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Occurrence of Human Immunodeficiency Virus Among Pregnant Women Attending Antenatal Clinic at the Braithwaite Memorial Hospital in Port Harcourt, Rivers State, Nigeria

GLORY BARINUAKA BAEKA & LOVINAH ISAIAH MBA
Rivers State University, Port Harcourt, Nigeria

ABSTRACT The presence of antibodies to the Human immunodeficiency virus (HIV) in pregnant women who have been previously tested with a rapid kit was investigated. The aim of this study is to test for the presence of antibodies to HIV in pregnant women who have been previously tested with a rapid kit attending the Braithwaite Memorial hospital antenatal clinic. The objectives of the study were collection of blood samples from pregnant women who had been previously tested with the rapid kit for the presence of the virus, using a fourth generation DIA. PRO serological kit for HIV Ag-Ab ELISA assay which is very sensitive to the virus; and, to present the information gotten to the health authorities and the public. Blood specimen was collected from a total of 91 pregnant women between May and June 2016 and was screened for HIV using the ELISA assay. Out of the 91 samples received from the Hospital, having the age range of 15-45 years that were tested, 26(28.6%) of them were positive for HIV while 65(71.4%) were negative. This shows that more aggressive awareness and sensitization campaigns needs to be carried out by Government and various organizations to educate the public to adhere to the caution given in order to prevent the spread of HIV.

Keywords: HIV, ELISA assay, Pregnant women, HIV Ag-Ab, Serology.

Introduction

Human Immunodeficiency Virus is the etiologic agent of Human Immunodeficiency Virus (HIV) infection and Acquired Immunodeficiency Syndrome (AIDS) which is a disease of the human immune system. (Hall et al., 2010; Sepkowitz, 2001). It is an Amphotropic virus, meaning that it exhibits a broad host range (able to infect cells not only of the natural host, but of heterologous [foreign] species as well), because, they recognize a receptor that is widely distributed and Xenotropic virus (because it infects and replicates in some heterologous cells not in natural host) (Jawetz et al., 2004). HIV belongs to the family Retroviridae, sub-family Orthoretrovirinae, genus Lentivirus and two species have been identified: HIV-1 and HIV-2 (Hall et al., 2010) (International Committee on Taxonomy of Viruses [ICTV], 2002). HIV is an enveloped, positive sense, single strand diploid RNA virus (Alimonti et al., 2003); HIV-1been responsible for most HIV infections throughout the world, whereas HIV-2 is found primarily in West Africa (Hall et al., 2010; Jawetz et al., 2004). The virus is

transmitted primarily via unprotected sexual intercourse (including anal and even oral sex), contaminated blood transfusions and hypodermic needles, and from mother to child during pregnancy, delivery, or breastfeeding (Markowitz et al., 2007). Some bodily fluids, such as saliva and tears, do not transmit HIV (CDC, 2003). The average per act risk of getting HIV by exposure route is: Blood Transfusion 90% (Smith et al., 2005), Childbirth (to child) 25% (Coovadia, 2004), Needle-sharing injection drug use 0.67% (Smith et al., 2005),Percutaneous needle stick 0.30% (Kepiela, 2007), Receptive anal intercourse 0.04–3.0% (Dosekun et al., 2010), Insertive anal intercourse 0.03% (Cunha, 2012),Receptive penile-vaginal intercourse 0.05–0.30 (Dosekun et al., 2010, Boily et al., 2009), Insertive penile-vaginal intercourse 0.01–0.38%,receptive oral intercourse 0–0.04% (Dosekun et al., 2010), Insertive oral intercourse 0–0.005% assuming no condom use. (Baggaley et al., 2008).

Prevention of HIV infection, primarily through safe sex and needle-exchange programs, is a key strategy to control the spread of the disease. There is no cure or vaccine; however, antiretroviral treatment can slow the course of the disease and may lead to a near-normal life expectancy. While antiretroviral treatment reduces the risk of death and complications from the disease, these medications are expensive and may be associated with side effects (UNAIDS, 2011).

Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors (Smith et al., 2006).

A majority of about 34 million people currently infected with HIV-1 live in developing countries. Approximately 2.5 million new infections and 2 million fatalities from Acquired Immunodeficiency Syndrome (AIDS) caused by the infection occur annually (Hall et al., 2010). Highly Active Antiretroviral Therapy (HAART) is the name of a treatment regiment that aggressively suppresses HIV replication and progression to AIDS. The usual HAART regiment, combines three or more anti-HIV drugs from at least two different classes to avoid development of resistant viral variants (Hall et al., 2010).

Two types of HIV have been characterized: HIV-1 and HIV-2 (Hall et al., 2010; ICTV, 2002). HIV-1 is the virus that was firstly discovered (and initially referred to also as LAV or HTLV-III). It is more virulent, more infective (Gilbert et al., 2003) and, also, the cause of most HIV infections globally (UNAIDS, 2011).

In June 1981 clinicians in the United States reported many cases of Pneumocystis carinii infection in homosexual men. The infections were linked to impaired immune response, which was initially called the Gay-Related Immune Deficiency Syndrome (GRIDS). Later, it was more appropriately renamed Acquired Immunodeficiency Syndrome (AIDS) (Hall et al., 2010) by CDC (Gallo, 2006), and the San Francisco Chronicle newspaper published a description of the "seven deadly symptoms" associated with the disease:

- Fever persisting for more than 4 or 5 days.
- Unexplained weight loss of 10 to 20 pounds in a few months.
- General aches and pains like an acute viral syndrome for more than 10 days.
- Sore or swollen lymph glands for more than a week.

- Appearance of blue or purplish spots on the skin (now recognized as Kaposi's sarcoma).
- Herpes sores that worsen and persist for more than 5 weeks.
- Loss of sensory or motor ability or defects in mental or neurological function (Hall et al., 2010; Entonu, 2007; Barre-sinossi, 2002).

AIDS is pandemic within the sub-Saharan Africa, which include Nigeria, according to a UNAIDS/WHO (2007) report. The first case of AIDS in Nigeria was reported in a sexually active 13 years girl in 1986 (Agwale, et al., 2002). Laboratory diagnosis of HIV infection generally focuses on detection of antibody to HIV. However, the new generation assays also incorporate HIV antigen detection to increase the sensitivity of the assay. In this way, a HIV antigen-antibody combination assay is helpful in closing the window period (the time between HIV infection and appearance of antibodies to HIV) as HIV antigen is present in the blood before antibodies to HIV can be detected (WHO, 2017).

Material and Methods

Study Area

Samples were collected from Braithwait Memorial Hospital (BMH), Harley Street, Old GRA, Port Harcourt, Rivers State, Nigeria in the months of May and June 2016 from pregnant women attending the antenatal clinic, who had been previously tested for the HIV, using a rapid kit with the result showing negative. That indicated that they were not infected with the virus.

Sample Collection

Total blood sample of 91 were collected from pregnant women through venipuncture and was put into EDTA bottles to prevent coagulation. From Braithwaite Memorial Hospital and the blood samples were centrifuged to separate the plasma from the red blood cells, the plasma was separated into another EDTA bottle.

Materials

A DIA. PRO kit for HIV ag-Ab which was used contains the following:

- Micro litre plate
- Wash buffer
- Enzyme conjugate
- Chromagen/substrate (mixture of tetramethyl-benzidine and hydrogen peroxide)
- Sulphuric acid
- Specimen diluements
- Control serum
- Plate sealing foil
- Package insert

Other materials used but not indicated in the kit include the following:

- Calibrated micro litre pipette (100ul and 50ul) and disposable plastic tips.
- Distilled water
- Timer
- Absoubent tissue paper.
- Thermostatic incubator
- Calibrated ELISA microplate washer.
- Calibrated ELISA Microwell reader with 450nm and with 620-630nm.

Principle Test

The Grenscreen ULTRA HIVAg-Ab is an enzyme immunoassay based on the sandwich technique for the detection of HIV antigen and the various antibodies associated with HIV-1 and/or HIV-2 virus in human serum or plasma. The solid phase is coated with:

- Monoilonal antibodies against P24 HIV-1 antigen.
- Purified antigens; gp160 recombinant protein a synthetic peptide mimicking theimmunodominantepilope of the HIC-2 envelope protein.

The conjugates are based upon the use of:

- Biotinylated polyclonal antibodies to HIVAg (conjugated)
- Streptavidin and HIV antigens peroxidase conjugate (gp41 and gp36 pepides mimicking the immunodominanteptopes of the HIV-1 and HIV -2 envelope glycoproteins and the same synthetic peptide mimicking a totally artificial HIV-1 group O-specific epitope used for the solid phase) (conjugate 2).

Preparation of Wash Solution:

- 400ml of the distilled water was dispensed into a 500ml measuring cylinder and 20ml of the buffer was added to the 400ml of water and then this solution was dispensed into a wash bottle to be used by the wash machine. The enzymatic reaction was stopped by dispensing 100ul of sulphuric acid into each well (the blank wells included).

The result was read with micro titre plate reader ar 0D450 (reading) the result in A and B, wells were positive control.

Quantitative Analysis

Conjugated (biotinlatedpolyclonaantibody to p24 HIV-1Ag) was added to the 96 microplate wells. Serum samples to be assayed and controls were pipette into their respective wells. If present, HIV antigens bind with the monoclonal antibody bound to the solid phase and the conjugate 1 and samples were validated through a colour change from yellow green to blue. After incubation at 37^0C, the plate was then washed using the ELISA washer. Conjugate 2 was added to the wells, Streptavidin reacts with biotinylated Ab-Ag-Ab complexes. Purified HIV-1 and HIV-2 antigens bind in turn to the LgG, 1gm or 1gA antibodies captured on the solid phase. The enzymatic reaction was stopped by dispensing 100ul of sulphuric acid into each well. The result was read with a micro titre plate reader at OD450 (reading).

Results

Table 1: show the work sheet with each box representing the wells for the calibrators and the samples of HIV among pregnant women.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84
B	PC	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77	S85
C	NC	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78	S86
D	NC	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79	S87
E	NC	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80	S88
F	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81	S89
G	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82	S90
H	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83	S91

KEY: PC = Positive Control
NC = Negative Control
S = Sample

Table 2: shows the OD450nm value gotten from the micro litre reader

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.118	0.221	0.161	0.781	0.298	0.249	3.54	0.575	0.186	1.3	0.422	0.388
B	0.289	0.171	0.161	0.254	0.279	0.194	0.238	0.247	0.175	3.452	0.239	0.239
C	0.157	0.169	0.153	0,169	0.196	0.229	0.204	0.173	0.177	0.186	3.225	0.332
D	0.152	0.221	0.177	0.333	0,312	0.199	0.382	0.219	0.264	3.171	0.313	3.544
E	0.158	0.201	1.35	0.468	0.305	0.227	0.229	3.27	0.215	0.416	0.752	0.76
F	0.172	0.187	1.927	0.232	0.315	0.218	0.21	0.204	0.275	0.182	3.07	3.548
G	0.147	0.182	0.993	0.197	0.4	0.22	0.193	0.207	0.223	1.532	3.611	0.472
H	0.206	0.228	0.197	0.328	0.531	0.223	0.245	0.221	0.243	3.569	2.963	0.592

Calculation

The calculation used was according to the manufacturers guide in order to get the cut-off point to determine the positive and negative samples.
The mean value of the 3 negative controls (C1, D1, E1) were calculated using the OD450nm reader.
From the result sheet
C1 = 0.157
D1 = 0.152
E1 = 0.158
Therefore, the mean value of the 3 negative controls = 0.156
Cut-off Value = 0.156 + 0.200 = 0.356

Therefore, samples with values lesser than the cut off values are negative.While, samples with values greater than the cut off value, are positive.

Table 3: shows result of those those who are positive and those who are negative using the cut off value.

	1	2	3	4	5	6	7	8	9	10	11	12
A		-	-	-	-	-	+	+	+	+	+	+
B		-	-	-	-	-	-	-	-	+	+	-
C		-	-	-	-	-	-	-	-	-	+	-
D		-	-	-	-	-	-	-	-	+	-	+
E		-	+	-	-	-	-	+	-	+	+	+
F	-	-	+	-	+	-	-	-	-	-	+	+
G	-	-	-	-	+	-	-	-	-	+	+	+
H	-	-	-	+	+	-	-	-	-	+	+	+

Table 4: Age distribution of the pregnant women, with the number of positive and negative patients and their percentage

Age range patients	Total tested	positive	% of positive patients	Negative	% of Negatives
15-20	*2*	*1*	*50*	*1*	*50*
21-25	*11*	*5*	*45.45*	*6*	*56.55*
26-30	*31*	*4*	*13*	*27*	*87*
31-35	*24*	*7*	*29*	*17*	*71*
36-40	*19*	*8*	*42*	*11*	*58*
41-45	4	1	25	3	75

Percentage for Negative and Positive
Total sample tested = 91
Total positive = 26
Total negative = 65
Percentage positive = 26/91x100 =29%
Percentage negative 65/91x100 =71%

Discussion

From this study, it was discovered that out of the 91 pregnant women who had been previously screened for the virus, using a rapid kit and certified negative i.e. non-reactive to the virus, 26 of them where found to be positive to the virus, which gives a percentage of 29% out of 100. This is quite high, given the amount of awareness created about this virus. This study agrees with the findings of Coovadia (2004), which states that around 25% of HIV is transmitted during child birth from the mother to the child.

Most of the positive patients, were between the ages of 15 and 25, which is the age at which most youths are sexually active (CDC, 2016). This further agrees with the finding that young women (16-25 years) report higher rate of boyfriend (12.6% and 15% respectively) compare to the 26-35 years group reporting the rate 3.0%.

This observation brings to bare the need for HIV testing of pregnant women, using a forth generation serological kit which is more sensitive than the rapid kits (WHO, 2017). These patients were non-reactive to the rapid kit, since these patients were in their window

phase and therefore the absence of antibody, which is the component that the rapid kits detect. But the serological kit was able to detect the antigen of the virus, which becomes present immediately infection is established. The benefits of using a serological kit, include the possibility of early detection of women who may be seropositive, to use this knowledge for early counseling and treatment and to utilize appropriate interventions to reduce the rate of mother to child transmission (MCT). Currently, over one million children are living with HIV, contracted predominantly through infection from their mothers. Knowledge of her HIV status, enables the woman to take decisions on continuation of the pregnancy and on future fertility. Awareness also enables the women to take precautions to help prevent transmission to sexual partner. (WHO/UNAIDS, 1998).

These findings of HIV seropositivity in pregnancy have a number of implications. This is serious in the potential of giving birth to children with the HIV virus if not provided with available antiretroviral therapy for the prevention of mother to child transmission of HIV.

Conclusion

This study has confirmed the high positivity of antibodies to HIV among pregnant women in Port Harcourt, Rivers State Nigeria. This calls for urgent and concerted efforts aimed at promoting behavioural and socio-cultural changes that could reduce or eliminate the current detection rate among Nigerian pregnant women. A combination of preventive strategies such as the use of condoms, breaking the chain of transmission within sexual networks by prompt treatment and reducing the amount of unsafe sexual behaviours, promoting sexual abstinence and behavioural change from high-risk behaviours are advocated. Also, the use of serological kits which are more sensitive than rapid kits should be used to test for the virus.

Correspondence
Glory Barinuaka Baeka
Rivers State University
Port Harcourt, Nigeria
Email: preetybiggy@yahoo.com

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