 45 years. Levels of cytokines were assayed using, an enzyme-linked immunosorbent assay technique, while levels of MDA, SOD, zinc, and selenium were determined using atomic absorption spectrophotometry.

Study site

This study was carried out in the ante-natal clinic (PMCT unit) of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi, Anambra State, Nigeria. Also, the laboratory analysis of cytokines and oxidative stress markers was done at the Chemical Pathology laboratory of NAUTH while zn and sel were analyzed at BIOTEC center, Nnamdi Azikiwe University, Awka.

Subject recruitment

A purposive sampling technique was employed. The subjects were pregnant women attending the PMCT clinic at NAUTH between October 2021 and April 2022 who gave their consent to participate in the study.

Inclusion and exclusion criteria:

Pregnant women between 18 and 45 years old were selected for the study. HIV-infected pregnant women with malaria co-1nfection and HIV-infected pregnant women without malaria infection were selected. Pregnant women with malaria infection were also included. Non-pregnant women without malaria co-infection were also included in the study. Pregnant women aged less than 18 and above 45 years were excluded. Participants who were active smokers, alcoholics, hypertensive, and diabetic patients were excluded. Participants who had tuberculosis and hepatitis were excluded from the study.

Collection of samples

Five milliliters (5ml) of venous blood was collected from each of the participants. 3ml and 2ml each were dispensed into well-labeled plain and EDTA containers. The plain sample was allowed to clot, retracted, centrifuged at 3000 rpm for 10 minutes, and separated. Serum was collected and transferred into the plain container and stored at -80 °C in the specialized laboratory of NAUTH for analysis of TNF-α and INF-ɣ, MDA, Zn, and Sel.

Laboratory analyses

HIV-1/2 assay was done according to the National algorithm using determine HIV-1/2 assay as was described by Masciotra, [11], Uni-gold HIV test kit as was described by Butler, [12] and Stat-pak HIV test kits as was described by WHO, [13].

Plasmodium falciparum malaria was identified using a Rapid detection test for *Plasmodium falciparum* malaria antigen [14] and Giemsa Stained Thick and thin blood film for microscopic detection of P. falciparum, parasites as was described by WHO, [15].

Determination of Serum levels of Zinc and selenium was done by Atomic Absorption Spectrophotometry (AAS) [16].

Determination of Malondialdehyde (MDA) and superoxide dismutase enzyme (SOD) was done using colorimetric method as were described by Gutteridge and Wilkins, [17] and Misra and Fredovich, [18] respectively.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used for the statistical analysis.

The data generated was analyzed using Analysis of variance (ANOVA) to compare more than two independent variables. Student's t-test was used for two independent variables. The Pearson correlation was used to correlate different parameters and values considered statistically significant if the p-value ≤ 0.05

Results

Some anthropometric parameters in HIV seropositive pregnant subjects with malaria co-infection and control participants

The mean value of BMI was significantly higher in pregnant women with malaria parasite infection (27.59 ± 6.83), compared with HIV seropositive pregnant women with malaria co-infection (25.18 ± 3.39), HIV seropositive pregnant women without mp (25.99±4.09), and control participants (24.01 ± 3.04) (P= 0.001).

The mean DBP value was significantly higher in HIV seropositive pregnant women with malaria co-infection (90.49±7.90), HIV seropositive pregnant women

(80.83±9.30), and pregnant women with malaria parasite infection (80.37±9.11)when compared with control participants (71.01 ± 5.17) (p=0.05 respectively).
The mean SBP value was significantly higher in HIV seropositive pregnant women with malaria parasite infection (139.18± 9.41), HIV seropositive pregnant women without malaria (13.1 ± 7.30), pregnant women with malaria infection (129.56±6.43), when compared with control participants (110.11 ± 5.08) (p=0.001) (table 1).

Table 1. Levels of some anthropometric parameters in HIV-infected pregnant women with malaria co-infection and control subjects

Group	BMI(kg/m^2)	DBP(mmHg	SBP(mmHg)
HIV seropositive pregnant women with mp (A) n=30	25.18±3 .39	90.49±7.90	139.18±9.41
HIV seropositive pregnant women without mp (B) n=30	25.99±4.09	80.83±9.30	132.1±7.30
pregnant women with malaria infection (C)n=30	27.59±6.83	80.37±9.11	129.56±6.43
Control (D) n=30	24.01±3.04	71.01±5.17	110.11±5.08
F- Value	6.853	11.19	20.74
P-Value type	0.000	0.000	0.000
A vs B	0.067	0.003	0.005
A vs C	0.000	0.001	0.003
A vs D	0.033	0.000	0.000
B VS C	0.024	0.637	0.277
B VS D	0.002	0.005	0.000
C VS D	0.000	0.000	0.001

Levels of serum TNF-α and INF-ɣ, in HIV seropositive pregnant women with malaria co-infection and control participants.

The mean levels of IFN-ɣ and TNF-α were significantly higher in HIV seropositive pregnant women with malaria (136.62±20.09, 14.24±3.11), HIV sero-positive pregnant women (86.83±11.49, 11.78±2.12), and pregnant women with malaria parasite infection (99.73±16.49, 8.81 ±2.05) when compared with control participants (64.68 ±8.89, 6.83± 1.84) (P = 0.001 respectively). The mean level of IFN-ɣ was significantly lower in HIV sero-positive pregnant women (86.83±11.49) compared with pregnant women with malaria parasite infection (99.73±16.49) (p=0.010) (table 2).

Table 2. Levels of serum TNF-α and INF-ɣ, in HIV seropositive pregnant women with malaria co-infection and control participants.

Group	IFN-ɣ(pg/ml)	TNF-α (pg/ml)
HIV seropositive pregnant women with mp (A) n=30	136.62±20.09	14.24±3.11
HIV seropositive pregnant women without mp (B) n=30	86.83±11.49	11.78±2.12
Pregnant women with mp (C) n=30	99.73±16.49	8.81 ±2.05
Non-pregnant women without HIV/mp (D) n=30	64.68 ±8.89	6.83± 1.84
F- Value	81.75	65.36
P- Value type	0.000	0.000
A VS B	0.000	0.000
A VS C	0.000	0.000
A VS D	0.000	0.000
B VS C	0.010	0.000
B VS D	0.000	0.000
C VS D	0.006	0.000

Key: IFN-ɣ = interferon gamma, TNF-α = Tumor necrosis factor alpha

Levels of serum zinc, selenium, MDA and SOD in HIV seropositive pregnant women with malaria co-infection and control participants.

The mean levels of zinc, selenium and SOD were significantly lower in HIV sero-positive pregnant women with malaria parasite infection (29.85±7.09, 37.89±5.16, 26.3±0.72), HIV seropositive pregnant women (39.81±8.12, 59.18±6.93, 31.35±1.67), pregnant women with malaria (68.10±8.91, 99.17±8.38, 36.65±2.84) when compared with control participants (77.21±12.72, 126.89±9.15, 49.89±2.98) ($P<0.05$ respectively). Similarly, the mean levels of zinc, selenium and SOD were significantly lower in HIV sero-positive pregnant women with malaria parasite infection (29.85±7.09, 37.89±5.16, 26.3±0.72) when compared with their counterparts without malaria parasite infection (39.81±8.12, 59.18±6.93, 31.35±1.67) ($p< 0.05$ respectively). The mean levels of zinc, selenium and SOD were significantly lower in HIV sero-positive pregnant women with malaria (29.85±7.09, 37.89±5.16, 26.3±0.72) and HIV seropositive pregnant women (39.81±8.12, 59.18±6.93, 31.35±1.67) when compared with pregnant women with malaria co-infection (68.10±8.91, 99.17±8.38, 36.65±2.84) ($p<0.05$ respectively)

However, the mean serum MDA level was significantly higher in HIV sero-positive pregnant women with malaria parasite infection (5.58±1.87), HIV seropositive pregnant women (3.80±1.16), pregnant women with malaria (2.94±0.59) when compared with control participants (1.93±0.45) ($p<0.05$ respectively) (table 3).

Table 3. Levels of serum MDA, SOD, zinc, and selenium in HIV-infected pregnant women with malaria co-infection and control subjects.

Group	IFN-ɣ(pg/ml)	TNF-α (pg/ml)
HIV seropositive pregnant women with mp (A) n=30	136.62±20.09	14.24±3.11
HIV seropositive pregnant women without mp (B) n=30	86.83±11.49	11.78±2.12
Pregnant women with mp (C) n=30	99.73±16.49	8.81 ±2.05
Non-pregnant women without HIV/mp (D) n=30	64.68 ±8.89	6.83± 1.84
F- Value	81.75	65.36
P- Value type	0.000	0.000
A VS B	0.000	0.000
A VS C	0.000	0.000
A VS D	0.000	0.000
B VS C	0.010	0.000
B VS D	0.000	0.000
C VS D	0.006	0.000

Key: D = Non pregnant women without HIV or malaria parasite infection, MDA = malondialdehyde, SOD = superoxide dismutase.

Correlation between TNF-α, INF-ɣ. Sel, BMI, and systolic blood pressure in HIV seropositive pregnant women with malaria co-infection and control participants

There was positive correlation between TNF-α and systolic blood pressure in HIV sero-positive pregnant women (r= -0.423, p=0.012). Similarly, there was a positive correlation between levels of INF-ɣ and BMI in control participants (r=0.504, p = -0.001). Similarly there was a negative correlation between INF-ɣ and selenium in control participants (r=-0.395, p=0.031) (table 4).

Table 4. Correlation between TNF-α, Sel, and some anthropometric parameters in HIV-infected pregnant women with malaria co-infection and control participants.

PARAMETERS	R- VALUE	P- VALUE
TNF-α vs SPB(B)n=30	-0.423	0.012
INF-ɣ vs Sel (D) n=30	-0.385	0.031
INF-ɣ vs BMI (D) n=30	-0.504	0.000

B = HIV seropositive pregnant women without malaria infection, D= Non-pregnant women without HIV or malaria parasite infections (Control). r = Pearson Correlation Co-efficient. Correlation is significant when P is <0.05.

Discussion

Oxidative stress and inflammation have a considerable influence on HIV disease progression especially in malaria-endemic regions. Unhealthy nutritional status in HIV infection has a significant influence on both innate and cellular function and this may result from imbalances between the oxidant and antioxidant molecules thereby causing oxidative stress as well as tissue, cell, and organ damage [19, 20].

The present study shows that HIV and malaria co-infection is associated with a significant elevation of IFN-ɣ and TNF-α levels in HIV-infected pregnant women with malaria co-infection when compared with their counterparts without malaria infection and control participants.

The finding was consistent with some earlier reports [3, 21, 22]. The authors documented increased IFN-ɣ levels in pregnant women with malaria infection irrespective of their HIV status.

The combined effect of HIV and malaria infection is associated with strong up-regulation of pro-inflammatory cytokines as a result of strong CD4+ cell activation with resultant free radicals due to stress[23]. Previous studies have reported the detrimental effects of cytokine imbalance on the peripheral blood and placentas of malaria-infected pregnant women as well as the importance of interferon (IFN)-γ parasite sequestration and placental malarial protection [2, 24].

The study observed a significant increase in serum MDA and uric acid with decreases in antioxidant (SOD, zinc, selenium) levels among pregnant women with or without HIV/mal co-infection. This indicates strong evidence of increased levels of oxidative stress which may result in lipid peroxidation and inflammatory reactions. This is in order with some previous findings [10, 25, 26]. However, the decreases in the antioxidant levels manifested more in HIV-infected women. Parasite clearance can increase leukocyte infiltration and stimulation of inflammatory cytokines which increases the parasite-infected red blood cells thereby resulting in oxidative stress [25, 27]. This may increase the chances of adverse pregnancy outcomes in those pregnant women [28, 29]. Increased oxidative stress observed in this study may be due to alterations in the oxygen radicals and antioxidant defense molecules in the entire body system in reaction to the malaria parasite load in the affected individuals [23, 30].The determination of serum MDA level has been documented as a strong immune modulator during malaria infection by enhancing inflammatory reactions [25, 27].

Furthermore, the decreased levels of antioxidants SOD, zinc, and selenium may have contributed greatly to the increased oxidative stress as observed in the study. Antioxidant molecules play a role in modulating oxidative damage by free radicals [31]. Zinc is shuttled into cellular compartments, where it is utilized for protein synthesis, neutralization of free radicals, and to prevent microbial invasion. The redistribution of zinc during inflammatory events seems to be mediated by

cytokines. Several studies have demonstrated how patients with acute illnesses present with hypozincemia along with elevated cytokine production [32]. The significant reduction in serum zinc levels in HIV/malaria co-infected pregnant women is in agreement with the findings of some previous studies [33, 34].

In this study, selenium concentration was significantly lower among the HIV/malaria pregnant women. This is similar to other findings elsewhere [35, 36].

During pregnancy, a lot of stress is experienced physiologically and pathologically. The physiological stress is due to changes resulting from increased demands for micronutrients, and changes in plasma volume. These increased demands eventually lead to a decrease in micronutrients, especially antioxidant micronutrients [37]. Earlier reports have shown the effects of micronutrients and vitamin deficiencies in increased mother-to-child transmissions as well as maternal HIV disease progression [33, 38]. Nutritional well-being of a healthy mother is of optimal importance and paramount for a healthy pregnancy outcome especially in HIV-pregnant mothers [39, 40].This is needed to avert the burden of the adverse pregnancy complications that herald the affected mothers due to depressed immunity and infections[39, 40].

Lower selenium levels as was observed in the present study may likely increase fetal mortality risk and it is also evident with decreased CD4 cell counts and increased cytokines levels. This may have also enhanced the oxidative stress recorded in this study, especially when there is a co-infection. Selenium has been shown to significantly impact DNA amplification, oxidative defenses, and infections [3, 37, 41]. Previous reports have also implicated selenium deficiency in pregnant mothers in the overall risk of maternal pregnancy complications and outcomes including preterm labor, fetal death, low birth weight, neural tube defects, miscarriages, and poor weight gain during pregnancy [38, 42]. Previous study has documented strong evidence of the immunoregulatory mechanism of Selenium supplementation in NF-kB activation and HIV replication [43].

The blood pressure of HIV-infected pregnant women was significantly higher than control pregnant women. This suggests that HIV- infected pregnant women have the tendency to develop hypertension and possibly preeclampsia. This is in line with previous works [3, 44, 45]. A negative correlation was established between TNF-α and systolic blood pressure in HIV seropositive-positive pregnant women. A positive correlation was established between INF-$_\gamma$ with HIV seropositive pregnant women with malaria co-infection.

Conclusion

HIV and malaria co-infection impacts serious significant oxidative stress and alterations in the levels of maternal inflammatory markers with resultant derangement in antioxidant molecules such as SOD, zinc, and selenium in the

affected pregnant women thereby conferring a bidirectional burden of both diseases in pregnancy due to reduced cellular immunity. The increased MDA, BMI, and blood pressure levels observed further established the evidence of enhanced lipid peroxidation and possible hypertension which could subsequently lead to preeclampsia, disease progression, and other adverse pregnancy outcomes. Antioxidant supplementations are strongly recommended in the management of HIV seropositive pregnant mothers irrespective of their co-infection status.

References

1. Onyenekwe CC, Ukibe N, Meludu SC, Ifeanyi M, Ezeani M, Onochie A, Ofiaeli N, Aboh N, Ilika A. (2008). Possible biochemical impact of malaria infection in subjects with HIV co-infection in Anambra state, Nigeria. J Vector Borne Dis. 45(2), 151-156.
2. Obase BN, Bigoga JD, Nsagha DS. (2023). Malaria and HIV Co-Infection among Pregnant Women in Africa: Prevalence, Effect on Immunity and Clinical Management: Review. International Journal of Translational Medicine. 3(2), 187-202.
3. Chukwuagwu IU, Ukibe NR, Ogbu II, Ikimi CG, Agu VO, Kalu OA, Ukibe SN, Awalu JC. (2020). Evaluation of Serum Interleukin 6, Tumor Necrosis Factor-Alpha, and Interferon-Gamma Levels in Relation to Body Mass Index and Blood Pressure in HIV Seropositive Pregnant Women Coinfected with Malaria. Interdiscip Perspect Infect Dis. 2424802.
4. World Health Organization. Official WHO updates - who. Int www.who.int › HIV. 2023.
5. Mirzohreh, ST., Safarpour, H., Pagheh, A.S. et al.(2022). Malaria prevalence in HIV-positive children, pregnant women, and adults: a systematic review and meta-analysis. Parasites Vectors 15, 324
6. Samad A.L., Denoeud N., Djimon M. Z., Achille M. (2015).American Journal of Tropical Medicine and Hygene93(2), 287–292
7. Abu-Raya B, Michalski C, Sadarangani M, Lavoie PM. (2020). Maternal Immunological Adaptation during Normal Pregnancy. Front Immunol. 11, 575197.
8. Obeng-Aboagye, E., Frimpong, A., Amponsah, J.A. et al. (2023). Inflammatory cytokines as potential biomarkers for early diagnosis of severe malaria in children in Ghana. Malar J 22, 220.
9. Nkengfack GN, Torimiro JN, Englert H. (2012). Effects of antioxidants on CD4 and viral load in HIV-infected women in sub-Saharan Africa - dietary supplements vs. local diet. Int J Vitam Nutr Res. 82(1), 63-72.

10. Ukibe RN, Manafa OP, Ozumba UA, Eleje UG, Kalu AO, Ochiabuto MTBO, et al. (2023). Variation in Redox Status of HIV Sero-Positive Pregnant Women in NAUTH, Nnewi, Nigeria. IJARESM,11(6) 2429.
11. Masciotra, S., Luo, W., Youngpairoj, A. S., Kennedy, M. S., Wells, S., Ambrose, K., Sprinkle, P., & Owen, S. M. (2013). Performance of the Alere Determine™ HIV-1/2 Ag/Ab Combo Rapid Test with specimens from HIV-1 seroconverters from the US and HIV-2 infected individuals from Ivory Coast. Journal of Clinical Virology: The Official Publication of the Pan American Society for Clinical Virology, 58(Suppl 1), e54.
12. Butler C. K. In Enzyme Immunoassay. Maggio E. T., editor. Boca Raton, FL, USA: CRC Press; 1980.
13. World Health Organization Department of Reproductive Health and Research. Family Planning: A Global Handbook for Providers: Evidence-Based Guidance Developed through Worldwide Collaboration (PDF) (Review and Updated Edition) Geneva, Switzerland: WHO and Center for Communication Programs; 2011.
14. Murray CK,Gasser RA,Magill AJ,Miller RS. (2008). Update on rapid diagnostic testing for malaria.Clinical MIMicrobiologyeviews. 21(1), 97-110
15. WHO. Malaria parasite counting. Malaria standard operating procedure. 2016. MM-SOP.09
16. Izquierdo AS, Castañón SG, Ruata ML, Aragüés EF, Terraz PB, Irazabal YG, et al. (2007). Updating of normal levels of copper, zinc and selenium in serum of pregnant women. J Trace Elem Med Biol. 21 Suppl 1, 49-52.
17. Gutteridge JM, Wilkins S. (1982). Copper dependant hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. FEBS Letters. 137, 327-330.
18. Misra HP and Fridovich I. (1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. Journal of Biological Chemistry. 247, 3170
19. Faintuch J Soeters PB, Osmo HG. (2006). Nutritional and metabolic abnormalities in pre-AIDS HIV infection. Nutrition. 22, 683-690
20. Nunnari G, Coco C, Pinzone MR, Pavone P, Berretta M, Di Rosa M et al. (2012). The role of micronutrients in the diet of HIV-1-infected individuals. Frontiers in Bioscience.E4, 2442-2456.
21. Ibitokou SA, Denoeud-Ndam L, Ezinmegnon S, Ladékpo R, Zannou DM, Massougbodji A, Girard PM, Cot M, Luty AJ, Ndam NT. (2015). Insights Into Circulating Cytokine Dynamics During Pregnancy in HIV-Infected Beninese

Exposed to Plasmodium falciparum Malaria. Am J Trop Med Hyg.93(2), 287-292.

22. Nmorsi O. P. G, Isaac C., Ukwandu N. C. D., Ohaneme B. A. (2021). Pro–and anti–inflammatory cytokines profiles among Nigerian children infected with Plasmodium falciparum malaria. Asian Pacific Journal of Tropical Medicine. 3(1), 41–44.
23. Megnekou R, Djontu JC, Bigoga JD, Medou FM, Tenou S, Lissom A. (2015). Impact of placental Plasmodium falciparum malaria on the profile of some oxidative stress biomarkers in women living in Yaoundé, Cameroon. PLoS One. 10:e0134633.
24. Djontu, J.C.; Siewe Siewe, S.; Mpeke Edene, Y.D.; Nana, B.C.; Chomga Foko, E.V.; Bigoga, J.D.; Leke, R.F.G.; Megnekou, R. (2016). Impact of placental Plasmodium falciparum malaria infection on the Cameroonian maternal and neonate's plasma levels of some cytokines known to regulate T cells differentiation and function. Malar. J. 15, 561
25. Punnath K, Chandrashekar VN, Dayanand KK, Achur RN, Kakkilaya SB, Kumari S. (2019). Differential oxidative stress and antioxidant responses in mild and severe malaria. IJSRR. 8, 3310- 3328.
26. Chandrashekhar VN, Punnath K, Dayanand KK, Kakkilaya SB, Jayadev P, Kumari SN, Achur RN, Gowda DC. (2022). Impact of oxidative stress in response to malarial infection during pregnancy: Complications, histological changes, and pregnancy outcomes. Trop Parasitol. 12(1), 21-33.
27. Suguitan AL, Jr, Leke RG, Fouda G, Zhou A, Thuita L, et al. (2003). Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria. J Infect Dis.188, 1074 -1082
28. Lekana Douki JB, Traore B, Costa FT, Fusaı T, Pouvelle B, Sterkers Y, et al. (2022). Sequestration of Plasmodium falciparum–infected erythrocytes to chondroitin sulfate A, a receptor for maternal malaria: Monoclonal antibodies against the native parasite ligand reveal pan reactive epitopes in placental isolates. Am J Hematol. 100, 1478- 1483.
29. Nwagha UI, Okeke TC, Nwagha TU, Ejezie FE, Ogbodo SO, Dim CC, et al. (2011). Asymptomatic malaria parasitemia does not induce additional oxidative stress in pregnant women of South East Nigeria. Asian Pac J Trop Med. 4, 229 -233
30. Takem EN, D'Alessandro U. (2013). Malaria in pregnancy. Mediterr J Hematol Infect Dis. 5:e2013010.
31. Dayanand KK, Punnath K, Chandrashekar V, Achur RN, Kakkilaya SB, Ghosh SK, et al. (2017) Malaria prevalence in Mangaluru city area in the Southwestern coastal region of India. Malar J. 16, 492.

32. Besecker BY, Exline MC, Hollyfield J, Phillips G, Disilvestro RA. (2011). A comparison of zinc metabolism, inflammation, and disease severity in critically ill infected and non-infected adults early after intensive care unit admission. Am J Clin Nutri. 93: 1356–1364.
33. Kassu, A., T. Yabutani, A. Mulu, B. Tessema, and F. Ota (2008). Serum zinc, copper, selenium, calcium, and magnesium levels in pregnant and non-pregnant women in Gondar. Northwest Ethiopia. Biological Trace Element Research. 122(2), 97–106.
34. Saad A.A., Doka Y.A., Osman S.M., Magzoub M., Ali N.I., Adam I. (2005). Zinc, copper and C-reactive protein in children with severe plasmodium falciparum malaria in an area of unstable malaria transmission in Eastern Sudan. Journal of. Tropical. Pediatric.59(2), 150– 153.
35. Ogbodo S. O., Okaka A. N., Nwagha U. I., Ejezie F. E., Okafor C. S. (2014). Oxidative Stress in Symptomatic Malaria Parasitemic Pregnant Women from Malaria Endemic Area of Nigeria American Journal of Medicine and Medical Sciences 4(5), 168-174
36. Okonkwo RI, Onyeabochukwu AD, Izuka EO, Duke-Onyeabo CP, Obiora-Izuka CE, Ejelonu UT, Nwagha UI. (2023). Association between maternal selenium levels and pregnancy outcome among human immunodeficiency virus-positive and human immunodeficiency virus-negative pregnant women in a tertiary health-care center in Owerri, Nigeria: A comparative cross-sectional study. Ann Afr Med. 22(3), 373-380.
37. Chukwuagwu, I. U., Ukibe, N. R., Ogbu, I. S., Agu, V. O., Awalu, J. C., Ehiaghe, F. A., & Onyema-Iloh, O. B. (2020b). Possible Impact of Select Trace Mineral Deficiency in HIV Seropositive Pregnant Women with/without Malaria Co-infection in NAUTH, Nnewi, Nigeria. Annual Research & Review in Biology,35(5), 53-62.
38. Ogbodo S.O., Nwagha U.I., Okaka N.C., Okeke A.C, Chukwurah E.F., Ezeonu P.O. (2012). Low levels of some nutritional parameters of pregnant women in a rural community of Southeast Nigeria: implications for attainment of the Millenium Developmental Goals. Annals of Medical and Health Science Research. 2, 49–55.
39. Kupka, R., G. I. Msamanga, C. Xu, D. Anderson, D. Hunter, and W. W. Fawzi (2007b). Relationship between plasma selenium concentrations and lower genital tract levels of HIV-1 RNA and interleukin type 1beta. European Journal of Clinical Nutrition, 61(4), 542–7.
40. Mala JO, Tuitock PJ, Odhiambo RA. (2012). Effect of dietary intakes on pregnancy outcomes: A comparative study among HIV-infected and

uninfected women at Nyanza Provincial General Hospital Kenya. Afr J Food Agric Nutr Dev. 12, 6776–6793.

41. Sunde RA. Selenium. In: Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler TR. editors. (2012). Modern Nutrition in Health and Disease. 11th ed. Philadelphia, PA: Lippincott Williams and Wilkins; pp. 225–37.
42. Makwe CC, Nwabua FI, Anorlu RI. (2015). Selenium status and infant birth weight among HIV-positive and HIV-negative pregnant women in Lagos, Nigeria. Nig Q J Hosp Med. 25, 209–215.
43. Stone CA, Kawai K, Kupka R, Fawzi WW. (2010). Role of selenium in HIV infection. Nutr Rev.68(11), 671-681.
44. Coloma A. G., Alvarez A. M., Roca-Cusachs C. A., Domingo P. P., Puig C. M. (2008). Prevalence of arterial hypertension and lipid profile in HIV patients. Medicina Clínica.131(18), 681–684.
45. Ukibe N. R., Onyenekwe C. C., Anojulu A. A., Ukibe S. N., Ikechukwu E. C. (2017). Possible changes in maternal plasma cortisol, adrenocorticotropic hormones, pregnancy-associated plasma protein-A and alpha-fetoprotein in HIV pregnant women at NAUTH Nnewi, Nigeria. Journal of Bioanalysis and Biomedicine. 9(5), 250–255.