

Full Length Research Paper

Sero-prevalance of anti-R7V antibody in HIV infected patients in the Federal Capital Territory (FCT), Nigeria

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Studies in some parts of the world have shown that Anti-R7V antibodies, which neutralize 100% of the different variant's panel (targeted against a beta2-microglobulin epitope acquired when the virus is released by budding) *in vitro*, are found in 30 to 50% of naïve HIV positive patients, but even more in so-called "long-term survivor" patients with a close to 90% correlation. The seroprevalence of Anti-R7V antibody was therefore investigated in HIV patients attending clinic within the Federal Capital Territory (FCT) and compared with HIV negative patients. Correlation between the presence of the antibody and the clinical status of patients was also investigated. The HIV positive patients were categorized into drug naïve and drug experienced subjects and their Anti-R7V antibody together with CD4 counts were determined using Anti-R7V ELISA kits and BD FACS count, respectively. About 47.2% of the HIV-infected patients tested positive for the Anti-R7V antibody while 25.2% were negative for this antibody. Patients with Anti-R7V antibody had a mean CD4 count (355 ± 19.2) significantly ($P < 0.05$) higher than that of Anti-R7V antibody negative patients (215 ± 42.6). Also it was observed that Anti-R7V antibody was significantly ($P < 0.05$) lower in drug experienced patients as compared to drug naïve patients. The significance of these findings is discussed. It was concluded that Anti-R7V antibody may be a natural immunity against HIV-infection in drug naïve HIV patients and that the synthesis and release of this antibody may decrease with ARD treatment.

Key words: HIV, AIDS, anti-R7V antibodies, Nigeria, CD4 count.

INTRODUCTION

Since the first reported cases of HIV/AIDS in the early 1980s, HIV has emerged as one of the most significant infectious agents, infecting approximately 40 million people worldwide (UNAIDS, 2002). The key to the pathogenicity of this virus is its genetic heterogeneity, which is the result of several features namely the error-prone enzyme reverse transcriptase (RT), which is estimated to introduce an average of one error per genome per replication cycle (Robert et al., 1988; Mansky and Temin,

1995) and the rapid turnover of HIV- 1 *in vivo* (Ho et al., 1995), recombination which occurs at a rate of about 2% per kilobase per replication cycle (Temin, 1993; Subbarao and Schochetman, 1996) with its selective immune pressures by the host (Micheal, 1999). At present HIV is classified on the basis of genetic difference and similarities into types, group and sub types (Reeves and Dom, 2002).

Since the first case of AIDS diagnosed in Nigeria, in a 13 year-old girl in 1986 (National Guideline on Prevention of Mother to Child Transmission in Nigeria, 2005), HIV prevalence has been on the increase from 1.8% in 1991 to 5.8% in 2001 and declined to 5.0% in 2003 and 4.4% in 2005. The 2005 survey showed State prevalence vary

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from as low as 1.6% in Ekiti State to as high as 10% in Benue state and with 15 state including the FCT having prevalence above the national average of 4.4%. Women are believed to be more severely affected than men (National HIV/Syphilis Sentinel Seroprevalence Survey of 2005).

The existence of asymptomatic patients showing non-progression for more than 10 years raises the question of a possible natural AIDS resistance mechanism. It has been reported that anti-R7V antibodies, which neutralize 100% of the different variant's panel (targeted against a beta2-microglobulin epitope acquired when the virus is released by budding) *in vitro*, are found in 30 to 50% of naïve HIV positive patients, but even more in so-called "long-term survivor" patients with a close to 90% correlation (Galea et al., 1996).

The CD4 lymphocyte count has demonstrated variability, even in the same patient, the variation can be more than 100/ μ L cells in the same day due to diurnal variations. Additional laboratory testing factors that play a role in measurement of CD4 lymphocytes include variations in total white blood cell count, lymphocyte percentage and CD4 percentage. Physiologic factors may include exercise as well as consumption of tobacco, alcohol and caffeine (Raboud et al., 1995).

The anti-R7V antibodies have been linked to non progression of HIV-infected patients to full blown AIDS. Correlation of the presence of the protective anti-beta2 microglobulin derived peptide antibodies with non progression to AIDS has been reported in France (Galea et al., 1996). However, no information has been documented regarding the prevalence of this antibody in long term non progressors in Nigeria. Thus, the objectives of this study were to determine the prevalence of neutralising anti-R7V antibodies in the sera of HIV- infected persons within the FCT and some neighbouring towns, determine the prevalence of neutralising anti-R7V antibodies in the sera of HIV-infected persons on anti-retroviral drugs, and to provide baseline information on anti-R7V antibody in the FCT.

MATERIALS AND METHODS

Subjects

A total of 176 randomly diagnosed HIV positive patients attending HIV clinic at Asokoro District Hospital were used for the study. The 176 HIV patients included those on Antiretroviral drugs (ARDs) and drug naïve. In addition, 88 randomly selected apparently healthy subjects who after being screened tested negative for HIV antibody were used as control.

Area of study

The study was conducted using data and blood specimen collected from patients attending HIV clinics at Asokoro District Hospital in the FCT. The FCT was chosen as the study area because of its location within a zone (North Central) that has the highest prevalence of HIV and the highest population growth rate (10% per annum) in Nigeria (WHO/UNAIDS, 2005).

Ethical consideration

Informed consent and approval was obtained from the Asokoro District Hospital's Management and its Ethical Committee. The hospital's ethical committee was assured of the confidentiality of the subject's identity.

Collection of specimens

Two to three millilitres (2 to 3 ml) of blood was collected from the median cubital vein of each subject into EDTA vacutainers and centrifuged to obtain the plasma. The plasma obtained was stored at -20°C until needed for assay.

Anti-R7V ELISA Kits

The Anti-R7V ELISA test was purchased from the manufactures, that is, IVAGEN S.A (Bernis, France) (anti-R7V ELISA ref IVR96000).

Experimental design

Blood samples were collected from HIV positive patients on anti retro viral drugs (ARV) and those recently diagnosed but not on ARV treatment. The following assays were carried out on blood samples obtained from each subject:

1. Screening for HIV.
2. CD4 count
3. Anti-R7V Antibodies.

Screening for HIV antibody

The screening for HIV was performed using 2 types of HIV test kits per sample namely CHEMBIO HIV1/2 STAT-PAK[®] ASSAY and Abbot Determine[™] according to the manufacturers' specifications.

CD4 count

The BD FACSCount[™] reagent kits which contain CD4 PE/CD3 PE-Cy5 tubes (green top), CD8 PE/CD3 PE-Cy5/CD3 tubes (clear top) and 220 reagent tube caps were used to enumerate the absolute counts of CD4 of unlysed whole blood, using the BD FACSCount instrument. This will provide information on the immune status of the patients with the human immunodeficiency virus. The CD4/ CD3 tube determines the absolute number of helper/inducer T lymphocytes. The CD8/CD3 tube determines the absolute number of suppressor/cytotoxic T lymphocytes. Both tubes measure the absolute number of total T lymphocytes (CD3).

ANTI-R7V antibody

The Anti-R7V ELISA is a serological test (90 min) based on an immunoenzymatic assay. The assay allows the detection of the presence of antibodies directed against the R7V epitope in serum or plasma samples of individuals detected as being seropositive for HIV infection. Microplate wells that were coated in a covalent and homogeneous way with peptidic fragments containing the sequence R7V allowed the specific capture of anti-R7V antibodies which were present in the samples of serum or plasma. Their colorimetric revelation was made after addition of an enzymatic conjugate (Goat

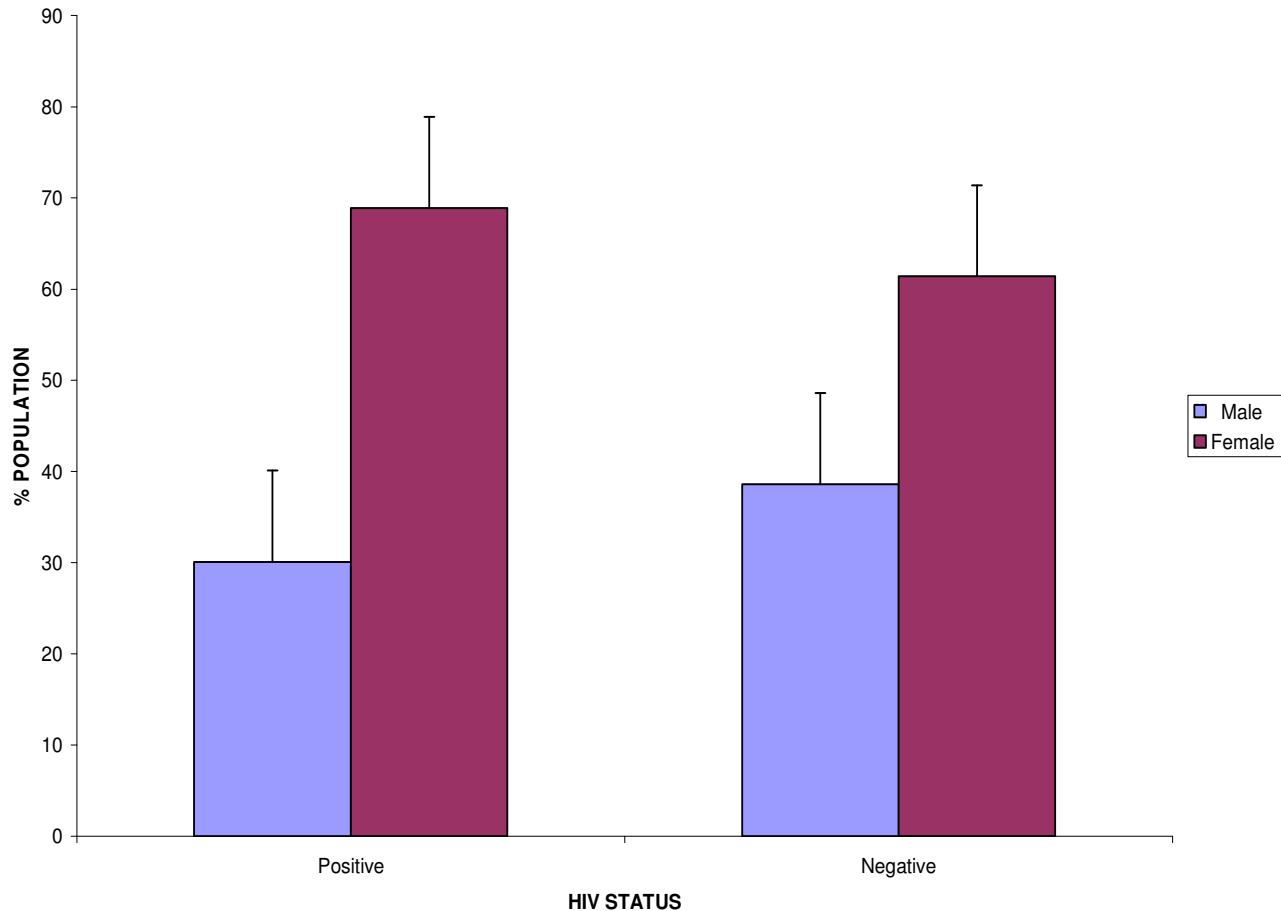


Figure 1. Distribution of HIV status according to sex.

anti-human IgG coupled to horse radish peroxidase containing 0.5% Proclin 300) and 12 mL of an enzymatic chromogen substrate (3,3',5,5' tetramethylbenzidine TMB). The optical density (OD) values were read at 450 nm within 15 min of adding the stop solution (diluted hydrochloric acid). After subtraction of the sample blank, the mean OD value observed for each of the patient samples was normalized on the mean OD value of the internal calibrator. The conclusion as for the presence or the absence of anti-R7V antibodies was based on the value of the antibody ratios obtained.

RESULTS

The distribution of the 176 subjects diagnosed as HIV positive as presented in Figure 1, shows that 123 (68.9%) were females while 53 (30.1%) were males. In the 88 HIV negative subjects 34 (38.6%) were males and 54 (61.4%) females.

Figure 2 shows the distribution of HIV positive patients according to their age groups. The data obtained showed that the age bracket 27 - 36 yrs had the highest number of HIV-infected patients representing 50% of the total population, this was followed by the age brackets 37 - 46, 17 - 26 yrs which had (23.3%) and (19.3%), respectively. It was observed that the age brackets of 47 - 56, 57 - 66

and 67 - 76 had HIV- infected patients' population of below 5% while the age group of 17-46 accounted for 92% of the infected population.

The result of the prevalence of anti-R7V antibody in the sera of HIV- infected patients is presented in Figure 3. Of the total population of the infected patients 83 (47.2%) tested positive for anti-R7V antibody while 45 (25.6%) were negative for this antibody. The remaining 48 subjects fell within the grey zone, that is, having titer value ratio of $(OD_{\text{sample}}/OD_{\text{CAL}})$ 0.8 - 1.0. Also, of the population that tested positive for anti-R7V antibody, females had remarkably higher percentage; that is, (61) 73.5% positive against (22) 26.5% positive males. There was however, no significant ($P > 0.05$) difference in anti-R7V antibody between male and female HIV- infected patients.

The prevalence of anti-R7V antibody in the sera of the 88 HIV negative subjects as shown in Figure 4 shows 1 (1.1%) positive, 2 (2.8%) grey and 31 (35.2%) negative for males against 2 (2.8%) positive, 3 (3.4%) grey and 49 (55.7%) negative females.

The relationship between anti-R7V antibody and CD4 count as presented in Figure 5, depicted patients that are anti-R7V positive had a mean CD4 count of 355.6 ± 19.2 .

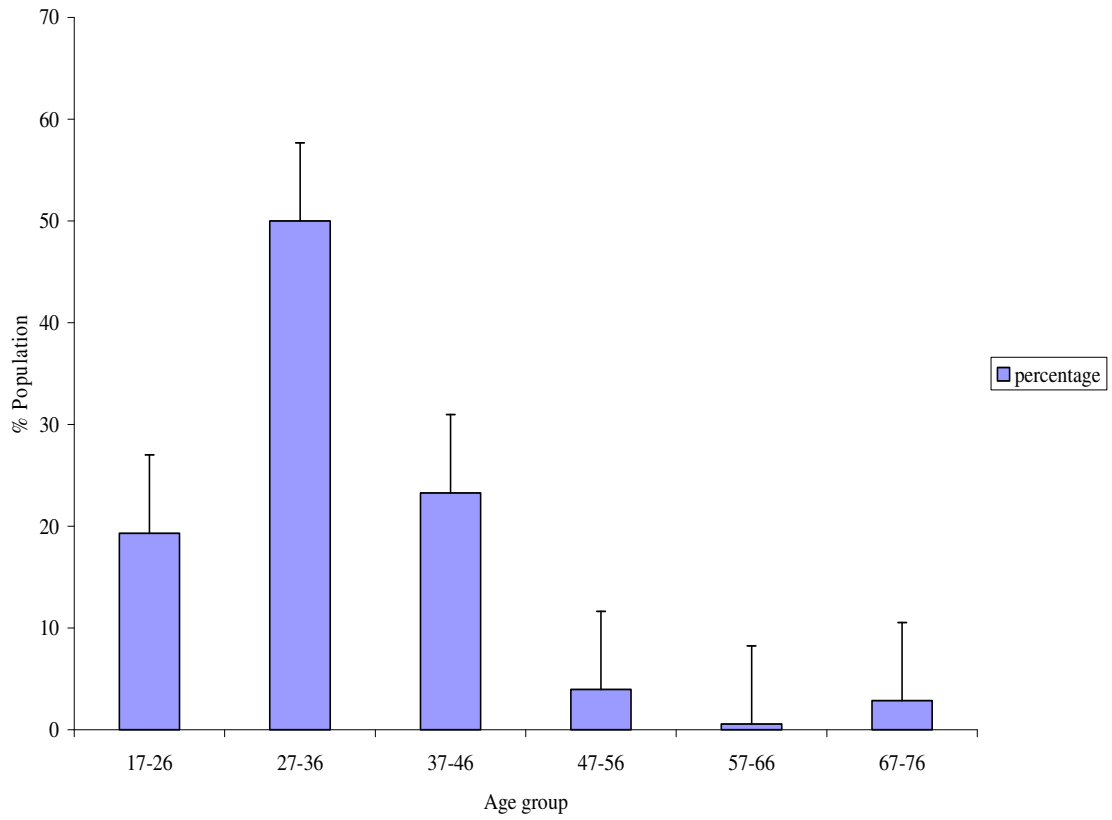


Figure 2. Distributions of HIV patients according to age.

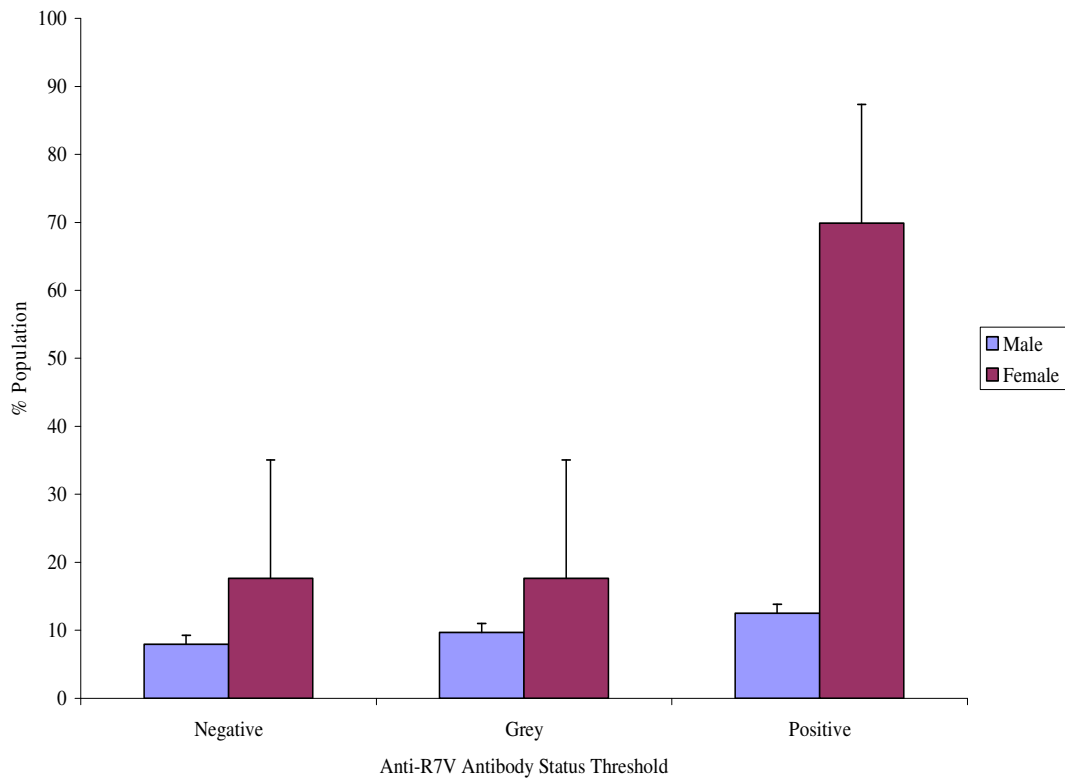


Figure 3. Seroprevalence of Anti-R7V antibody in HIV infected patients.

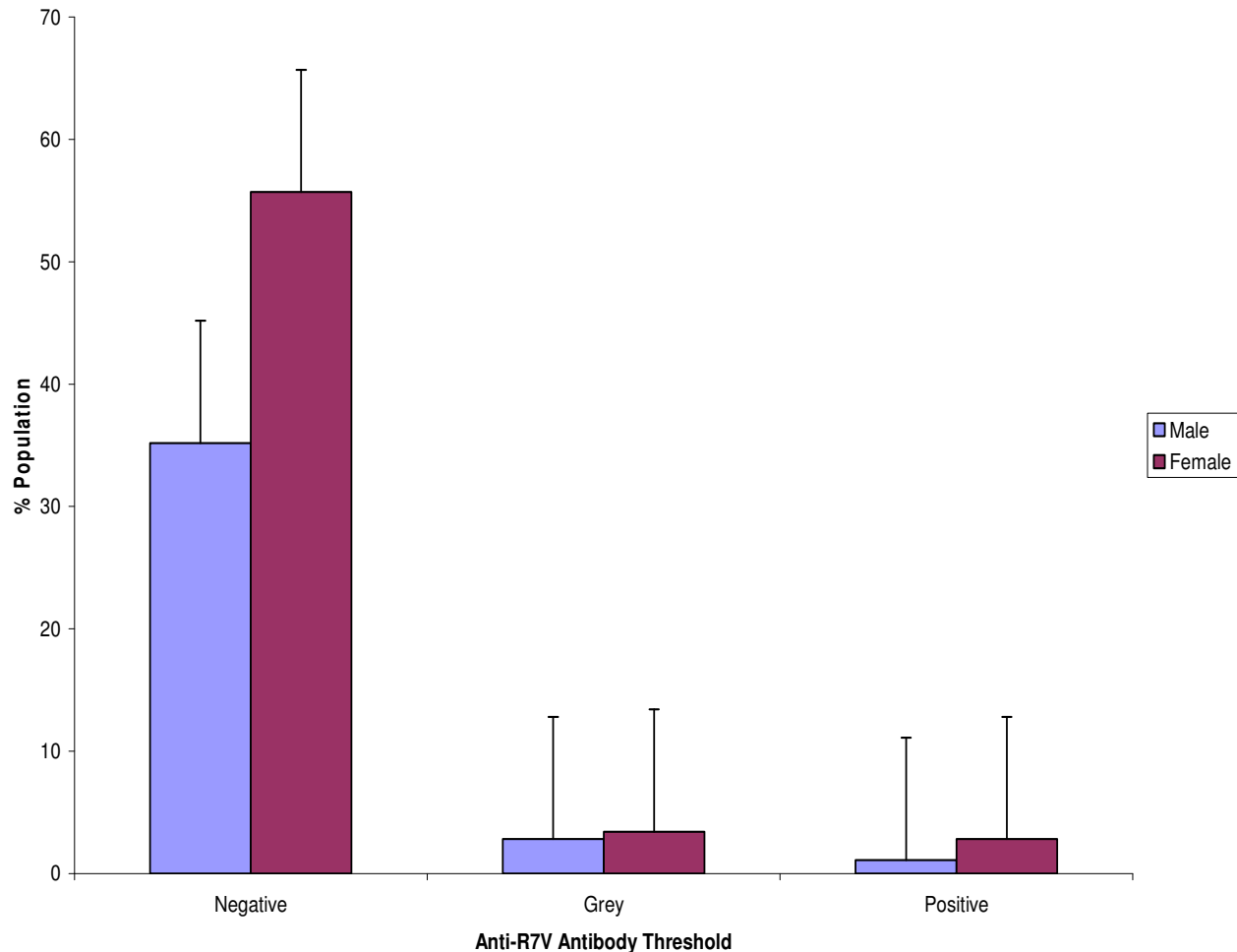


Figure 4. Seroprevalence of Anti-R7V antibody in HIV negative subjects.

The data showed that 19 (22.9%) of the anti-R7V positive antibody patient had a CD4 count of below 200 while 52 (62.7%) had CD4 count of 200 - 499 and 12 (14.5%) had CD4 count of 500 and above. On the test of independence of Anti-R7V antibody and CD4 count, the significant value of 0.08 may be adjudged significant since 92% confidence coefficient is high enough to indicate that CD4 count depends on Anti-R7V

The distribution of mean CD4 count according to age groups as shown in Figure 6 indicates the age bracket 17 - 26 yrs having the highest mean CD4 count of 404.9 ± 37.5 followed by 26 - 36 and 37 - 46 yrs with mean CD4 count of 356.9 ± 27.9 and 329 ± 24.5 , respectively. However the age bracket 27 - 36 had the highest number of patients 18 patients (10.6%) with CD4 count of above 500 and also those with CD4 count of between 200 - 499, that is, 36 (21.2%). Patients in the age bracket 17 - 26 yrs had 7 (4.1%) with CD4 count of above 500 and 19 (11.2%) with CD4 count of between 200 - 499. Similarly the age group 27 - 36 had the highest number of patients with CD4 count of below 200, 33 subjects (19.4%) followed by the age group 37 - 46 with 16 subjects

(9.41%) and 17 - 26 having 8 subjects (4.71%).

The relationship between CD4 count and sex is shown in Figure 7. The male patients had a mean CD4 count of 349.2 ± 26.1 against the female mean of 371 ± 22.2 . Furthermore the female with CD4 count of between 200 - 499 were 53 (30.3%) patients and 26 (14.9%) with CD4 count of above 500. In the male patients those with CD4 count of between 200 - 499 were 28 (16.0%) while 9 (5.1%) had CD4 count of above 500. The test for dependence of CD4 count and sex was not significant ($P > 0.05$), that is, CD4 count does not depend on sex.

Figure 8 shows the relationship between the presence of the Anti-R7V antibody with treatment status, that is, on drugs (ARDs) and drug naïve patients. There were only 12 (14.5%) drug experienced patients who tested positive for the presence of the anti R7V antibody as against 73 (85.5%) who were drug naïve patients.

DISCUSSION

The results of this study showed the population of female

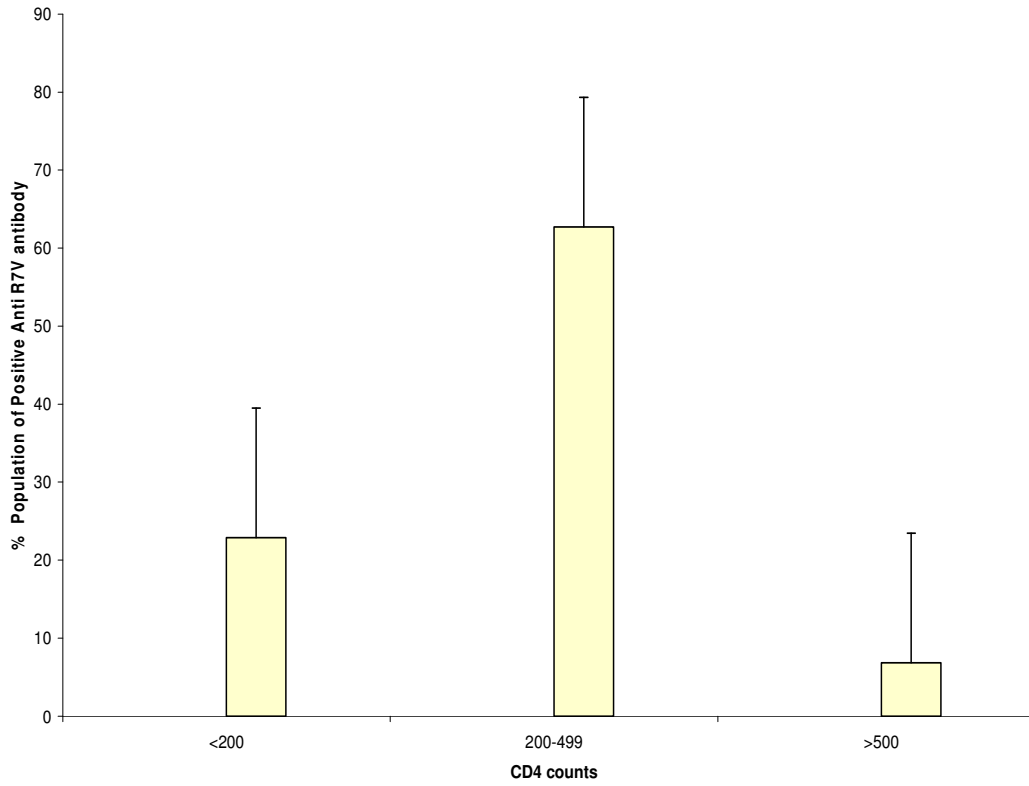


Figure 5. Relationship between Anti-R7V antibody with CD4 count.

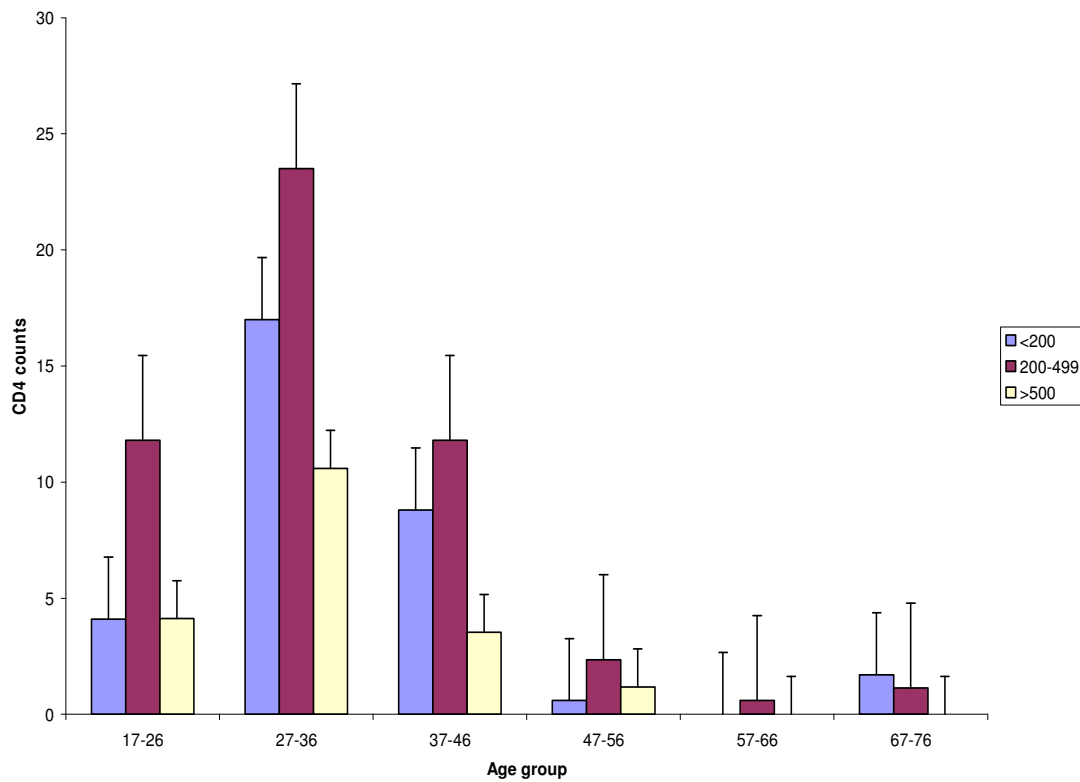


Figure 6. Relationship between age and CD4 count.

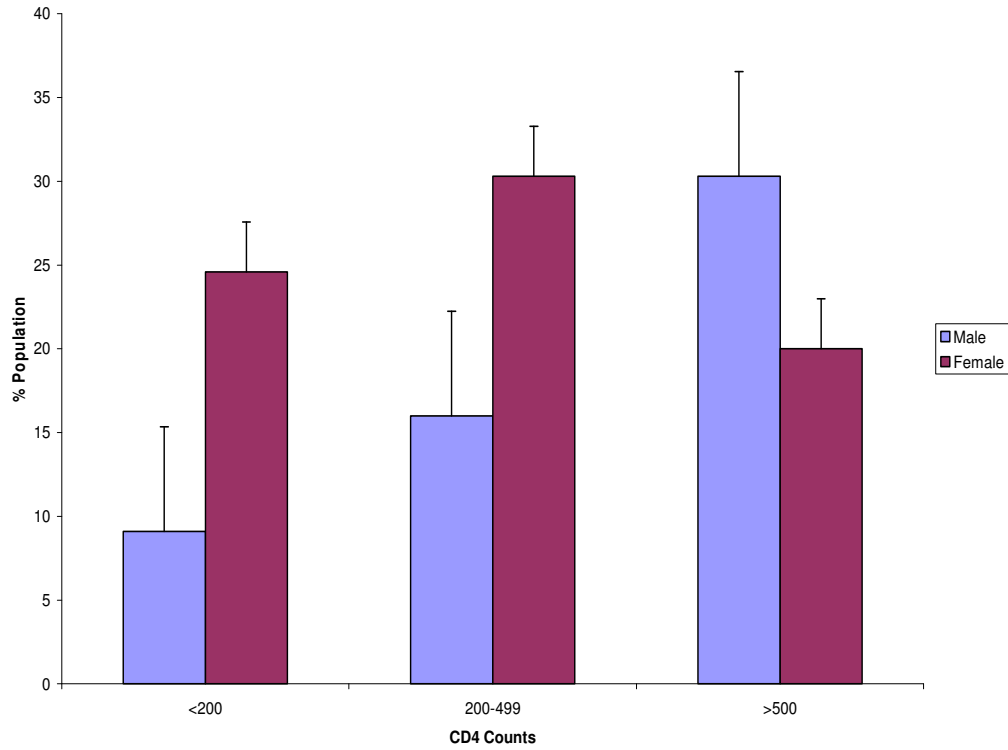


Figure 7. Relationship between sex and CD4 count.

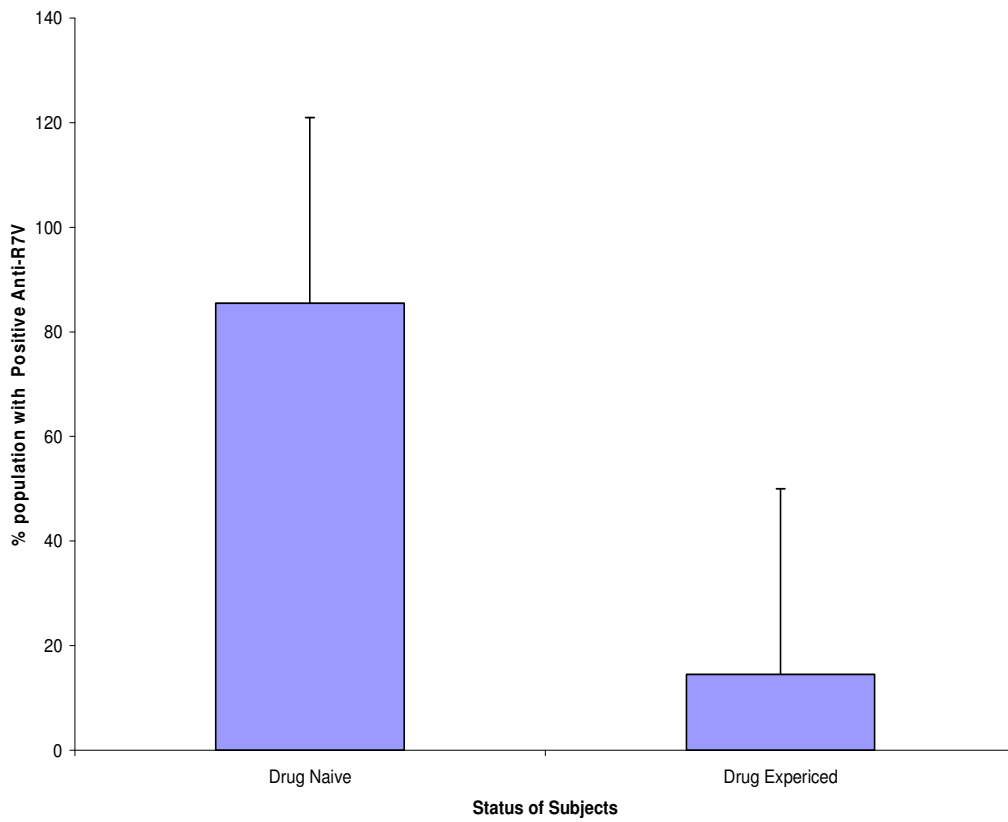


Figure 8. Relationship between status of patients and Anti-R7V antibody.

is far more affected when compared to males and the age group with the highest prevalence rate falls between the age brackets of 17 - 46 years. This is in agreement with the WHO statistics for Nigeria in 2005 which showed that the age bracket 15 - 49 yrs had the highest percentage distribution of HIV positive patients (WHO/UNAIDS, 2005).

Merson (2006) from his work stated that "At the start of the 21st century, 95% of new HIV infections and deaths occurred in developing nations and two thirds of persons living with HIV infection resided in sub-Saharan Africa. The age group most affected, young persons from 15 to 24 years of age, accounted for 40% of new HIV infections Worldwide". While Quinn (1996) showed that over half the victims of AIDS are women and a consequence of this is perinatal infection resulting in a significant number of children born with HIV infection.

About 48% of the population had the Anti-R7V antibody. This result is in line with the work of Chermann (2002) who found that only 35 - 65% of HIV- infected patients seem to develop specific antibody against the R7V epitope. From his work in France, 30% of the HIV population carry anti-R7V antibodies, in Malaysia 20% of the 200 tested patients had the antibody, while in the US, they found 50% of black HIV patients, 38% of Haitians, 30% of Caucasians and Hispanics and 25% Asians had the Anti-R7V antibodies.

The presence of the anti-R7V antibodies in individuals was correlated with treatment status of patients. The result from this study shows that in the treated population 14.8% had the antibody as against 85.2% of drug naïve population having the antibody. The observation of a significantly ($P < 0.05$) high percentage population of patients who are sero positive to anti-R7V antibody in drug naïve patients, may suggest that the synthesis of this antibody and its secretion into blood circulation may be related to the viral load. In the patients under treatment the drug may have reduced the viral load, that is, degree of infection. Consequently the patient's immune system may have reduced the rate at which the antibody is synthesized and released into circulation. This finding is in line with previous report of Chermann (2002) that the Anti-R7V antibody disappears with treatment. The disappearance of this antibody is attributed to the fact the anti-R7V antibody is a natural immunity that is acquired when the body is exposed to the HIV.

The CD4 count is a reflection of how many functional CD4 T-cells is circulating in the blood. CD4 cell tests are normally reported as the number of cells in a cubic millimeter of blood, or mm^3 . A standard reference range is not available for this test because the value bounces a lot as factors like, time of day, fatigue, stress, and test method can affect the test results and also numeric test results have different meanings in different laboratory.

The normal CD4 cell count for most laboratories is a mean of 800 to 1050/ mm^3 , with a range representing two standard deviations of approximately 500 to 1400/ mm^3

(Hughes et al., 1994). The wide range in normal values reflects the fact that the CD4 cell count is the product of three variables: the white blood cell count, the percent lymphocytes and the percent lymphocytes that bear the CD4 receptor. CD4 cells are reduced precipitously with acute HIV-infection, but may rebound over several weeks and then progressively decline over several years. CD4 cells determination is important in the stage evaluation of patients with HIV infection as it provides guidelines for differential diagnosis of complications and to guide both antiviral therapy and prophylaxis for opportunistic pathogens. The CD4 cell count is also a relatively consistent indicator of the response to antiretroviral therapy (Graham et al., 1995)

The Center for Disease Control and Prevention (CDC) defined a set of guidelines and recommendations for HIV-infected adolescents and adults on the basis of clinical conditions associated with the HIV infection and CD4 T lymphocyte counts (CDC, 1988; CDC, 1993; CDC, 1997). The system is based on 3 ranges of CD4 T-lymphocyte counts (> 500 , $200 - 499$, < 200) or CD4% and 3 clinical categories and is represented by a matrix of 9 mutually exclusive categories. This complex yet comprehensive case definition of AIDS enables the clinician to view HIV disease as a spectrum ranging from primary acute phase to advanced clinical disease and thus plays an important role in AIDS surveillance (CDC, 1993)

This study went further to correlate anti-R7V antibody with CD4 count and the result showed a mean CD4 count of 350 ± 19.2 with the presence of the Anti-R7V antibody. This result is in agreement with preceding studies (Galea et al., 1996; Galea et al., 1999; Haslin and Chermann, 2006; Maggiore, 2007) that postulated that subjects who have anti-R7V antibodies have lower likelihood of progressing to AIDS, because R7V epitope with which the antibody combines, is acquired by the HIV virus while budding from an infected cell. The anti-R7V antibody adheres to the R7V epitope on its surface and as the structure of the HIV envelope is changed it prevents the virus from entering healthy immune system cells thereby neutralizing the HIV.

This study found no relationship between presence of anti R7V antibody and age or sex. Information is scarce in the literature on the relationship of Anti-R7V antibody with sex and age of patients.

Conclusion

1. It is concluded from this study that the seroprevalence of Anti-R7V antibody is higher in HIV patients who are drug naïve when compared with drug experienced and HIV negative individuals.
2. It is also concluded that that there is a positive correlation between stable CD4 counts of HIV infected patients with Anti-R7V antibody.
3. Anti-R7V antibody may be a natural immunity against

HIV-infection in drug naïve HIV patients and that the synthesis and release of this antibody may decrease with ARD treatment.

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