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Level Of Albumin in Malaria and HIV Co Infected Pregnant Women, Attending The Antenatal Clinic Of Nnamdi Azikiwe University Teaching Hospital, Nnewi, South Eastern Nigeria

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Abstract: *The purpose of the study was to measure the amount of albumin in pregnant HIV-positive malaria-infected women. Participants in the study were 18 to 40 (36.98 ± 5.49) years old, HIV stage 1 and 2 seropositive pregnant women. 80 HIV-positive pregnant women with 40 co-infected with malaria and 80 HIV-negative pregnant women with 40 co-infected with malaria comprised the participant groups. Blood samples were obtained from the subjects for malaria parasite identification using the thick and thin film methods, and HIV status determination using the immunochromatographic approach. The bromocresol green albumin binding method was used to measure the amount of albumin, and the resultant solution was measured spectrophotometrically at 628 nm. In all cases, there was a statistically significant difference in albumin levels between the HIV seronegative group with and without malaria infection ($p<0.05$) and HIV seropositive group with malaria infection. The mean albumin level of HIV-positive pregnant women who have malaria infection was statistically higher than that of HIV-positive pregnant women who do not have malaria but statistically lower than those of HIV-negative pregnant women with and without malaria infection. The study suggests that pregnant HIV-positive women do not experience a decrease in albumin levels because of malaria infection.*

1. INTRODUTION

One kind of protein present in human blood is called albumin. About half of serum protein is made up of this protein, which is the most prevalent in human blood plasma. 25% of the proteins that the liver synthesises are made up of it (Haefliger, Moskaitis, Schoenberg and Wahli, 1989). Mutlu, Mutlu and Keshavarzinent (2006), describe it as a highly soluble monomeric protein in globule form that is soluble in water. Among its many roles, albumin buffers pH, carries hormones, fatty acids, and other substances, and keeps the oncotic pressure constant (Haefliger *et al.* 1989 . Malaria and the HIV/AIDS virus are both highly prevalent worldwide, with most cases occurring in Sub-Saharan Africa, Southeast Asia, and the Indian Subcontinent. The global geographic distribution of HIV and malaria, as well as their co-infection rates overlap. Major public health problems are raised by these interactions (World Health Organisation (WHO), 2007 a, b; United Nations Programme on HIV/AIDS (UNAIDS), 2007). UNAIDS (2007) reports that HIV and malaria together were responsible for over 3 million fatalities in 2007. HIV/AIDS and malaria are more common in the impoverished population (USSAID,2022). Female Anopheles mosquitoes carry the Plasmodium protozoan parasite, which causes malaria. In the majority of the world's tropical and subtropical regions, it is endemic. Humans can contract parasite infections from four different species: Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, and Plasmodium ovale (Kublin, Patnaik, and Jere 2005). Plasmodium falciparum is the most pathogenic of the parasites (*Osuji et al.*, 2012). Most of the malarial morbidity and mortality are caused by this deadliest parasite, Plasmodium falciparum. An estimated 1.2 billion people are susceptible to malaria infection, which causes 500 million cases and over 1 million fatalities annually (WHO, 2008). According to the latest World malaria report, there were 249 million cases of malaria in 2022 compared to 244 million in 2021. The estimated number of malaria deaths stood at 608 000 in 2022 compared to 610 000 in 2021 (WHO, 2023). Normal serum albumin concentrations is within the reference range of 35–50 g/L (3.5–5.0 g/dL). Its half-life in serum is around 21 days. 66.5 kDa is its molecular mass (Li, Cao, and Geng, 2017). Mutations in the albumin gene, which is found at location 4q13.3 on chromosome 4, can produce abnormal proteins. It aids in maintaining blood fluid levels and is produced by the liver. Low albumin levels may be a sign of renal or liver disease (Busher, 1990). Among the main plasma proteins, albumin and C-reactive protein are special in that they don't include any carbohydrate residues (Yu, *et al*., 2021). According to Yu *et al*. (2021), many different compounds, especially lipid-soluble anions, can be bound by albumin. At a normal concentration of 1-2 molecules per albumin molecule, most of the long-chain fatty acids in plasma that are in circulation are bound to a hydrophobic site by albumin. (He and Carter ,1992).

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 South Eastern 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.

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.

MALARIA PARASITES COUNT AND DETECTING PLACENTAL MALARIA

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 will be 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. X 100 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.

ALBUMIN ESTIMATION

One well-known property of albumin is its capacity to bind a wide range of chemical substances, including organic colours. The maximum absorbance of bromocresol green (BCG) is altered when albumin binds to it (Jean and Payne, 1988). The amount of albumin present can be ascertained by spectrophotometrically measuring this change. Reagent blank: a spectrophotometer zeroing solution (set to 100% T); Enough 13 x100 mm test tubes were labelled for all samples. Other materials were controls, calibrators, and reagent blanks. I pipette 2.5 millilitres of BCG reagent into each tube using a 5-millilitre serological pipette. Next, I pipette 10 µL each of calibrator, control, or sample into the relevant tubes. The reagent blank tube was filled with 10 µL of distilled water. Every tube was mixed by inversion. Each tube's absorbance was measured at 628 nm against the reagent blank, and the outcomes was noted on the data sheet. Using the absorbance/concentration proportion method, the albumin concentration of each sample was determined in g/L.

$$\text{Albumin concentration} = \frac{\text{Absorbance of Standard}}{\text{Absorbance of unknown}} \times \text{Standard concentration (mg/dl)}.$$

STATISTICAL ANALYSIS

The result was analysed statistically. The means were compared using the Students't-test and one-way analysis of variance (ANOVA). P <0.05 was regarded as statistically significant. The analysis was carried out using the Statistical tool for Social Sciences (SPSS) statistical software tool, version 13.0.

RESULT

Table 1.1**Mean ± SD of Albumin 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)**	**Albumin** **(g/L)**	**Weight (Kg)**	**Height (m)**	**BMI (kg/m2)**	**Gestation (wks)**
(a) Pregnant women with HIV but without malaria infection	30.05 ± 5.00	34.98 ±1.59	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	36.98±5.49	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	68.63 ±6.25	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	62.88 ±7.84	81.22 ±10.48	1.51 ±0.61	35.94 ±6.47	20.45 ±6.18
F-value	16.472	476.049	4.352	3.002	3.442	0.339
P-value	0.000	0.000	0.06	0.032	0.018	0.797
A v B	0.000	0.000	0.139	0.014	0.964	0.663
A v C	0.000	0.000	0.004	0.256	0.060	0.444
A v D	0.05	0.000	0.001	0.731	0.011	0.941
B v C	0.012	0.66	0.188	0.205	0.082	0.861
B v D	0.000	0.000	0.096	0.011	0.017	0.527
C v D	0.075	0.01	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 presented a comparison of the mean ± standard deviation of albumin levels between pregnant HIV-positive women with and without malaria infection and pregnant HIV-negative women with and without malaria infection. The outcome demonstrates that the albumin levels of the test groups and the control group differ statistically significantly at ($p<0.05$). When comparing the mean ± SD albumin levels between the groups, a statistically significant difference was observed at $P<0.05$. A comparison within the group revealed that the mean ± SD levels of albumin in pregnant women infected with HIV and malaria (36.98 ± 5.49) were considerably lower than those in participants not infected with HIV

and malaria (62.88 ± 7.84). considerably lower than those of participants who have malaria but without HIV (68.23± 6.25). The level of albumin in women with HIV and malaria infection (36.98± 5.49) was found to be statistically significantly different when compared to the level of albumin in pregnant women with HIV but without malaria (34.98± 1.59) at P<0.05. This was found to be statistically higher than the level in pregnant HIV seropositive women without malaria.

DISCUSSION

When compared to pregnant HIV seronegative women with malaria infection and pregnant HIV seronegative women without malaria infection, the level of albumin in pregnant HIV seropositive women with malaria infection was shown to be considerably lower. The results of previous studies (Goselle, Onwuliri C.O. E and Onwuliri V.A, 2007) agreed with this finding. The level of albumin in pregnant HIV seropositive women with malaria infection was found to be higher than that of pregnant HIV seropositive women without malaria infection. This demonstrates how HIV infection progressively depletes pregnant women's albumin levels, causing a noticeable drop in them (Goselle, Onwuliri C.O. E and Onwuliri V.A, 2007). However, it has been found in this study that pregnant women's albumin levels are not significantly affected by malaria infection. Previous research revealed that pregnant women with co-infections of HIV and malaria had reduced levels of albumin (Goselle, Onwuliri C.O. E, and Onwuliri V.A, 2007). This study agrees with previous research carried out on the effect of HIV on albumin levels in pregnant HIV seropositive women (Dao *et al*, 2011). The observed higher level of albumin in pregnant HIV seropositive women with malaria infection when compared to the level in pregnant HIV seropositive women without malaria infection indicates that malaria infection has negligible effect on the level of albumin in pregnant women. This shows that the observed lower level of albumin in pregnant HIV seropositive women with malaria infection when compared to that of pregnant HIV seronegative women with and without malaria is largely due to HIV and not malaria infection. Furthermore to buttress this finding, we observed that the level of albumin in pregnant HIV seronegative women with malaria is statistically higher than that of the control ; pregnant HIV seronegative women without malaria infection.

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