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Levels of Maternal Serum Alpha-fetoprotein and Beta Human Chorionic Gonadotropin in HIV Seropositive Pregnant Women Attending Antenatal Care at Nnamdi Azikiwe University Teaching Hospital Nnewi, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

HIV infection remains a worrisome pandemic especially in developing countries like Nigeria, with an increasing number of infected women becoming pregnant, with added risk of pregnancy complications such as intrauterine fetal death, neural tube defects, and vertical transmissions. Hence this study assessed the levels of maternal serum alpha-fetoprotein (MSAFP) and beta human chorionic gonadotropin (β-hCG) in HIV seropositive pregnant women, and their implication

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for maternal and fetal health. A total of 86 patients were recruited for the study from the Antenatal Clinic of Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi. Forty three were HIV seropositive (Test group), and forty three were HIV seronegative (Control group). Maternal serum alpha-fetoprotein and beta human chorionic gonadotropin levels were assayed by Enzyme Linked Immunosorbent Assay. The results showed that MSAFP levels (3.14±2.80 ng/ml) were significantly lower (P < .05) in HIV infected pregnant women when compared to HIV uninfected pregnant women (4.75±3.68 ng/ml). There was no significant difference in the levels of β-hCG (217.88±63.85mIU/mL) in the test group (P = 0.629) as compared to controls (223.75±47.71 mIU/mL). In the test group, increased CD4 counts were significantly associated with increased MSAFP and β-hCG levels in a positive fashion (P < .05). Increase in Gestational age was also significantly associated with increased β-hCG levels (P < .05).
The measurements of MSAFP and β-hCG levels may be used alongside CD4 count in the assessment of maternal and fetal wellbeing. Human Immunodeficiency Virus by reducing the levels of MSAFP in HIV infected pregnant women increases the likelihood of pregnancy complications such as intrauterine fetal death.

Keywords: Acquired immunodeficiency syndrome; alpha-fetoprotein; beta-human chorionic gonadotropin; HIV infections; pregnancy; Down syndrome.

1. INTRODUCTION

Human immunodeficiency virus infection/ Acquired immune deficiency syndrome (HIV/AIDS) is a disease of the human immune system caused by infection with HIV [1]. The virus is transmitted primarily through unprotected sexual intercourse, contaminated blood transfusions, hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding [2]. It is a retrovirus that primarily infects components of the human immune system such as CD4 T-cells, macrophages and dendritic cells [3]. It directly and indirectly destroys CD4 T-cells. Basically, HIV causes AIDS by depleting CD4 T-cells. This weakens the immune system and allows for opportunistic infections [4].

Several publications have shown that maternal HIV infection and antiretroviral treatment (ART) modify the values of the biochemical markers used for second trimester screening for Down Syndrome (i.e. β-hCG, MSAFP, and unconjugated estriol) [5]. Unexplained levels of maternal serum AFP and β-HCG are associated with an increased risk of most pregnancy complications [6]. Beta-hCG has been implicated in modifying Kaposi sarcoma in HIV positive patients and in reducing HIV load in human lymphocytes and human choriocarcinoma cells. These anti-HIV effects of β-hCG may contribute to the limited maternal-fetal transmission of HIV infection (25%-35%) without treatment [7]. Such inhibitory action of β-hCG may be present at varying levels throughout gestation based upon the circulating levels of β-hCG and its production by the placenta [8].

Human alpha-fetoprotein is a tumour associated fetal glycoprotein (oncofetal) that is associated with certain malignant neoplasms and with fetal defects during ontogenic growth and differentiation [9]. During pregnancy, AFP plays a role in the development of the embryo and fetus, and has been employed as a biomarker in fetal, prenatal, and pediatric disorders such as Beckwith-Wiedemann syndrome, Fanconi's anemia, and pancreatic blastomas [10,11]. Serum maternal AFP is first detectable in ($\approx 5\mu$g/L which is also equivalent to 5ng/mL) at about the 10thweek of gestation. The concentration increases about 15% per week to a peak of approximately 35 μ g/L [12]. The maternal serum concentration then subsequently declines slowly until term. After birth, the maternal serum AFP rapidly decreases to less than 2μg/L in an infant, serum AFP declines exponentially to reach adult concentrations by the 10th month of life [13].

Beta-Human Chorionic Gonadotropin is a pregnancy associated immunodulating hormone, which has been recently shown invitro to suppress reverse transcriptase activity in chronically infected lymphocytes and monocytes, and to block viral transmission resulting from cell-cell contact between virus carrying lymphocytes and placental trophoblasts [7]. Beta-hCG is synthesized in the syncytiotrophoblast cells of the placenta. Minute amounts are also made in the pituitary of men and non-pregnant women, and like many other pituitary hormones, it is secreted in a pulsatile fashion [14].

An increasing number of HIV-infected women are becoming pregnant [15]. Among women infected

with HIV, 80% to 90% of them are of reproductive age [16]. HIV infected pregnant women are at increased risk of most pregnancy complications such as intrauterine fetal death, harbouring babies with trisomy syndromes (involving neural tube defects), and transmitting this infection to their unborn babies [6]. In most sub Saharan African countries including Nigeria, HIV infection has become a leading medical complication of pregnancy and cause of maternal and neonatal morbidity and mortality [17]. It therefore becomes necessary to assess any possible role HIV has to play in the serum concentrations of AFP and β-hCGin HIV seropositive pregnant women.

2. MATERIALS AND METHODS

2.1 Study Design

A total of 86 pregnant women aged 18 to 50 years were conveniently recruited for this study, comprising of 43 individuals as test group (i.e. HIV infected pregnant women), and 43 as control (Apparently healthy pregnant women who were not diagnosed of any diseased condition as at the time of sample collection). The sample size was calculated from the formula: $N= [T^2 \times P(1-P)]/M^2$; where N is the sample size, T is the confidence interval of 95% (1.96), P is 6.0% and is the prevalence rate of HIV seropositive pregnant women in Nnewi Metropolis [18], M is the margin of error (5% = 0.05).

Hence $N = [1.96^2 \times 0.06(1-0.06)]/0.05^2$
Therefore N = 86.

2.2 Specimen Collection and Analytical Methods

Bio-data, and medical history – gestation age (weeks), Age (years), BMI, Parity, Systolic and Diastolic blood pressure, and CD4 count (cell/μL) were obtained from maternal health records file. BMI was calculated as weight (in kilograms) divided by the square of height (in metres). A single non-fasting blood sample was used for MSAFP and β-hCG analyses. About 5mls of blood samples were drawn from the ante-cubital space into labeled plain containers, allowed to clot, and centrifuged at 5000rpm for 5 minutes. The sera were carefully transferred into labeled plain containers, and stored frozen. The assay was done 3 weeks after collection.

2.3 Study Site

The study was carried out at the antenatal clinic of Prevention to mother-to-child transmission of HIV clinic unit of Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria. The analyses of MSAFP and β-hCG was done using ELISA method at the department of Chemical Pathology, NAUTH, Nnewi Nigeria.

2.3.1 Inclusion criteria

HIV infected pregnant women aged between 18-50 years, as well as age-matched HIV uninfected pregnant women were recruited for this study.

2.3.2 Exclusion criteria

Pregnant women with hypertension, diabetes, hepatitis B or C co-infections, tuberculosis, and other chronic systemic infections were excluded from this study.

2.4 Statistical Analysis

The data from this study were subjected to statistical analysis using SPSS package, version 23 and presented as mean ± standard deviation. Student's t-test (independent t-test) was used to test difference in mean values. The results obtained were presented in tables for clarity. The p- value ≤ 0.05 was considered statistically significant. Correlation was performed using the Pearson's correlation.

3. RESULTS

Table 1 shows the gestational age, age, BMI, parity, blood pressure, CD4 count, AFP and β-hCG in HIV positive and negative pregnant women in the study. The results are expressed in Mean±SD. There was no significant difference (P > .05) in the gestational age (in weeks), age (in years), parity, systolic blood pressure of HIV seropositive pregnant women when compared with HIV seronegative pregnant women. The diastolic blood pressure, AFP and β-hCG of HIV seropositive pregnant women was significantly lower (P < .05) when compared with HIV seronegative pregnant women.

Table 2 shows that in HIV seropositive pregnant women, that there was a very weak positive relationship between Gestational age and AFP concentration, which was not significant (P = 0.439). There was a very weak positive relationship between Gestational age and β-HCG concentration, which was significant (P = 0.014). There was also a weak positive relationship between CD4 count and AFP level,

Table 1. The levels of gestational age, age, BMI, parity, blood pressure, CD4 count, AFP and β-HCG in HIV positive and negative pregnant women (Mean±SD)

PARAMETER	HIV positive pregnant women (N = 43)	HIV negative pregnant women (N = 43)	t- value	Sig.
Gestational age (weeks)	26.02±7.77	26.45±8.49	0.245	0.807
Age (years)	30.77±3.85	29.47±4.90	-1.368	0.175
BMI (kg/m^2)	27.56±4.75	27.40±4.42	-0.162	0.872
Parity	3.20±1.42	2.53±1.32	-2.266	0.026
Systolic BP (mm/Hg)	106.27±10.43	109.81±12.09	1.454	0.150
Diastolic BP (mm/Hg)	65.84±8.30	71.02±9.63	2.672	0.010*
CD4 (cells/µL)	559.18±285.50			
AFP (ng/ml)	3.14±2.80	4.75±3.69	-2.30	0.024*
β-hCG (mIU/mL)	217.88±63.85	223.75±7.71	-0.49	0.629

*N: Number of subjects, BMI: Body mass index, BP: Blood pressure, CD4: Cluster of differentiation 4, AFP: Alpha fetoprotein, β-hCG: Beta human chorionic gonadotropin. $P \leq .05$: *significant differences between groups (Sig.)*

Table 2. Correlation between Gestation age, CD4, BMI with AFP and β-HCG

Variables	Study (N = 43)		Control (N = 43)	
	r	Sig.	r	Sig.
Gestation age vs. AFP	0.120	0.439	0.196	0.208
Gestation age vs. β-hCG	0.368	0.014*	-0.039	0.805
CD4 vs. AFP	0.349	0.047*		
CD4 vs. β-hCG	0.415	0.016*		
BMI vs. AFP	-0.036	0.814	0.122	0.435
BMI vs. β-hCG	0.090	0.562	0.137	0.383

*N: Number of subjects, BMI: Body mass index, BP: Blood pressure, CD4: Cluster of differentiation 4, AFP: Alpha fetoprotein, β-hCG: Beta human chorionic gonadotropin. $P \leq .05$: *statistically significant (Sig.), r: Pearson's correlation coefficient*

which was statistically significant (P = 0.047). Furthermore, there was a weak positive relationship between CD4 count and β-hCG concentration, which was statistically significant (P = 0.016). There was a very weak negative relationship between BMI and AFP, which was not significant (P = 0.814). Also, there was a very weak negative relationship between BMI and β-hCG, which was not significant (P = 0.090). However, in HIV seronegative pregnant women, there was a very weak positive relationship between gestational age and AFP levels, which was not significant (P = 0.208). There was also a very weak negative relationship between gestational age and β-hCG levels, which was not significant (P = 0.805). There was a very weak positive relationship between BMI and AFP, which was not significant (P = 0.435). Also, there was a very weak positive relationship between BMI and β-hCG, which was not significant (P = 0.383).

4. DISCUSSION

Human immunodeficiency virus is a retrovirus that infects and depletes components of the human immune system such as CD4 T-cells and macrophages, hence predisposing the infected individual to opportunistic infections [4]. Maternal HIV infection modify the values of the biochemical markers used for second trimester screening for Down Syndrome (i.e. β-hCG, AFP, and unconjugated estriol) [5]. Chandra et al. [6] reported that unexplained levels of maternal serum AFP and β-hCG are associated with an increased risk of most pregnancy complications. Gross et al. [19] reported elevations in maternal serum AFP levels in HIV infected pregnant women as compared to controls. Ukibe et al. [20]

demonstrated that there was no significant difference between MSAFP in HIV infected pregnant women as compared to controls. Spencer [21] reported no elevations in the maternal serum levels of both AFP and β-hCG.

In the present study, the mean MSAFP level in HIV infected pregnant women was significantly lower than that of the control group, thus contradicts the reports by Gross et al. [19] and Ukibe et al. [20]. Since normal circulating levels of maternal AFP is an indication of good fetal health [5], the decrease in MSAFP levels among HIV infected pregnant women as observed in this study, may indicate that these women are at increased risk of pregnancy complications [6], such as intrauterine fetal death. Although Le Meaux et al. [22] reported that HIV infected pregnant women had lower median MSAFP levels than did control women, but with no significant difference, this study reported a statistically significant difference between the mean MSAFP levels in the test and control groups.

Although Yudin et al. [5] showed that β-hCG multiples of the median (MoM) were significantly higher in HIV infected pregnant women as compared to uninfected women, Spencer [21] and Le Meaux et al. [22] reported that β-hCG MoM levels did not differ between HIV infected pregnant women and the control subjects. The present study agrees with the findings of Spencer [21] and Le Meaux et al. [22], which showed that the difference between the mean serum levels of β-hCG in the test and control subjects was not statistically significant. This may be because of the significant role which β-hCG plays in the reduction of viral load in HIV infected pregnant women [7,8], hence these women continue to secrete β-hCG as a way of preventing vertical transmission from mother to fetus.

Gross et al. [19] reported a negative correlation between maternal serum β-hCG level and CD4 count. The above finding contradicts our finding, as there was a moderately positive correlation between CD4 counts and β-hCG in HIV infected pregnant women, and this positive correlation was significant. There was also from this study a significant positive correlation between CD4 counts and MSAFP levels in HIV seropositive pregnant women. Hence the measurement of AFP and β-hCG can be used alongside CD4 count for the assessment of maternal and fetal wellbeing.

The systolic and diastolic blood pressure of both the test and control groups was within the normal range (120/80), although it was lower in the test group.

The mean CD4 status of the HIV-infected pregnant women was appreciably normal, suggesting a healthy immune system, perhaps due to antiretroviral therapy.

5. CONCLUSION

HIV infection caused a significant reduction in MSAFP levels of infected individuals as compared to the age and sex-matched controls. The difference in MSAFP levels so obtained may have resulted from the HIV infection and may indicate danger to both maternal and fetal health. Since normal circulating levels of MSAFP is an indication of good fetal health, it can be concluded from this study that HIV infection by causing decreased MSAFP levels in HIV seropositive pregnant women, may have a potential of posing increased risk of pregnancy complications such as intrauterine fetal death.

CONSENT AND ETHICAL APPROVAL

The ethical approval for this research was obtained from the Human Research Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State. The procedures were explained to the subjects, and written informed consent obtained from each subject before specimen collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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