EFFECT OF NUTRITIONAL STATE ON INFLAMMATION IN PREGNANT WOMEN INFECTED WITH MALARIA AT THE YARIMAN BAKURA SPECIALIST HOSPITAL IN GUSAU, ZAMFARA STATE.

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**ABSTRACT**

Inflammation is a part of the body's defense mechanism in which the immune system recognizes and removes harmful and foreign stimuli and initiates the healing process. Populations residing in malaria-endemic areas generally live under low socioeconomic conditions, leading to poor nutritional status. The aim of this study was to assess the nutritional status of inflammatory and malaria-infected pregnant women who were receiving antenatal care at the Yariman Bakura specialist Hospital in Gusau, Zamfara State. Blood samples were collected to check the packed cell volume (PCV) level, and malaria status was analyzed using microscopy. (82.9%) women fell under the category of "Normal" mid upper arm circumference (MUAC) measurements, and (17.2%) categorized as "Severe Malnourishment. The antenatal clinic had a (23%) prevalence of malaria. (54.3%) packed cell volume (PCV) and (37.1%) hemoglobin (Hb) levels reported. High plasmodium lactate dehydrogenase (pLDH) levels were observed in (71.4%), (n= 39), whereas minor to elevated C - reactive protein levels were observed in (81.5%) (n=49). There was no statistical significance between plasmodium lactate dehydrogenase (PLDH) and dietary diversity; however, C-reactive protein showed statistical significance with mid-upper arm circumference (MUAC), packed cell volume (PCV) and plasmodium lactate dehydrogenase (PLDH), (p= 0.042, 0.001 and 0.011 respectively). This study revealed that poor nutritional status, hemoglobin levels, and packed cell volume (PCV) levels affect inflammation in pregnant women infected with malaria. Therefore, urgent nutritional interventions and medical attention are needed for such individuals.

Key Words: Malaria status, Plasmodium lactate dehydrogenase, packed cell volume, hemoglobin, mid-upper arm circumference, C-reactive protein

INTRODUCTION

Inflammation is a part of the body's defense mechanism. The process by which the immune system recognizes and removes harmful and foreign stimuli begins the healing process (Zhang *et al*, 2019). Inflammation can be acute or chronic; Acute Inflammation can be induced by tissue damage caused by trauma or microbial invasion. It starts rapidly and becomes severe in a short time, and symptoms may last for a few days, for example cellulitis or acute pneumonia (Pahwa *et al*, 2023). Chronic inflammation, on the other hand, occurs when this response lingers, leaving the body in a constant state of alert. Chronic inflammation may have a negative impact on tissues and organs (Pahwa *et al*, 2023).

 Malaria is a highly inflammatory disease. In response to the presence of the parasite, the host’s immune system produces proinflammatory cytokines, including IL-6, IL-8, IFN-γ, and TNF, which play a pivotal role in controlling the growth of the parasite and its elimination. Regulatory cytokines such as transforming growth factor- (TGF) β and IL-10 maintain the balance between pro- and anti-inflammatory responses (Shomyseh, et, al., 2010). Some studies argue that due to the effects of inflammatory cytokines on mitochondria, there is an inability to use available oxygen, leading to ATP deficiency in malaria (Clark *et al*, 2006).

Acute-phase protein is a nonspecific protein released during infection, tissue damage, tissue injury, and inflammation. C-reactive protein (CRP), which is one of the acute-phase proteins, is a classic marker for inflammation and is synthesized by liver cells. Previous studies showed that C-reactive protein (CRP) could be used as a predictor of chronic diseases, including diabetes and cancer (Yousuf, *et al,* 2013*)*. C-reactive protein (CRP) has also been used as a biomarker for neonatal sepsis and bacterial pneumonia, which helps in differentiating malaria from bacterial infection (Diez *et al, 2010)*. A study by Taylor suggested that the detection of increased levels of circulating acute-phase proteins may act as a useful surrogate marker of high-level parasitemia (Taylor-Robinson, 2000). Studies have also demonstrated the strong binding of C-reactive protein (CRP) to infected erythrocytes, which activates the complement pathway leading to erythrocyte clearance and hemolysis, which is the causative factor of anemia, one of the severe manifestations of malaria (Ansar *et al,* 2006). Inflammation is one of the body’s immune responses to internal or external stimuli.

Pregnancy malaria (PM) is linked to a proinflammatory immune response characterized by elevated levels of cytokines and chemokines, such as C-reactive protein (CRP), tumor necrosis factor (TNF–α) and interferon-γ (Fried and Misore, 1998). CRP is an acute phase protein produced by the liver. It is involved in the process of immune activation in infected red blood cells by binding infected erythrocytes and helping to clear the infection. Usually, the blood containing C-reactive protein (CRP) level is not elevated except when inflammation occurs. Studies have shown that C-reactive protein (CRP) is an effective biomarker for assessing malaria severity.  In highly endemic regions where usual disease symptoms like fever may be absent, even in patients with high parasite loads (Rudrajit, et, al., 2012). It has long been acknowledged that populations living in malaria-endemic areas generally live under socioeconomic conditions that lead to poor nutritional status. The groups at the highest risk of the adverse effects of malaria, (children, and pregnant women) are also most affected by poor nutrition. Although it has been suspected that nutrition may influence susceptibility to disease or alter its course, (Shankar 2000, Genton *et al* 1998). Therefore, in regard to the crucial role of nutrition in pregnancy, there is a need to study the relationship between inflammation and malaria infection in pregnant women.

**MATERIALS AND METHOD**

This was a cross-sectional study designed in which the subject is recruited and samples were collected at a certain time. Data were collected during antenatal visits from pregnant women who gave their consent to participate in the research. Screening was conducted to determine the eligibility of participants based on the inclusion criteria for the study. The participants were sensitized to the purpose of the study and its importance by nursing staff at antenatal care (ANC). Data were collected from 263 pregnant women with malaria. Materials used in this study included MUAC tape, a centrifuge machine, a spectrophotometer, and a microtiter plate reader.

**Blood Samples Collection**

Blood samples were collected using standard procedures as described by the World Health Organization (WHO, 2010). 5mls of blood was collected by certified laboratory technicians working in the hospital facility. The blood sample was transferred into an ethylenediaminetetracetic acid (EDTA bottles). 3.5mls of each sample was taken for analysis of malaria infection, while the remaining portion was centrifuged at 3000 rpm for 5 min, plasma was removed and stored at -10oC in a plane bottle until analysis.

**Determination of C-reactive protein (CRP) levels**

An enzyme-linked immunosorbent assay was used for the quantitative detection of human C-reactive protein in pregnant women infected with malaria. The human C-reactive protein sandwich ELISA was designed to measure the amount of target bound between matched antibody pairs. A target-specific antibody was pre-coated in the wells of the supplied microplate. Samples of serum were added to the wells and bound to the immobilized antibody. The sandwich was formed by the addition of a second (detector) antibody, and a substrate solution was added that reacted with the enzyme-antibody-target complex, which produced color change. The intensity of this color change was directly proportional to the concentration of the malaria parasite present in the serum. Data collected from this study were analyzed using SPSS BM software version 21. Assays were analyzed, and data were represented as mean ± standard deviation. Descriptive statistics were used to summarize data appropriately. Chi-square test was used to establish relationships between MUAC, PCV, hemoglobin, malaria infection, and CRP levels. A P-value of ‹0.05 was considered statistically significant.

**RESULTS**

The results of the nutritional status and inflammatory infections in pregnant women who were receiving antenatal care at the Yariman Bakura Specialist Hospital in Gusau, Zamfara, are presented as followed.

Figure 4. 3: Nutritional Status (MUAC) of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=239)

Figure 4.4: Prevalence of malaria in pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=239)

Figure 4.5: Anemia makers (PCV and Hb) of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=239)

Table 4.10: Association between Anemia makers (PCV and Hb) and Nutritional status of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=239)

|  |  |  |
| --- | --- | --- |
| Variables | PCV (%) | Hb(mg/dL) |
| Normal | Low | p-value | Normal | Low | p-value |
| **Dietary Diversity** |   |  |  |  |  |  |
| Diversified | 26(49.1) | 27(50.9) | 0.021\* | 34(64.2) | 19(35.8) | 0.049\* |
| Not diversified | 17(48.6) | 18(51.4) |  | 14(40.0) | 21(60.0) |  |
| **MUAC** |  |  |  |  |  |  |
| Severe Malnourishment | 0(0.0) | 2(100) | 0.001\* | 2(100) | 0(0.0) | 0.061 |
| Moderate Malnourishment | 2(20.0) | 2(80.0) |  | 6(60.8) | 4(40.0) |  |
| Normal | 32(49.2) | 33(50.8) |   | 38(58.5) | 27(41.5) |   |

Table 4:11: pHLD and CRP levels of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=54)

|  |  |  |
| --- | --- | --- |
| Variables | Frequency | Percentage (%) |
| **pHLD(mg/dL)** |
| Normal | 15 | 28.6 |
| High | 39 | 71.4 |
| *Mean* | *3.6±1.9* |
| **CRP (mg/dL)** |
| Normal | 10 | 18.5 |
| Minor Elevation | 7 | 13.0 |
| Moderate Elevation | 17 | 31.5 |
| Marked Elevation | 13 | 24.1 |
| Severe Elevation | 7 | 13.0 |
| *Mean* | *15.6±12.33* |

Table 4:12: Association between pLDH and nutritional status of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=54)

|  |  |
| --- | --- |
| Variables | pLDH(mg/dL) |
| Normal (%) | High (%) | p-value |
| Dietary Diversity | Diversified | 27.3 | 72.7 | 0.349 |
| Not diversified | 50.0 | 50.0 |  |
| MUAC | Severe Malnourishment | 0.0 | 0.0 | 0.348 |
| Moderate Malnourishment | 25.0 | 75.0 |  |
| Normal | 40.0 | 60.0 |  |
| PCV (%) | Normal | 66.7 | 33.3 | 0.016\* |
| Low | 0.0 | 100.0 |  |
| Hb(mg/dl) | Normal | 66.7 | 33.3 | 0.016\* |
| Low | 0.0 | 100.0 |   |

\* Chi-square statistics are significant at the .05 level.

Table 4:13: Association between CRP levels and nutritional status of pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=54)

|  |  |
| --- | --- |
| Variables | CRP(mg/dL) |
| Normal | Elevated | p-value |
| Dietary Diversity | Diversified | 14.3 | 48.5 | 0.021\* |
| Not diversified | 26.3 | 73.7 |  |
| MUAC | Severe Malnourishment | 0.0 | 100 | 0.042\* |
| Moderate Malnourishment | 0.0 | 100 |  |
| Normal | 21.1 | 78.9 |  |
| PCV (%) | Normal | 30.2 | 69.8 | 0.001\* |
| Low | 11.1 | 88.9 |  |
| Hb(mg/dl) | Normal | 27.3 | 72.7 | 0.001\* |
| Low | 9.1 | 90.9 |   |

\*The Chi-square statistic is significant at the .05 level.

Table 4.14: Association between CRP, pLDH, PCV, and Hb in malaria-positive pregnant women receiving antenatal care at Yariman Bakura Specialist Hospital, Gusau, Zamfara state (n=54)

|  |  |
| --- | --- |
| Variables | CRP(mg/dL) |
| Normal(%) | Elevated(%) | p-value |
| pLDH | Normal | 86.7 | 13.3 | *0.011\** |
| High | 20.5 | 79.5 |  |
| PCV(%) | Normal | 30.2 | 69.8 | *0.001\** |
| Low | 11.1 | 88.9 |  |

Chi-square test is significant at the 0.05 level, pLDH:, PCV: Pack Cell Volume: Hb: Hemoglobin CRP: C-reactive protein

**DISCUSSION**

A total of 239 pregnant women attending antenatal care in Yariman Bakura Specialist Hospital, Gusau, Zamfara, were recruited for this study. MUAC is a measure of nutritional status, a thinner arm indicating wasted lean mass. The MUAC range ranged from > 25cm to < 23cm. MUAC range used was 29.75 to >25cm for normal, <25 to >23cm for moderate and <23cm for severe malnourishment. (82.9%) of the women had normal MUAC, and the remaining (27.1%) ranged between moderate to severe malnourishment, which is similar to a work reported by (Ahshanul, et, al., 2021). (2.9%) of the pregnant women were severely malnourished, and it is a potential health concern. This category indicates a critical level of malnutrition that poses significant risks to both mothers and their unborn babies.

According to work carried out by the Nigeria Millennium Development Goals Information System, Zamfara state recorded a high prevalence of malaria parasitemia at about (37%) and in our study, out of the 239 women enrolled, a malaria prevalence of (23%) (n= 54) was recorded, which is in contrast with the Nigeria Millennium Development Goals Information System report (DHS, 2021). The variation in prevalence is possibly due to the season, which is similar to our study, between the months of February and April, which falls into the low peak season for malaria (DHS, 2021).

The total number of women who tested positive for the plasmodium parasite via microscopy was 54. Blood samples were analyzed to determine the level of the malaria marker plasmodium lactate dehydrogenase (pLDH). This finding showed that the majority of pregnant women (71.4%) who tested positive had elevated plasmodium lactate dehydrogenase (PLDH) levels, which is a confirmatory test for the presence of the parasite. C-reactive protein levels were typical among all recruited pregnant women. Only (13%) of the patients had a minor elevation. This is an indication of a high level of inflammation, which consequently increases the severity of the infection, which is similar to a study conducted by (Linlin, et, al., (2017).

Women with higher level of plasmodium lactate dehydrogenase (PLDH) also showed elevated level of C-reactive protein (CRP), signifying more severe inflammation in this category. The severity of inflammation is therefore linked to the presence of the plasmodium parasite. The level of inflammation among women with severe malnourishment (100%) tended to be higher well nourished, (78,9%). This finding highlights the effect of severe malnutrition on inflammation, as reviewed in previous studies (Franceschi, and Campisi, 2014, Ferrucci, and Fabbri, 2018).

Packed cell volume was also markedly associated with inflammation. Women with low packed cell volume (PCV) had higher inflammatory marker levels than those with normal packed cell volume (PCV) level. This represents a potential health challenge. The findings in our study are in line with a study reported by (Ahshanul, et, al., 2021)

**Conclusion**

The findings of this study reflect the significant prevalence of undernutrition among pregnant women. This could lead to an increase in anemia, a higher risk of intrauterine growth restriction (IUGR), impaired immune function, and infant stunts within the first few months after birth. Therefore, we can conclude that poor nutritional status affected the level of inflammation among pregnant women receiving antenatal care at the Yariman Bakura Specialist Hospital in Zamfara State.

REFERENCES

Ahshanul, H, M, d, Nuzhat, C, Fahmida, D, F,Mohammad, A,Mohammad, J, R,Tanvir, A, S. M,Sheikh, S, R,Towfida, J. S.,Abu, S, G, F,and Tahmeed, A., (2021). Determinants of maternal low mid‐upper arm circumference and its association with child nutritional status among poor and very poor households in rural Bangladesh., Maternal Child Nutrition, Published online 2021 May 20. doi: [10.1111/mcn.13217](https://doi.org/10.1111/mcn.13217). 17(4): e13217.

Ansar, W., Bandyopadhyay, S. M., Chowdhury, S., Habib, S. H., and Mandal, C., (2006). Role of C-reactive protein in complement-mediated hemolysis in malaria. *Glycoconj Journal.*2006;23:233–40.

Clark, I, A., (2006). Suggested importance of monokines in the pathophysiology of endotoxin shock and malaria. Klin Wochenschr. 2006, 60: 756-758.

Dozie, U. Ekeh, N. Iwuoha, G. Nwaokoro, C. , Asuzu, N. and Dozie, I. (2020). The efficacy of rapid diagnostic test in the Diagnosis of Malaria among adults as compared to microscope in a Hospital in Imo State, South Eastern Nigeria.

DHS, (2021). Nigeria Malaria Indicator Survey. Final Report National Malaria Elimination Program Abuja, Nigeria National Population Commission Abuja, Nigeria. Maryland, USA, November 2022

Fried, M., Muga, R.O., and Misore, A.O. (1998). Duffy PE. Malaria elicits type 1 cytokines in the human placenta: IFN- and TNF- are associated with pregnancy outcomes. *Journal of Immunology*. 1998 Mar 1;160(5):2523-30. PMID: 9498798.

Ferrucci, L, and Fabbri, E., (2018). Inflammageing: chronic inflammation in aging, cardiovascular disease, and frailty. *Nat Rev Cardiol.*2018;15(9):505–522.

Franceschi, C, and Campisi, J., (2014). Chronic inflammation (Inflammaging) and its potential contribution to age-associated diseases. *Journal of Gerontology. A Biological Science of Medical Sciences.*2014;69(S1):S4–S9.

Genton, B., Al-Yaman, F., Ginny, M., Taraika, J, and Alpers, M, P., (1998). Relationship between anthropometry and malaria morbidity and immunity in Papua New Guinean children. America Journal of Clinical Nutrition. 1998 Sep;68(3):734-41. doi: 10.1093/ajcn/68.3.734. PMID: 9734755.

Linlin, C., Huidan, D., Hengmin, C., Jing, F., Zhicai, Z., Junliang, D., Yinglun, L,  Xun, W, and Ling Z., (2017). Inflammatory responses and inflammation-associated diseases in organs., Published online 2017 Dec 14. doi: [10.18632/oncotarget.23208](https://doi.org/10.18632/oncotarget.23208). 9(6): 7204–7218.

Pahwa, R., Goyal, A, and Jialal, I., (2023). Chronic Inflammation. [Updated 2023 Aug 7]. In: StatPearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2023. PMID: **29630225**. Bookshelf ID: [NBK493173;](http://www.ncbi.nlm.nih.gov/books/nbk493173/)

[Rudrajit, P.](https://pubmed.ncbi.nlm.nih.gov/?term=Paul%20R%5BAuthor%5D), [Pradip, K., Sinha](https://pubmed.ncbi.nlm.nih.gov/?term=Sinha%20PK%5BAuthor%5D), [R., Bhattacharya](https://pubmed.ncbi.nlm.nih.gov/?term=Bhattacharya%20R%5BAuthor%5D), [Amit, K., Banerjee](https://pubmed.ncbi.nlm.nih.gov/?term=Banerjee%20AK%5BAuthor%5D), [Pradip, R.,](https://pubmed.ncbi.nlm.nih.gov/?term=Raychaudhuri%20P%5BAuthor%5D) and [Jayati, M.,](https://pubmed.ncbi.nlm.nih.gov/?term=Mondal%20J%5BAuthor%5D) (2012). Study of C-reactive protein as a prognostic marker in malaria from Eastern India. Advance Biomed Res 2012; 1: 41. Published online on August 28, 2012. doi: [10.4103/2277-9175.100140](https://doi.org/10.4103/2277-9175.100140)

Shomyseh S, Lauren, A, Z, Masahito, K, and Richard, A. F., (2010). Anti- and Pro-inflammatory Roles of TGF-β, IL-10, and IL-22 In Immunity and Autoimmunity. 2010; Published online 2009 May 29. doi: [10.1016/j.coph.2009.04.008](https://doi.org/10.1016/j.coph.2009.04.008). [9(4): 447–453.](https://www.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&retmode=ref&cmd=prlinks&id=19481975)

Shankar, A, H., (2000). Nutritional modulation of malaria morbidity and mortality. J Infect Dis. 2000;182 Suppl 1:S37-53. doi: 10.1086/315906. PMID: 10944483.

Taylor-Robinson, A. W. (2000). Increased production of acute phase proteins corresponds to peak parasitemia in primary malaria infection. *Parasitology international*, *48*(4), 297–301.

WHO, (2010). Guidelines on drawing blood: best practices in phalebotomy. Geneva: World Health Organization; 2010. 3 blood sampling systems. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK138666/>

Yousuf, O., B. D. Mohanty, S. S. Martin, P. H. Joshi, M. J. Blaha, K. Nasir, R. S. Blumenthal, and M. J. (2013). High-sensitivity C-reactive protein and cardiovascular disease: a resolute belief or an elusive link?. *Journal of the American College of Cardiology*, *62*(5), 397–408. https://doi.org/10.1016/j.jacc.2013.05.016

Zhang, X., Wu, X., Hu, Q., Wu, J., Wang, G., Hong, Z., Ren, J., & Lab for Trauma and Surgical Infections (2019). Mitochondrial DNA in liver inflammation and oxidative stress. *Life Sciences* *236*, 116464. <https://doi.org/10.1016/j.lfs.2019.05.020>