THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Determination of CD4 T-Cells Counts In HIV/HBV/HCV Co-Infected Pregnant Women on Haart in Lafia, Nasarawa State, Nigeria

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Abstract:
HIV/HBV and HIV/HCV co-infections places patients at high risk of liver-related morbidity and mortality and the interaction of these viruses further complicate treatment. Pregnant women are particularly at higher risk for increased morbidity and mortality with the prospect of vertical transmission of the viruses to their new born. A total of one hundred and fifty pregnant women were enrolled for the study, out of which fifteen 15(10%) of the pregnant women were sero-positive for HBV and ten 10(6.9%) sero-positive for HCV. The immunologic status of the HIV/HBV and HIV/HCV co-infected pregnant women were 361cells µ/l and 342cells µ/l which was lower when compared with those mono-infected with 419cells µ/l and 418cells µ/l CD4 mean count. The study also discovered that liver enzymes were higher among mono-infected (ALT 29 U/L) and (AST 28 U/L) when compared with those co-infected with HIV/HBV and HIV/HCV (ALT 16 U/L, AST 18 U/L) and (ALT 12 U/L, AST 9 U/L) respectively. The 10% prevalence of HBV and 6.9% prevalence of HCV reported in this study confirms the endemicity of HIV/HBV and HIV/HCV co-infections in Nigeria, and this support the calls for screening hepatitis B and C as a routine laboratory diagnosis in antenatal care.

Keywords: *Alanine Transaminase, Aspartate Transaminase, Human immunodeficiency Virus, Hepatitis B Virus, Hepatitis C virus, cluster of differentiation four, Hepatitis B surface Antigen, High active antiretroviral therapy*

1. Introduction

Hepatitis is an inflammation of the liver caused by hepatitis virus, which can be acute, or chronic (Ryder and Beckingham, 2001). And the infection during pregnancy is a major cause of high risk maternal complications and vertical transmission, which causes foetal and neonatal hepatitis with an attendant consequences leading to maternal mortality (Elinav et al., 2006), also a common cause of jaundice in pregnancy (Hill et al., 2002).Hepatitis B virus (HBV) and Hepatitis C virus (HCV) are the major cause of chronic diseases of the liver worldwide and commonest cause of hepatic dysfunction in pregnancy (Fenton, 2007a).

HIV infection and acquired immune deficiency syndrome (HIV/AIDS) is a spectrum of conditions caused by infection with human immunodeficiency virus (HIV) (Sepkowitz, 2001). The sero-prevalence of HIV in Nigeria has witnessed a slight decline from 4.4% in 2008 to 4.1% in 2010 (Federal Ministry of Health Report, 2010). The case was not the same in Nasarawa state; FMH reported HIV sero-prevalence of 7.5% in the State, which is higher than the average national prevalence of 4.1% (FMHR, 2010).

Co-infection of these viruses (HIV, HBV and HCV) is common because they share similar routes of transmission (Soriano, Barreiro and Nunez, 2006).Detecting HBV and HCV in HIV positive pregnant women is very important in view of the morbidity and mortality of the host and the risk of vertical transmission from mother-to-child. The contaminated newborn may often remain a chronic carrier and serve as source of infection to others, with attendant consequences of liver cirrhosis and hepatocellular carcinoma (Olokoba et al., 2011). The co-infection pattern of these viruses revealed that 10% of HIV infected population are estimated to have chronic HBV infection and estimated 3% to have chronic HCV infection worldwide (Fenton, 2007b). From the report on 18 sub-Sahara African countries, it was discovered that the prevalence of HBV and HCV in HIV infected individuals ranged from 3.9-7.3% and 6.9% respectively (Barth, Quirine and Jantjie, 2010). Sero-prevalence of 6.67% HBV was reported in pregnant women in Nasarawa state by Pennap and her colleagues (Pennap, Osanga and Ubam, 2011).

Although there is continued decline in the rate of morbidity and mortality from HIV/AIDS due to Highly Active Antiretroviral Therapy (HAART), liver diseases due to chronic HBV and HCV infections become a leading cause of death (Konopnicki et al., 2005).There is also a growing concern about the possible hepatotoxicity in HIV patients co-infected with HBV/HCV receiving HAART and how this could result in increased mortality in HIV patients (Health, 2000). Hepatic toxicity is due to increased rate of cytolysis and significantly elevated serum transaminase level. It is usually a common

complication in HIV infected patients undergoing ART (Anthony, 2001), and approximately found in 6-30% of treated subjects (Beisel, 1996).

A study conducted by Forbi et al, (2007) on individuals co-infected with HIV, HBV, and HCV in north central Nigeria, showed that co-infection with these triple infections appears to decrease the CD4 T-cells counts.CD4 T-cells are subset of T-lymphocyte that generally functions as helper T-cells. It constitutes 65% of the blood T-cells (Akinbami et al., 2014).Though similar studies have been conducted on co-infection with HIV, HBV, and HCV and their impact on CD4 T-lymphocytes in different parts of Nigeria, information regarding pregnant women is very sparse especially in north central part of the country to the best of my knowledge as of the time this research was about to be conducted.

Therefore,in view of this, the research work is aimed at determining the impact of co-infection (HIV, HBV and HCV) on CD4 T-cells counts and morbidity of the liver in pregnant women already on HAART, which will serve as a platform for proper management of the subject by policy makers and health care providers.

2. Material and Method

2.1. Study Area

This study was conducted at Dalhatu Araf Specialist Hospital (DASH), Lafia. The hospital has 200 bed capacities, a tertiary health facility located in Lafia, Nasarawa State, North Central Nigeria. It is a government owned hospital and serves a large population from both within the state and neighbouring states including Benue, Plateau, Taraba, Kaduna, and Federal Capital Territory (FCT). Although, a tertiary health centre, it provides both secondary and tertiary health services due to lack of a secondary health facility in the state capital. The following health care centres were used for samples collection, they include; primary health care Doma road, primary health care Akurba, primary health care new market, primary health care Shabu and primary health care Tudun Kuari. Samples were analysed in DASH, Lafia.

2.2. Study Population

The study was conducted among HIV positive ethnic Nigerian pregnant women between the age of 18-43 years exposed to HAART, who attended the antenatal and Prevention of Mother-to-Child Transmission (PMTCT) units of DASH, PHC Doma road, PHC new market, PHC Shabu, PHC Akurba, and PHC Tudun Kuari, in Lafia, Nasarawa State, Nigeria. Relevant information such as age, occupation, and tribal affiliation, trimester of pregnancy, educational qualification and state of origin was obtained using pre-test structured questionnaire.

2.3. Study Design

This was a cross sectional prospective study carried out among confirmed HIV positive pregnant women already on HAART at DASH and other primary health care centres between the months of June to September 2015.

2.3.1. Inclusion Criteria

All confirmed HIV positive pregnant women on HAART

2.3.2. Exclusion Criteria

Those who refused to give informed consent

2.3.3. Ethical Clearance

This was obtained from the ethical committee of the hospital (DASH).

2.3.4. Research Instrument

Structured questionnaire and consent form

2.3.4. Counselling

The study participants were counselled by the nurses at the ante natal and PMTCT units.

2.3.5. Data Collection

Data were collected using structured questionnaire

2.4. Sample Size

Sample size was determined using the formula:

$n=Z^2(pq)/d^2$ by (Araoye., 2003)

Where:

n= minimum sample size

z= standard error (1.96) at 95% confidence limit

p= local prevalence= 8% (0.08) (WHO, 1990)

q= (1.0-p)

d= degree of accuracy (5%) = (0.05)

$n= (1.96)^2 \times 0.08 \times 0.92 / (0.05)^2$

$n= 0.2827/0.0025 = 113.08$

Using the formula above, the minimum sample size was 113.08.
A total of 150 samples were collected, with 10% attrition rate = 165 samples

2.5. Biosafety

HIV, HBV and HCV belong to biosafety level 2 (BSL 2) agents that pose moderate hazard to personnel and the environment.
The following precautions required for handling BSL 2 agents were observed;

2.5.1. Standard Microbiological Practices

- Hands were washed properly after handling sample
- Used needles and other sharp objects were disposed properly
- Work surfaces were disinfected using appropriate disinfectant after each work

2.5.2. Special Practices

- Samples were collected using leak proof container
- Needle injury or exposure to infectious material was treated immediately
- Used equipments were routinely decontaminated whenever there are splashes and spills
- Procedure that will generate aerosol was done within biological safety cabinet

2.5.2 Safety Equipment (Primary Barriers and Personnel Protective Equipment)

- Protective laboratory coat was worn while carrying out analysis and remove before leaving the laboratory
- Eye and face were protected using goggled, mask, and face shield
- Gloves were worn to protect hands from exposure to infectious agents
- Gloves were changed when contaminated
- Gloves were removed immediately after work
- Disposable gloves were not wash or reuse

2.6. Sample Collection

Before sample collection, consent of each participant was sought with the help of the nurses at the antenatal clinic, after which their information was collected using structured questionnaire.
5ml of venous blood was collected aseptically using plain and ethylene diamine tetracetic acid (EDTA) vacutainer tube, for the determination of HBV, HCV, liver enzyme level and CD4 counts from each participant.

2.7. Laboratory Analysis

The blood samples collected in the plain tube were centrifuged at 3000 revolution per minutes for 5 minutes to separate the serum which was used for the detection of HBV, HCV and liver enzymes test, for the presence of HBsAg, Anti.HCV and ALT, AST respectively. The blood samples collected in the EDTA vacutainer were used for CD4 T-cells enumeration.

2.8. Quality Control

The standard operational procedures were strictly adhered to, for the quality control check. Both HBV and HCV kits used were tested using known HBsAg and Anti.HCV antibody positive and negative control samples.
Also, the quality of CD4 reagents where been monitored by running control beads before carrying out the actual work. Liver enzyme reagents were checked to determine the effectiveness of the working reagent before performing any analysis.

2.9. Statistical Analysis

Data were analyzed using statistical package for social sciences (SPSS) version 23.0. The Pearson Chi-square test and student's t-test were used to test for differences between groups in the case of normally distributed data. Statistical significant was $p<0.05$ at 95% confidence interval.

3. Result

3.1. Socio-Demographic Characteristics

A total of 150 participants (pregnant women) were enrolled into this study, the lowest and highest age were 18 and 43 years respectively. Age group 25-29 constitute majority of the study participants 55(36.7%), while 124(82.7%) are of Nasarawa state origin. Eggon ethnic extraction constitute majority 45(30%) of the study participants, 148(98.7%) of the pregnant women were married, with 65(43.3%) of them had business as their occupation. 49(32.7%) of them had secondary education, while 59(39.3%) are in their third trimester of pregnancy. (Table 1)

Variables	Number of participants (%)
Age Group	
18-24	33(22.0)
25-29	55(36.7)
30-34	43(28.6)
35-39	16(10.6)
40-44	3(2.0)
State of Origin	
Nasarawa	124(82.7)
Benue	4(2.7)
Plateau	6(4.0)
Others	16(10.7)
Tribes	
Eggon	45(30)
Alago	16(10.7)
Mada	6(4.0)
Afo	3(2.0)
Hausa/Fulani	17(11.3)
Igbo	6(4.0)
Others	57(38.0)

Table 1: Socio-Demographic Characteristics of HAART Exposed Pregnant Women in Lafia, Nasarawa State

Variables	Number of participants (%)
Marital Status	
Single	2(1.3)
Married	148(98.7)
Occupation	
Farming	9(6.0)
Civil Servant	27(18.0
House Wife	43(28.7)
Student	6(4.0)
Educational Qualification	
Primary	29(19.3)
Secondary	49(32.7)
Tertiary	44(29.3)
Non	28(18.7)

Table 2: Socio-Demographic Characteristics of HAART Exposed Pregnant Women in Lafia, Nasarawa State

Hepatitis Serology	Number Tested	Number Positive (%)
HIV/HBV150	15(10.0)	
HIV/HCV150	10(6.9)	

Table 3: Prevalence of Co-Infection of HIV/HBV, HIV/HCV amongst HAART Exposed Pregnant Women in Lafia, Nasarawa State

Variables	CD4mean ± SD(μl)	Normal CD4	Value(μl)p-value
HIV alone	419±226	547-1327	0.351
HIV/HBV	361±243	547-1327	
HIV alone	418±226	547-1327	0.312
HIV/HCV	342±241	547-1327	

Table 4: CD4 T-cell counts and its association with HIV/HBV, HIV/HCV amongst HAART exposed pregnant women in Lafia, Nasarawa State
Analytical tool: T-test at 95% confidence limit

Variables	HIV alone (U/L)	HIV/HBV (U/L)	p-value	HIV alone (U/L)	HIV/HCV (U/L)	p-value
ALT mean ± SD	29±8	16±10	0.010	29±8	12±5	0.000
Normal value	49	49		49	49	
AST mean ± SD	28±17	18±14	0.168	28±17	9±4	0.003
Normal value	46	46		46	46	

Table 5: Co-Infection (HIV/HBV, HIV/HCV) And Its Impact on the Morbidity of the Liver amongst HAART Exposed Pregnant Women in Lafia, Nasarawa State

4. Discussion

HBV and HCV co-infection with HIV in pregnancy is emerging as an increasingly important health challenge. Though more attention has been given to HIV for the purpose of preventing Mother-To-Child-Transmission (MTCT), co-infection with HBV and HCV which shared similar route of transmission with HIV have been reported to be higher in Nigeria, especially HBV with higher possibility of developing chronic liver disease (Piliero and Faragon, 2002; Buseri, Muhibi and Jeremiah, 2009). This called for a pragmatic approach, considering the danger this co-infections pose to the mother and the unborn child's health, possibility of becoming a source of disease transmission (carrier), and a risk of developing heap to cellular carcinoma.

Studies were conducted in different part of Nigeria, Africa and across the globe to ascertain the prevalence rate of the co-infections among the pregnant women. Pennap, Osanga and Ubam (2011), reported 6.67% HBV among HIV negative pregnant women attending antenatal clinic in Keffi, North Central Nigeria. The 10% in this study differed from 6.67% found in the same environment, suggesting that the situation with HBV infection in the environment has changed. Other studies in Nigeria have reported different prevalence rates of HBV infection. In a study carried out in Jos, North Central Nigeria within the same geopolitical zone where this study was conducted, the prevalence rate of HIV/HBV co-infection was 11.5% (Imade et al., 2004), in Federal Capital Territory (FCT), Abuja Nigeria, the prevalence rate of 7.1% was reported (Bassey et al., 2009), in Yola, North East Nigeria, a prevalence rate of 8.2% was reported among pregnant women (Olokoba et al., 20011). Also, a prevalence rate of 8.9% was reported by Adesina et al (2010) in Ibadan, South West Nigeria among pregnant women. Different prevalence rate has been reported in other parts of Africa. Pirillo et al (2007) reported a prevalence rate of 4.1% among pregnant women in Uganda, while 10.4% prevalence rate with almost similar figure with the one in this report, was reported among pregnant women in Malawi (Chasela et al., 2009).In Europe, 4.9% prevalence rate was reported among HIV infected pregnant women and low co-infection rate of 1.5% was reported among pregnant women in America (Landes et al., 2008; Patricia Santiago Munoz et al., 2005).

The prevalence of 10% HBV found in this study varies from some studies above; the reason could be due to geographical variation, differences in cultural and religious practices. Therefore, Lafia is a hyper-endemic area for HBV infection according to WHO classification for HBV endemicity, which is defined as HBsAg prevalence greater than 7% in an adult population (Uneke et al., 2005).The 6.7% prevalence of HCV infection in the tested population of HIV positive pregnant women was found to be higher when compared to other studies carried out in Nigeria, such as 1% reported by Agarry and Lekwot among pregnant women in FCT, Abuja Nigeria which share boundary with Nasarawa state where this study was conducted. In Maiduguri, North East Nigeria, 2.5% was reported among pregnant women (Baba et al., 1999). A prevalence rate of 6.7% similar to the one reported in this study, was reported by Omokayode and Ojiezeh (2015) in Ogun, South Western Nigeria among pregnant women. Also compared with findings from other African countries, a prevalence rate of 6.8% which is almost the same with this study was reported among Egyptian pregnant women (Khaled et al., 2010), but low prevalence rate of 2.6% was reported in Cote d'Ivoire (Zuccotti, 2006). A lower prevalence rate of 0.01% which is the lowest was reported in the United Kingdom (GBD, 2004).

With respect to the immune status of the study participants, there was no statistical significant difference between the mean CD4 count of HIV mono-infected and HIV-HBV, HIV-HCV co-infected study participants. However, pregnant women co-infected with HIV-HBV has mean CD4 count of 361cells/µl, but statistically, there was no association between HIV-HBV co-infection and the immune status of the women ($p>0.05$). Compared with the study carried out in Jos, Lar and his colleagues reported a CD4 count of 300cells/mm^3 and below, among pregnant women co-infected with HIV-HBV (Lar et al., 2013). When the mean CD4 count of HIV mono-infected (419cells/µl) is compared with the HIV-HBV co-infected (361cells/µl) study participants, there was decrease in the mean CD4 count of HIV-HBV co-infected pregnant women. This is almost similar with the report of Forbi and his colleagues, who reported a lower mean CD4 count of 377cells/µl in patients with HIV-HBV co-infection when compared with HIV mono-infection (478cells/µl) patients in North Central Nigeria. Another study by Otegbayo et al (2008), observed lower CD4 counts in HIV-HBV co-infected pregnant women in Ibadan, South Western Nigeria. Idoko et al (2009) also reported same in Jos. However, Hoffman and his colleagues reported that HBV does not affect CD4 counts in people infected with HIV (Hoffman et al., 2008).

Pregnant women co-infected with HIV-HCV had mean CD4 count of 342cells/µl. However, there was no statistical association between HIV-HCV co-infection and immune status of the pregnant women ($p>0.05$). The mean CD4 count of 342cells/µl in this study can be compared to similar report by Forbi et al (2007) who reported a mean CD4 count of 373cells/µl in patients co-infected with HIV-HCV.HIV-HCV co-infected pregnant women had relatively lower mean CD4 value (342cells/µl) than HIV mono-infected (418cells/µl). Forbi and his colleagues reported that the rate of increase in CD4 cells post HAART does not change in HIV and hepatitis co-infection but HCV appears to hinder virological response to therapy (Forbi et al., 2007). There was a significant rise in the mean serum level of ALT between mono-infected and HIV-HBV co-infected women ($p<0.05$), but none in the mean serum level of AST between mono-infected and HIV-HBV co-

infected pregnant women (p>0.05).Statistically, there was a significant rise in the mean serum levels of ALT and AST between mono-infected and HIV-HCV co-infected pregnant women (p<0.05).When the mean serum levels of ALT, AST of mono-infected and co-infected (HIV-HBV, HIV-HCV) women was compared, there was a rise in mean serum level of mono-infected than co-infected pregnant women. This is incomparable with the work of Oluboyo and his co-worker, who reported a rise in ALT and AST in HBV and HCV infected pregnant women in South Eastern Nigeria (Oluboyo et al., 2014). The reason could be that their study was done on HIV negative pregnant women not exposed to HAART, and it was envisaged that anti-HIV drugs can also bring about heap to cellular damage (Sulkowski, 2004). Therefore, the reason for the rise in the enzymes level of mono-infected against the co-infected pregnant women could be as a result of drug induced liver injury associated with antiretroviral therapy. To ascertain whether anti-HIV drugs are responsible for liver injury, further study can be carried out on the impact of different type of anti-HIV drugs on the liver.

4.1. Recommendations

This study has disclosed the presence of HBV and HCV, its effects on CD4 T-lymphocytes and morbidity of the liver amongst HIV infected pregnant women on HAART. Based on these findings, the following points are forwarded thus:

- HIV positive pregnant women should be mandatorily and routinely screened for HBV and HCV infection as part of antenatal care services in their booking to prevent mother- to- child transmission (MTCT).
- Infants and new born should be immunized against HBV and HCV infection to prevent further spread.
- There should be increase availability to antenatal care for early detection of HBV and HCV.
- HIV infected pregnant women on HAART should be monitored closely for liver enzymes along with their CD4 count.
- Public awareness and immunization against viral hepatitis in both urban and rural pregnant women should be instituted as a national health policy.

4.2. Conclusion

Co-infection of HIV with HBV and HCV is still a major health challenge in Nigeria as established from the findings in the study. Therefore, more still needs to be done by policy makers and health care providers to address the challenge.

4.3. Limitation

This study employed the use of HBsAg and Anti. HCV for the detection which was not able to distinguished between recent and past HBV and HCV infection. Screening for HBsAg alone does not fully reflect the epidemiology of the disease as it could indicate a carrier state or chronic hepatitis.

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