DOI: https://doi.org/10.61841/xhkt8k29 Publication URL: https://nnpub.org/index.php/PBS/article/view/2204

LEVELS OF C-REACTIVE PROTEIN AND TOTAL PROTEIN IN MALARIA AND HIV CO INFECTED PREGNANT WOMEN, ATTENDING THE ANTENATAL CLINIC OF NNAMDI AZIKIWE UNIVERSITY TEACHING HOSPITAL, NNEWI, SOUTHEASTERN NIGERIA.

[1] Ezeugwunne I.P., [1] Emeka G.C., [1] Ugwu C.E., [1] Oguaka V.N.

1. Department of Human Biochemistry, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria.

Corresponding Author: Ip.ezeugwunne@unizik.edu.ng

ABSTRACT: *The goal of the study was to quantify the levels of C reactive protein and protein in pregnant HIV-positive malaria-infected women. Pregnant women in HIV stages 1 and 2 who were 18 to 40 (36.98 ± 5.49) years old participated in the study. The participant groups consisted of 80 HIV-positive pregnant women with 40 co-infected with malaria and 80 HIV-negative pregnant women with 40 co-infected with malaria. Blood samples were obtained from participants for the thick and thin film methods of counting and identifying malaria parasites and the immunochromatographic method of determining HIV status. The total protein content of the serum was determined using the Biuret method, and the violet-coloured solution that was produced was measured spectrophotometrically at 540 nm. The Enzyme linked Immunosorbent assay using the Human CRP kit method was employed to measure the level of CRP in the participants' blood sample and the developed yellow coloured solution read at 450 nm using a spectrophotometer. In all cases, there was a statistically significant difference in protein and CRP levels between the HIV seropositive group with malaria infection ($p<0.05$) and HIV seropositive group without malaria infection. The mean protein and CRP level of HIV-positive pregnant women who have malaria infection was statistically lower than that of HIV-positive pregnant women who do not have malaria but statistically higher than those of HIV-negative pregnant women with and without malaria infection. The study suggests that pregnant HIV-positive women do experience a heightened increase in protein and CRP levels majorly due to HIV. However, malaria infection also contributes to the level of increase seen in these parameters in pregnant women with the coinfection.*

1 INTRODUCTION

Human immunodeficiency virus (HIV) and Plasmodium falciparum malaria are two of the major lethal infectious diseases in sub-Saharan Africa which can infect pregnant women with detrimental effects to both the mother and the foetus (WHO, 2008 a,b ; UNAIDS,2007). The global geographic distribution of HIV and malaria overlap, as well as the resultant rates of co-infection. The interactions between them poses major public health concerns (UNAIDS, 2007; WHO, 2007 a, b). Malaria is caused by the Protozoan parasite Plasmodium and is transmitted by female Anopheles mosquitoes. Approximately 1.2 billion people are at risk for malaria infection, leading to 500 million infections and more than 1 million deaths every year. There are 39 million people living with HIV worldwide, with 25.6 million in sub-Saharan Africa alone (WHO,2023). Malaria has been shown to increases rate of HIV disease progression and mother-to-child transmission of HIV (MTCT).

C-reactive protein, also known as Pentraxin 1, is a non-glycosylated protein in the Pentraxin family that also includes Pentraxin 2/SAP and Pentraxin 3/TSG-14. CRP is a well-known soluble pattern recognition molecule that responds to infections (Du Clos, 2013) C-reactive protein is an acute phase reactant, a protein made by the liver and released into the blood within a few hours after tissue injury, the start of an infection, or other cause of inflammation (Boras et al.,2014) . A high level of CRP in the blood is a sign that there may be an inflammatory process occurring in the body (Thiele et al.,2015). C-reactive protein normally increases during gestation Belo et al. (2005), returning to basal concentrations shortly after delivery (Cicarelli, Perroni, Zugaib, de Albuquerque and Campa, 2005). C-reactive protein also helps the body recognize the presence and severity of infections Lubell et al. (2016) and it is used as an indicator of low-grade inflammation in chronic infections and chronic diseases (Sullivan, Wong, Ndung'u, Kasprowicz and Bishai, 2015). Significantly high c-reactive protein of more than 350 milligrams per liter (mg/L) are nearly always a sign of a serious underlying medical condition (Trial, Potempa and Entman, 2016). Inflammation as a response to pregnancy condition is indicated by the increase in the levels of C reactive protein. Owing to modulation in such responses, in a pregnant woman; the immunologic cells are kind of suppressed to accommodate the foetus and avoid attack by the body immune system. This becomes a serious issue when the pregnant woman is co-infected with both malaria and HIV. Which further depletes the immune system exposing both the woman and the foetus to opportunistic infections etc which may end up killing both the mother and the foetus or cause preterm labour as well as low birth weight (Ersoy et al., 2016). Serum or plasma proteins are primarily synthesized in the liver; a smaller percentage due to immunoglobulins is produced by lymphocytes and plasma cells. Serum total protein consists of albumin, globulins, and fibrinogen (in plasma only). Proteins function to control oncotic pressure, transport substances (hemoglobin, lipids, calcium), and promote inflammation and the complement cascade. Changes in total protein levels are due mostly to changes in albumin concentration (Ter Kuile, Parise, and Verhoeff, 2004). Serum proteins play a limited role in extracellular buffering, whereas intracellular proteins play an important role in the total buffer response of the body. Of the plasma proteins, albumin is much more important than the globulins. The buffer value of albumin is 0.12 to 0.14 mmol/g/pH unit, whereas that of globulins is 0 to 0.08 mmol/g/pH unit. Studies show that a high serum protein indicates an inflamatory disease condition such as HIV etc (Osuji et, al.,2012). The albumin/ globulin ratio serves as a preliminary diagnostics step. A total protein and albumin/globulin (A/G) ratio test measure the total amount of protein in the blood. There are two major types of protein in the blood:

- Albumin, which helps keep blood from leaking out of blood vessels. It also helps move hormones, medicines, vitamins, and other important substances throughout the body. Albumin is made in the liver.
- Globulins, which help fight infection and move nutrients throughout the body. Some globulins are made by the liver. Others are made by the immune system. (Ter Kuile, Parise, and Verhoeff, 2004).

If the total protein levels or A/G ratio results are not normal, it can be a sign of a serious health problem. This can easily occur due to reduced immunity Renia and Potter (2006.), which also enhances co-infections such as Malaria especially in places of high endemic transmission (Ter Kuile, Parise, and Verhoeff, 2004).

2. MATERIALS AND METHODS

A random selection was made of 160 pregnant women, ages 18 to 40, who visited the antenatal clinic of the Nnamdi Azikiwe University Teaching Hospital in Southeastern Nigeria who were between 12 and 30 weeks gestation. The participants were split up into 1 control group and 3 test groups, as shown below: There were 40 HIV seropositive pregnant women with malaria infection, 40 pregnant women who were solely HIV seropositive, 40 pregnant HIV seronegative women with malaria infection and 40 pregnant HIV seronegative women without malaria infection were in the control group. Using a one-step pregnancy test strip made by ACON Laboratories Inc. in the USA, research participants had their urine tested for Human Chorionic Gonadotropin (HCG) to determine whether they are pregnant.

MALARIA PARASITES COUNT AND PLACENTAL MALARIA DETECTION

Zakama, Ozarslan, and Gaw, S.L (2020), described the procedure collecting samples for Placental malaria detection. The placental malaria parasite analysis was done using a 5ml sample of heparinized maternal venous blood. Peripheral maternal venous blood samples were collected (with or without symptoms suggestive of malaria). "Symptomatic" patients were defined as those with asexual forms of Plasmodium spp. on a blood smear and with fever, chills, headache, and/or joint pain.

The Gold Standard was used to screen for the malaria parasite. This entails examining blood smears stained with giemsa under a microscope (WHO, 2008). As soon as blood was collected, duplicate thin and thick blood films were produced on the same slide and suitably labelled. Thin smears were used to identify species, whereas thick films were utilised to identify malaria parasites and measure parasite density. For thick films, 12 µl of blood was spread over a diameter of 15 mm, while 2 µl of blood was used for thin films. Following a brief fixation in 100% methanol, the thin film was allowed to air dry. The slides were appropriately labelled, and the blood films were stained with 3% Giemsa stain after 24 to 48 hours. X100 oil immersion microscope was used to do a microscopic examination using a 3% Giemsa dye solution at pH 7.2. The dipstick fast chromatographic immunoassay, which can identify pan-Plasmodium aldolase antigens and proteins unique to Plasmodium falciparum up to two weeks after the infection has resolved, was used for confirmation. The test strip shows two different coloured bands, which indicates the presence of the malaria parasite. A negative outcome is shown by one line in the control region and another line in the test region (T). Smears of placental blood was analysed to check for pigment and parasites.

PARASITES COUNT

On thick films stained with giemsa, the density of malaria parasites in the placenta and peripheral regions was measured in comparison to 200 white blood cells (WBC) (Adu-Gyasi., 2012). When the number of parasites on the slide was less than 200, it was deemed positive and negative after 200 high-power fields were examined. Women who have placental malaria are classified as having mild parasitaemia if there are less than 20,000 parasites/µl, or severe parasitaemia if there are more than 20,000 parasites/µl. Peripheral and/or placental parasitaemia after delivery was referred to as malaria parasitaemia at delivery.

HIV DETECTION

After pre-test counselling WHO (2012), and obtaining informed consent, sterile blood lancets were used to take a finger-prick blood sample from each participant. Two rapid in vitro test kits were used to screen for HIV-1/2 antibodies in the subjects' blood samples in accordance with the manufacturer's instructions: GENIE–II (Sanofi, Pasteur, France), and determine HIV-1 and HIV–2(Abbot Laboratories Japan), which is an immunochromatographic test used for the qualitative detection of antibodies to HIV-1 and HIV–2. To identify HIV positive samples, a 95% confidence interval between the two tests was developed. A positive result on both quick tests was therefore considered to be an indication of HIV infection. Subjects who tested positive for HIV were classified in accordance with World Health Organisation (WHO) criteria for classifying HIV patients into asymptomatic (stage-1) and symptomatic (stage-2) states (WHO,2007). Participants in stages three and four were not allowed to continue with the study.

TOTAL PROTEIN ESTIMATION

Serum protein reacts with cupric ion in an alkaline medium to produce a violet color (Biuret's method). The intensity of the colour, which has maximum absorption at 540 nm, is proportional to the protein concentration (Busher,1990). Fresh, clear, haemolyzed serum gotten from the 5ml of the participants blood was used along with Sodium chloride 0.9% solution (Saline solution), Stock Biuret reagent. Biuret solution and 4- Standard protein solution (Bovine albumin 6 to 7 g/dl). 45g of Rochelle salt was dissolved in about 400 ml of 0.2N NaOH and 15g of $CuSO_4$ added wile stirring continuously. 5g of potassium iodide (KI) was added and made up to 1Liter with 0.2N NaOH to get stock biuret reagent. 200ml of the stock reagent was diluted to 1Liter with 0.2N NaOH which contains 5g of potassium iodide per Liter to obtain biuret solution to use. I Pipette 0.1 ml of serum into the test tube labelled test. Pipette 0.1 ml of standard into the test tube labelled standard. Pipette 2.9ml of 0.9% saline solution into the three test tubes. Pipette 3ml of biuret solution into the three test tubes. I mixed then well and put in water bath (37°C) for 10 minutes. I Read the violet colour that developed at 540 nm.

CALCULATIONS

$$\text{Concentration Of Total Protein (g/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Conc. of standard (g/dl)}}{1}$$

Normal range: 6.0 - 8.2 g/dl.

C-REACTIVE PROTEIN ESTIMATION

The amount of c-reactive protein in the serum will was measured using an ELISA kit for human C-reactive protein. The enzyme immunoassay (EIA) kit for measuring human c-reactive protein (CRP) uses a double polyclonal antibody sandwich design. Sample dilution test tubes, 2-1000 mL precision pipettes, a microplate reader with a 450 ± 10 nm filter, software programme that makes data gathering and analysis easier, washer for microtitration plates, absorbent substance to wipe the microwell plate with Deionized (distilled) water, an adhesive microplate cover, and a clean 250–500 mL wash bottle for the buffer were utilised. The polyclonal anti-human CRP antibody-coated microtitration wells were used to incubate standards, quality controls, and sera samples. Following a thorough washing, the immobilised antibody-CRP complex was incubated with the wells containing polyclonal anti-human CRP antibody labelled with horseradish peroxidase (HRP). The leftover HRP-conjugated antibody was then allowed to react with the substrate and tetramethyl benzidine after going through one more washing cycle. After adding an acidic solution to halt the reaction, the absorbance of the yellow-colored product was measured at 450 nm using spectrophotometry (Takiguchi, Fujinaga, and Naiki, 1990). The absorbance and CRP concentration are proportionate. Plotting absorbance values against CRP concentrations of standards resulted in the construction of a standard curve, which was then used to calculate the concentrations of unknown samples.

STATISTICAL ANALYSIS

A statistical analysis was done on the outcome. One-way analysis of variance (ANOVA) and the Students' t test were used to compare the means. A statistically significant result was defined as $P < 0.05$. Version 13.0 of the statistical software programme; Statistical Tool for Social Sciences (SPSS) was used to conduct the analysis.

RESULT

Table 1.1: Mean ± SD of protein and c reactive protein in pregnant HIV Positive women (a) without malaria infection (b) with malaria infection (c) HIV negative with malaria infection (d) HIV negative without malaria infection.

Variables **N= 40**	**Age (years)**	**Protein** **(g/L)**	**CRP** **(mg/L)**	**Weight (Kg)**	**Height (m)**	**BMI (kg/m2)**	**Gestation (wks)**
(a) Pregnant women with HIV but without malaria infection	30.05 ± 5.00	68.28 ±6.73	8.93 ± 6.39	88.8 ±9.77	1.51 ± 4.92	39.26 ± 4.86	20.35 ± 5.76
(b) Pregnant women with HIV and Malaria infection	36.98 ±5.49	17.99 ±16.25	5.46 ±1.27	85.30 ±11.11	1.48 ±5.42	39.21 ±5.47	19.60 ±5.77
(c) Pregnant women without HIV but with Malaria Infection	34.28 ±3.80	10.84 ±9.37	4.10 ±1.56	82.13 ±10.25	1.49 ±5.60	37.04 ±5.55	19.38 ±5.57
(d) Pregnant women without HIV and without malaria infection	32.83 ±3.38	13.02 ±1.21	4.42 ±1.68	81.22 ±10.48	1.51 ±0.61	35.94 ±6.47	20.45 ±6.18

F-value	16.472	205.962	16.415	4.352	3.002	3.442	0.339
P-value	0.000	0.000	0.000	0.06	0.032	0.018	0.797
A v B	0.000	0.000	0.001	0.139	0.014	0.964	0.663
A v C	0.000	0.000	0.000	0.004	0.256	0.060	0.444
A v D	0.05	0.000	0.000	0.001	0.731	0.011	0.941
B v C	0.012	0.018	0.000	0.188	0.205	0.082	0.861
B v D	0.000	0.141	0.002	0.096	0.011	0.017	0.527
C v D	0.075	0.404	0.378	0.699	0.178	0.420	0.416

Statistically significant at $p<0.05$.

Key:

F (p): ANOVA was used to compare pregnant women with malaria infection and HIV seropositive vs pregnant women with malaria infection and HIV seronegative

AvB: Using the student t test, pregnant HIV-positive women without malaria infection and pregnant HIV-positive women with malaria infection were compared.

AvC: Using the student t test, pregnant HIV seropositive women without malaria infection were compared to pregnant HIV seronegative women with malaria infection.

AvD: The student t test was used to compare pregnant HIV seropositive women who were free of malaria and pregnant HIV seronegative women who were free of malaria.

BvC: Using the student t test, pregnant HIV seropositive women with malaria infection were compared to pregnant HIV seronegative women with malaria infection.

BvD: Pregnant HIV-positive women who were infected with malaria and pregnant HIV-negative women who were not infected with malaria were

CvD: The student t test was used to compare pregnant HIV-negative women who were infected with malaria to pregnant HIV-negative women who were not infected with malaria.

Table 1.1 compared the mean $\pm$ standard deviation of the levels of C reactive protein and protein in pregnant HIV-positive women with and without malaria to pregnant HIV-negative women with and without malaria. The results show that there is a statistically significant difference ($p<0.05$) in the levels of protein and C reactive protein between the test groups and the control group. There was a statistically significant difference ($P<0.05$) between the groups when comparing the mean + SD protein and c reactive protein levels. The mean $\pm$ SD protein level in pregnant women infected with HIV plus malaria (17.99 $\pm$16.25) was significantly lower than that of individuals infected with HIV without malaria (68.28 $\pm$6.73), according to a comparison within the group. The mean + SD protein level in pregnant HIV seropositive women with malaria infection was found to be higher than those of pregnant HIV seronegative women with malaria infection (10.84 $\pm$ 9.37) and pregnant HIV negative women without malaria (13.02 $\pm$ 1.21). Similarly, mean $\pm$ SD CRP levels in HIV-positive pregnant women with malaria infection (5.46 $\pm$1.27) were shown to be statistically lower than those in HIV-positive participants without malaria (8.93 + 6.39). However, the CRP level in pregnant HIV-positive women with malaria was significantly higher than those of pregnant HIV seronegative women with malaria infection (4.10 $\pm$1.56) and pregnant HIV negative women without malaria (4.42 $\pm$1.68).

DISCUSSION

The result of this study demonstrates that pregnant HIV-positive women who have malaria have significantly lower protein levels than pregnant HIV-positive women who do not have malaria. Nonetheless, a significant increase in the mean $\pm$ SD protein level was seen when comparing pregnant HIV seropositive women with malaria infection to those of pregnant

HIV seronegative women with and without malaria infection. This is consistent with the findings of earlier studies (Osuji, et al., 2012). According to this study, pregnant women with HIV experienced higher levels of inflammation than those with malaria. This conclusion was drawn considering the noticeably higher protein levels seen in pregnant HIV-positive individuals who did not have malaria than in those who did. Additionally, it was demonstrated that pregnant HIV-positive women who also have malaria, have lower levels of CRP than pregnant HIV-positive women who do not have malaria. When compared to pregnant HIV seronegative women with and without malaria infection, the results show that pregnant HIV seropositive women with malaria have a higher CRP level. This is consistent with the results of earlier studies (Thiele et al., 2015; Epelboin et al., 2012). Conditions that cause inflammation leads to rise in CRP as an inflammation marker. The rise in C reactive protein level is indicative of inflammation in response to pregnant conditions (Du Clos, 2013). Pregnant HIV-positive women without malaria infection had higher levels of CRP when compared to pregnant HIV seropositive women with malaria infection and pregnant HIV seronegative women with and without malaria. The higher level of CRP seen in pregnant HIV seropositive women without malaria infection suggests that HIV is primarily to blame for pregnant women's increased inflammation. Worthy of note is that malaria also plays a role in pregnant women's inflammatory response, as evidenced by the fact that pregnant HIV-negative women who have malaria have greater levels of CRP than pregnant HIV-negative women who do not have malaria (control).

REFERENCES

[1]. Adu-Gyasi D, Adams M, Amoako S, Mahama E, Nsoh M, Amenga-Etego S, Baiden F, Asante KP, Newton S, Owusu-Agyei S. Estimating malaria parasite density: assumed white blood cell count of 10,000/µl of blood is appropriate measure in Central Ghana. Malar J. 2012 Jul 23;11:238. doi: 10.1186/1475-2875-11-238. PMID: 22823983; PMCID: PMC3411500.

[2]. Belo L, Santos-Silva A, Rocha S, Caslake M, Cooney J, Pereira-Leite L (2005). Fluctuations in c-reactive protein concentration and neutrophil activation during normal human pregnancy. *Eur J Obstet Gynecol Reprod Biol*, 123(1), 46–51.

[3]. Boras E, Slevin M, Alexander M .Y, Aljohi A, Gilmore W, Ashworth J (2014). Monomeric c-reactive protein and Notch-3 co-operatively increase angiogenesis through PI3K signalling pathway. *Cytokine* 69,165–79.

[4]. Busher J.T (1990). Serum Albumin and Globulin; In Walker HK, Hall WD, Hurst JW (eds.). Clinical methods : the history, physical, and laboratory examinations (3rd ed.) Chapter 101: Boston: Butterworths. ISBN 978-0409900774.

[5]. Cicarelli L.M, Perroni A.G, Zugaib M, de Albuquerque P.B, Campa A (2005). Maternal and cord blood levels of serum amyloid A, C-reactive protein, tumor necrosis factor-alpha, interleukin-1Beta, and interleukin-8 during and after delivery. *Mediators Inflamm*. 2, 96–100.

[6]. Du Clos T.W. Pentraxins (2013): Structure, function, and role in inflammation. *ISRN Inflamm*. 2013, 37-40.

[7]. Epelboin L, Hanf M, Dussart P, Ouar-Epelboin S, Djossou F, Nacher M (2012). Is dengue and malaria co-infection more severe than single infections? A retrospective matched-pair study in French Guiana. *Malar J,* 11,142-46.

[8]. Lubell Y, Althaus T, Blacksell SD, Paris DH, Mayxay M, Pan-Ngum W (2016). Modelling the impact and cost-effectiveness of biomarker tests as compared with pathogen-specific diagnostics in the management of undifferentiated fever in remote tropical settings. *PLoS One*. 11(3), e0152420.

[9]. Osuji F.N, Onyenekwe C.C, Ifeanyichukwu M, Ahaneku J.E, Ezeani M, Ezeugwunne I.P (2012)**.** Antioxidant activity in HIV and malaria co-infected subjects. *Asian Pac J Trop Med*. 7,45-50.

[10]. Renia L, Potter (2006). Co-infection of malaria with HIV: an immunological perspective, *Parasite Immunology*, 28 (11), 589–595.

[11]. Takiguchi M, Fujinaga T, Naiki, M (1990): Isolation, characterization, and quantitative analysis of c-reactive protein from horses. *Am J Vet Res* 51:1215–1220.

[12]. Ter Kuile F. O, Parise M. E, Verhoeff F. H (2004). The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene*, 71 (1): 41–54.

[13]. Thiele J.R, Zeller J, Bannasch H, Stark G.B, Peter K, Eisenhardt S.U (2015). Targeting C-reactive protein in inflammatory disease by preventing conformational changes. *Mediators Inflamm.* 2015,372-432.

[14]. Trial J, Potempa LA, Entman ML (2016). The role of C-reactive protein in innate and acquired inflammation: new perspectives. *Inflamm Cell Signal* 3(2), e1409.

[15]. UNAIDS (2007). AIDS epidemic update, Geneva, Switzerland,.

[16]. World Health Organization (2007), *HIV Clinical Trials*. 8(4), 246–53.

[17]. World Health Organization (2007), United Nations Programme on AIDS. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector: progress report. April, Geneva. ISBN 978. 924595391.

[18]. World Health Organization (2007), United Nations Programme on AIDS. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector: progress report. April, Geneva. ISBN 978. 924595391.

[19]. World Health Organization (2008). World Malaria Report 2008, WHO Press, Geneva, Switzerland.

[20]. World Health Organization (2008). HIV/AIDS in the South-East Asia region. March. New Delhi, WHO, Regional Office for South-East Asia.

[21]. World Health Organization (2010). Recommendations on the diagnosis of HIV infection in infants and children. Geneva, WHO.

[21]. World Health Organization (2012). Couples HIV testing and counselling including antiretroviral therapy for treatment and prevention in serodiscordant couples. Geneva.

[22]. World Health Organization (2020), World Malaria Report. Geneva.

[23]. World Health Organisation (2023), world malaria report. Geneva

[24]. Zakama, A.K., Ozarslan, N. Gaw, S.L (2020). Placental Malaria. Curr Trop Med Rep 7, 162–171 . https://doi.org/10.1007/s40475-020-00213-2