*Full Length Research Paper*

# Effects of 12 weeks cycle exercise programme on CD4 count and viral load in HIV sero-positive patients in Kano, Nigeria

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This study investigated the effects of 12 weeks bicycle ergometer exercise programme on CD4 cell count and viral loads in 40 male HIV sero positive patients in Kano, Nigeria, aged 39.2 years who were randomly assigned to experimental (n = 20) and control (n = 20) groups. The patients' pre and post-test anthropometric measurements as well as CD4 cell counts and viral loads were obtained using standard biochemical techniques. Following 5 min warm up, the experimental group participated in 30 min incremental cycle (mechanical brake type) exercise, thrice a week for 12 weeks at 50 to 60% of their maximum heart rate (HR max). Significant increase in pre and post-test values of CD4 cell counts (28%) and reduction of viral loads (34%) were found in the exercise group in contrast to the control group (p < 0.05). It was concluded that regular participation in sub- maximal structured exercise programme could lead to beneficial changes in human immunodeficiency virus (HIV) positive patients.

**Key words:** Human immunodeficiency virus (HIV), sub-maximal exercise, CD4 cell count, viral loads.

## INTRODUCTION

Discovered in 1983, human immunodeficiency virus (HIV) is a retrovirus identified as the etiologic agent for acquired immunodeficiency syndrome (AIDS). AIDS is characterized by changes in the population of T-cells lymphocytes that play a key role in the immune defense system (Centre for Disease Control (CDC), 2008). In the infected individual, the virus causes a depletion of subpopulation of T-cells, called T-helper cells or CD4 cells. Within three to six weeks of exposure to HIV, infected individuals generally develop a brief, acute syndrome characterized by flu-like symptoms associated with high levels of viremia in the peripheral blood. In most infected individuals this is followed by an HIV-specific immune response and a decline of plasma viremia, usually within four to six weeks of the onset of the symp-

toms (Marston et al., 2007).

The asymptomatic period is characterised by persistent, low level plasma viremia and a gradual depletion of CD4+lymphocytes leading to severe immunodeficiency, multiple opportunistic infections, malignancies and death (Asamoah-Odei et al., 2004). CD4 cells or T-helper cells are white blood cells which organise the immune system's response to some microorganisms, including bacteria, fungal infections and viruses (O' Brien S. Nixion A Tynan and Glazier R, 2004). The CD4 count is the measurement of the number of CD4 cells, in a cubic millimeter of blood. This is sometimes written as CD4 cells/mm$^3$.

The CD4 count of a person who is not infected with HIV may lie anywhere between 500 and 1200 (Bartholomew,

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2001). HIV can infect CD4 cells and use them to produce more HIV copies. Even when a person with HIV feels well and has no symptoms, millions of CD4 cells are infected by HIV and are destroyed each day, and millions more CD4 cells are produced to replace them. If CD4 count falls below 250 to 200, it is recommended that the individual starts treatment with anti-HIV drugs because this is the level at which the risk of AIDS-related illnesses is greatly increased (Agbogboroma, 2008). The CD4 count can give an overall trend of the immune system's health in terms of whether it is declining or improving. It can fluctuate in response to infections, stress, smoking, exercise, the menstrual cycle, contraceptives, and the time of day and even the seasons of the year (Glencross et al., 2002).

Viral load is the term used to describe the quantity of HIV in the blood. The more the HIV in the blood, the faster the CD4 cells are likely to disappear, and the greater the risk of developing symptoms or further illness within the next few years (Valdiserri, R.O, Holtgrave, D.R, West, G.R, 1999). Viral load measurements can rise and fall from one blood sample to the next but this may have no long-term impact on the health of the person being tested (Kannangai et al., 2001).

Every day, over 6,800 persons become infected with HIV and over 5,700 persons die from AIDS, mostly because of inadequate access to HIV prevention and treatment services (Nigerian Federal Ministry of Health NFMH, 2005). The HIV pandemic remains the most serious of all infectious diseases and it poses the most serious challenge to public health (World Health Organization (WHO), The Joint United Nations Programme on HIV/AIDS (UNAIDS), The United Nations Children's Fund (UNICEF), 2007; Utulu, S.N., Lawoyin, T.O, 2007). Aerobic exercise has been found to be beneficial in many disease conditions and its application can never be over-emphasized (CDC, 2007). Studies have shown that regular physical activity can beneficially increase CD4 count and lowered viral loads levels, few investigations, if any, have been carried out on HIV patients in this part of Nigeria.

With increased prevalent rates of HIV/AIDS patients in Kano metropolis and the difficulty of some patients getting anti-retroviral drugs motivated the need to investigate the effects of 12 weeks cycling exercise programme on HIV positive patients in Kano, northern Nigeria. It is therefore hypothesized that the exercise programme would yield beneficial changes in HIV patients CD4 count and viral load, thus serving as a non-pharmacological means of improving the overall quality of life of the patients.

## METHODOLOGY

### Research design

This study was conducted at the outpatient unit of Fatima specialist hospital Kano, Nigeria, using a pre-test-post-test randomized-

controlled trial group design in which the dependent variables (CD4 count and viral load) were measured before and after cycle exercise programme. The initial phase of the study involved a comparison of subjects receiving antivirus treatment care (control group), with subjects receiving antivirus treatment care plus cycle exercise (exercise group) for 6 weeks. Following this initial phase of the study, subjects randomly assigned to the control group performed cycle exercise for 6 weeks, while the experimental group continued to perform cycle exercise for an additional 6 weeks (12 weeks total). The design was utilized for ethical reasons, given that all cycle sessions performed by the exercise group during the initial 6 weeks of the trial were performed in the presence of those subjects randomly assigned to the control group.

Using a randomised, controlled study design, 40 HIV positive males (n = 40) with an average age of 35 years were stratified into groups A or B (A: CD4 less than 200/µl, B: CD4 of 300 to 500/µl). They were randomly assigned to either exercise (n = 20) or control group (n = 20). The treatment group participated in a supervised 12 weeks aerobic cycle exercise programme level out thrice a week. The control group did not receive any exercise intervention initially but during the 2nd phase, the control group also participated in cycle exercise for 6 weeks. To assess CD4 cell counts and viral loads, bicycle ergometer exercise testing was used according to CDC (2007) classification and the progress of illness was carefully monitored.

### Selection criteria

Participants included HIV seropositive patients who had a CD4 count of 200 cells/µl or less and those with CD4 count between 300 to 500 cells/µl, most of who were on antiretroviral drug treatment. All participants were assembled at Fatima Hospital Kabuga Kano where the experiment was conducted. Each patient completed the physical activity readiness questionnaire (PAR-Q) (American College of Sports Medicine ACSM, 2007) and gave consent after the purpose, procedure, possible risks and benefits of the study were carefully explained to them (UNAIDS/WHO, 2007; ACSM, 2007). They were constantly briefed about their health condition throughout the experimental period as requested by Kano State branch of people living with HIV/AIDS (PLWHA).

### Experimentals

#### Physical measurements

The patients' anthropometric measurements were taken using the protocol of the International Society for the Advancement of Kinanthropometry (ISAK) (Martell-Jones et al., 2006). These included height and weight from which body mass index (BMI) calculated as a ratio of body mass to stature and expressed as wt $kg/m^2$, was derived. UNAIDS/WHO (2007) guidelines were followed during the laboratory procedure to estimate the CD4 count and viral load in sero positive patients. CD4 lymphocyte was constructed for each subject by modeling log CD4 count against time in days. These included: specimen selection, collection, storage, and testing, HIV testing technologies and strategies, selecting and evaluating testing technologies, quality assurance measures, ethical issues, factors affecting CD4 lymphocytes and viral loads, for example, infections, stress, and time of the day, smoking and others were carefully considered.

#### Measurement of viral load

Viral load was measured through RNA polymerase chain reaction (PCR) (standard method) using AMPLICOR HIV-1 MONITOR Test,

version 1.5 (Roche Diagnostics, USA). The protocol can detect viral load from 400 to 750,000 copies. Viral load blood samples (10 ml) were taken from the patients' antecubetal vein at 9 am using swab spirit to clean the area and applying the tenniquoit above the area. The vacutainer was used before exercise as described by Mustafa (2008). Blood was then put into ethylenediaminetetraacetic acid (EDTA) bottle label with identification code and centrifuged for 25 min at 1600 rpm. It was then aliquot into 3 tubes.

### Sample preparation

Extraction buffer was mixed with quantitative standard and 600 μl of extraction buffer was added into separate tubes labeled as high positive, low positive and negative controls. Each patient's sample was then put into 9 different tubes making a total of 12 tubes for each set of test. For the control tubes, 200 μl of normal human plasma and 50 μl of the control was added to each respective tubes and vortexes. For sample tubes, 200 μl of patient plasma were added in each tube and vortexes, and then incubated at room temperature for 10 min. After incubation, 800 μl of 100% isopropanol was added to each tube and vortexed immediately. Then, all samples and controls were centrifuged for 15 min at 12,500 rpm. The supernatant was aspirated and 1 ml of 70% ethanol was added and centrifuged for 5 min at 12,500 rpm. The supernatant was then centrifuged to spine down excess ethanol. 400 μl of diluents were subsequently added and vortexes noted for 3 s. This gave ribonucleic acid (RNA) for amplification.

### Amplification

700 μl of master mix reagent was mixed with 100 μl of manganese and invented to mix. Then 50 μl of the working master mix was dispensed into reaction tubes. 50 μl of extraction sample was added into the reaction tubes and then placed into the thermocycler (Apply Biosystems B No 9700, USA) and incubated for 1 h 30min. Subsequently, it was taken out from the thermocyler and 100 μl of denaturation solution was added.

### Detection

100 μl of hybridisation solution was added into the micro well plate after which, 25 μl of denatured amp icons was also added into the micro well plate. Serial dilution of 1 in 5 was made and incubated for 1 h at 37°C. After 1 h, it was washed by the micro well plate washer for 5 min, 100 μl conjugate was added and incubated further for 15 min and micro well plate was washed 5 times. 100 μl of substrate A and B was added and incubated at room temperature in the dark for 10 min. Thereafter, 100 μl of stop solution was added and the absorbance was read using micro well plate reader. This gave the optical density (OD). Finally, a soft wave (KC4 Roche, USA) was used to interpret the optical density (OS) base on the number of viral load copies.

### Measurement of absolute CD4 count

The CD4 count blood samples were taken from the patients' antecubetal vein at 9 am just before the exercise using vacutainer/needle, tenniquoit and a swab spirit. The swab spirit was used to clean the area where blood was to be taken from the antecubital vein with the tenniquoit tied just above the antecubital area and 10 ml of blood was taken as described by (Albert,

Abrahamsson, Nagy, 1990). 20 μl of whole blood sample was mixed with 20 μl of CD4 easy count antibody in a level rohren tubes, then incubated in the dark at room temperature for 15 min. 800 μl of CD4 easy count no lyse buffer was diluted then read using cyflow SL green. After reading, it was gauged and the count noted per ml. To find the CD4 count in μl, the report was created using a report template (software, cyflow SL Green, Partec, Germany) to arrive at CD4/μl.

### Exercise training

A bicycle ergometer (mechanical brake type, Model no. HG 5013, Hang Zhou Tianhai Holding Group Co. Ltd., China), which includes indicators of fractional resistance was used for the exercise programme according to ACSM (2007) guidelines. Rpm was measured using a micro switch device and counter. It was regularly calibrated to ensure accuracy. Each exercise session was preceded by a 5 min warm up session followed by gradual exercise loads administered incrementally based on 50 to 60% heart rate response, typically at 25 watts with power increments of 10 watts per stage of 10 min for 30 min. Participants in the experimental group exercised three times a week for 12 weeks. The control group was advised to continue with their normal day to day activities without partaking in any structured physical exercise. They however, received periodic health talks on HIV/AIDS, dieting and ways to avoid HIV complications and opportunistic infections. Approximately 75% of participants in the experimental group complied (> 60% attendance) with the exercise programme, and analyses of exercise relapse data indicated that obesity and smoking status, but not exercise-associated illness, differentiated compliant from noncompliant exercisers. During the study, two patients in the control group with CD4 count less than 200 cell/micro liter later died and another became seriously ill and could not continue with the study. Their data were subsequently excluded from the analysis.

### Pilot test

The PAR-Q was first tested for content validity. Subsequently, it was pilot tested within two weeks interval with 15 HIV positive patients in Infectious Diseases Hospital, Kano in June, 2008. Pearson's r of 0.65 to 0.8 was obtained.

### Statistical analysis

Statistical analyses included means, standard deviation and t-test to describe the patients' CD4 count and viral load values. The analyses also examined if any significant differences existed between the experimental and control group before and after the exercise programme.

## RESULTS

As provided in Table 1 are the means and standard deviation of subjects' age and physical characteristics. Their mean height was 157.4 ± 3.39 cm, while the average body weight was 39.77 ± 8.79 and 59.77 ± 8.79 kg for the pre-and post-test measures, respectively. Pre and post test body mass index (BMI) values were 19.4

**Table 1.** Subject's physical characteristics (N=40)

| Variables | Mean | SD | SE |
|---|---|---|---|
| Age (years) | 39.2 | 12.75 | 3.29 |
| Height (cm) | 1.57.4 | 3.39 | 0.932 |
| **Weight (kg):** | | | |
| Pre-test | 54.72 | 9.52 | 2.27 |
| Post test | 59.767 | 8.79 | 1.27 |
| **BM1:** | | | |
| Pre-test | 19.4 | 5.76 | 1.49 |
| Post-test | 19.9 | 4.76 | 1.39 |

N = Total number of subjects; SD = standard deviation; SE = standard error.

**Table 2.** Pre-and post-test descriptive statistics of CD4 count and viral load (experimental group; N= 20).

| Variables | Mean | SD | %Δ |
|---|---|---|---|
| **CD4 counts (cells/mm$^3$)** | | | |
| Pre-test | 269.67 | 77.7 | 28 |
| Post-test | 297.73 | 87.9 | |
| **Viral load (copies/ml):** | | | |
| Pre-test | 474000.2 | 198027.7 | -26 |
| Post test | 409666.5 | 203134.7 | |

N= Total number of subjects; %Δ = percentage change; SD = standard deviation.

**Table 3.** Descriptive statistics of CD4 counts and viral loads (Control group; N=20)

| Variables | Mean | SD | %Δ |
|---|---|---|---|
| **CD4 counts (cells/mm$^3$)** | | | |
| Pre-test | 207.667 | 83.96 | -7 |
| Post-test | 265.267 | 87.88 | |
| **Viral load (copies/ml)** | | | |
| Pre-test | 460333.3 | 202100.25 | 9.6 |
| Post test | 470000.0 | 21580414 | |

N = Total number of subjects; %Δ = percentage change; SD = standard deviation.

and 18.9 kg/m$^2$, respectively. There were no significant differences in the patients' body weight and BMI for pre and post test, respectively.

At the end of the 12 weeks exercise programme, the subjects had the following mean values: CD4 count (297.73 ± 87.9 cell/mm$^3$), viral loads (409666.5 ± 205134.7 copies/ml) (Table 2). In calculating the percentage change between the pre and post-test measurements in CD4 count, the following formula was used: post-test – pre-test × 100 and pre-test – post-test × 100 for viral load. This yielded an increment in CD4 (28%), and decrease in viral load (-34%) (Table 2). A high standard deviation (SD) was found for CD4 and viral load which suggests that the group had considerable variability in these measurements. The results showed that after the exercise programme the control group had the following profile: CD4 count (265.3 ± 87.8 cells/m$^3$); and viral loads (470000.0 ± 2158 copies/ml) (Table 3). The corresponding pre-post percentage changes CD4 count (-7%) and increase viral load (9.6%). These indicate a decrease in CD4 count by -7% and an increase in viral loads by 9.6% in the control group.

Table 4 summarizes the differences in both pre-test and post-test in CD4 count and viral load values. The correlation between exercise and CD4 count and viral loads were 0.996 for CD4, 0.895 for viral loads in the experimental group, while the calculated t values were 8.536 (CD4) and 11.481 (viral load) for experimental group. The control group had t values of 0.985 (CD4) and 0.824 (viral load), respectively. There was a substantial increase in CD4 count and a decrease in viral loads in the experimental group while in the control group there was a decrease in CD4 count and an increase in viral load, respectively. The study demonstrated a significant deference in CD4 count between the pre experimental and post experimental groups after the exercise programme (t = 8.3, p < 0.05) (Table 5). This suggests that 12 weeks cycle ergo-meter exercise programme can substantially increase CD4 counts in HIV/AID positive patient.

Variation in the patients' pre and post-test CD4 count is illustrated in Figure 1. The highest CD4 count of 500 cells/mm$^3$ (pre-test) was found in respect of subject 17, while subject 5 had the lowest value (180 cells/mm$^3$). Regarding post-experimental CD4 count, subject 17 had the highest value (580 cells/mm$^3$) whereas lowest value (170 cells/mm$^3$) was noted in respect of subjects 5 and 14. The percentage difference between the two groups was 28%. The result of this study revealed a significant difference between the experimental and control group with regards to viral load after the bicycle exercise programme (t = 11.5; p < 0.05) (Table 6). This shows that the exercise programme had a beneficial effect on viral load in the HIV patients. Variations in patient's viral load are further illustrated in Figure 2.

The highest viral load of 750, 000 copies (pre-test) is shown in subjects 5, 12, 13, 14, 16 and 20 and the lowest viral load copies of 100, 000 copies was found in patient 7. For the post-experimental viral load, subjects 5, 12, 13 and 20 had the highest value of 700,000 copies while the lowest value of 80,000 copies was recorded for subject

**Table 4.** Differences within groups in CD4 count and viral loads for post exercise interventions in experimental and control groups (N = 40).

| Variables | Baseline | post-test | df | t | Correlation | %Δ |
|---|---|---|---|---|---|---|
| **Experimental group (N=20)** | | | | | | |
| CD4 count (Cells/m$^3$) | 269.67±77.7 | 297.77±87.97 | 19 | 8.536 | 0.996 | 28 |
| Viral load (copies/ml) | 4740000.0±98 | 439666.5± 205 | 19 | 11.481 | 0.895 | -26 |
| **Control Group (N=20)** | | | | | | |
| CD4 count (cells/m$^3$) | 272.67±84 | 265.27±87.89 | 19 | 0.985 | - | -7 |
| Viral load (copies/ml) | 460333.3±202 | 470000±216 | 19 | 0.824 | - | 9.6 |

N= Total number of subjects, df-degree of freedom, t= test of significance, %Δ= percentage change.

**Table 5.** Student t-test for post-treatment CD4 count (N = 40).

| Group | Mean | SD | df | t-value | %Δ |
|---|---|---|---|---|---|
| Pre Experimental | 265.27 | 85.2 | 40 | 8.3 | 21 |
| Post Experimental | 297.7 | 87.9 | | | |

Critical value = 2.04. SD = Standard deviation; d f= degree of freedom; %Δ = percentage change; p ≤ 0.05.

17. The percentage difference between the two groups was 26%. It was discovered that patients with a low CD4 count of 200 and below did not respond substantially to the exercise programme unlike those with CD4 of 300 to 500 cells/mm$^3$.

The result of study shows greater increase in CD4 count and decrease in viral loads substantially in patients within group B: (CD4 count 300 to 500 cell/mm$^3$) than group A: CD 200 and below. Also, a general beneficial increase of 28% in CD4 count and 34% decrease in viral load in the experimental group than the control group was found. There was a decrease CD4 of -7% and increase of 9.6% viral loads in the control group. There were substantial correlations between low intensity exercise, CD4 count and viral loads of 0.91 and 0.82, respectively. Although, biological condition of the HIV and AIDS patients in the experimental group improved generally, no physical improvement on the patients' body weight and BMI was found. The fitness level of patients in the experimental group also improved in contrast to the control group. The increase in CD4 count during the exercise might be due to enhancement of exercise on the T heaper cells as well as on the immune system (George et al., 2000).

## DISCUSSION

The present study examined the effects of 12 weeks bicycle ergometer exercise programme on CD4 cell count and viral loads in male HIV positive patients. The findings of the study demonstrate significant differences between the experimental and control groups in CD4 cell count and viral loads as shown in Table 4. The beneficial increase in CD4 count following a structured exercise programme as found in this study is consistent with previous reports (Mustafa, 2008; Macarthur et al., 1993; Schlenzy et al., 1992; Shinkai et al., 1992). For instance, Mustafa (2008) reported a slower progression of HIV to AIDS at 1, 2, 3 and 4 year hazard ratio (HR) of 0, 0.96, 1.18, and 1.36, respectively. Exercising 3 to 4 times/week had a more protective effect than daily exercise. Exercise was found to increase CD4 count during a year by a factor of 1.07. In another study, Macarthur (1993) reported a moderate increase in CD4 count in 25 individuals with severely immuno compromised form of HIV infection. It was found that in 24 weeks of habitual exercise, there was evidence of a training effect on CD4 count and viral loads. CD4 reportedly increased by 12%.

In another study aimed at evaluating the long term benefit of exercise on the biological condition of HIV/AID patients as well as on the course of illness, Schlenzig et al. (1992) reported that within 12 weeks of aerobic exercise by seropositive patients, the overall biological condition of the HIV and AIDS patients as well as course of illness improved. A delay of AIDS related complications was noted which was due to an improvement in CD4 count and viral loads by 15 and 25%, respectively in the experimental group. Regular exercise though seemed to be correlated with increase in T4 cell count and decrease in viral loads. However, the present findings contrast with those of previous published reports (Terry et al., 1999; Stringer et al., 1998; Vergel et al., 1998) which demonstrated no change in CD4 count and viral loads after moderate to high intensity aerobic exercise pro-gramme on patients with CD4 count below 200 cells/mm$^3$. Rather, exercise created complications in HIV/AIDS patients leading to deterioration of their biological conditions and course of the illness.

The present study showed a beneficial effect on CD4 count and viral load with exercise, with more improvements found in early HIV infected patients CD4 count 300 to 500 cell/mm$^3$. Those with long time HIV/AID infection (that is, CD4 count 200 cells mm$^3$ or below) should participate in clinical supervised exercise pro-gramme. The attrition experienced in the control group during the study might be due to patients having already
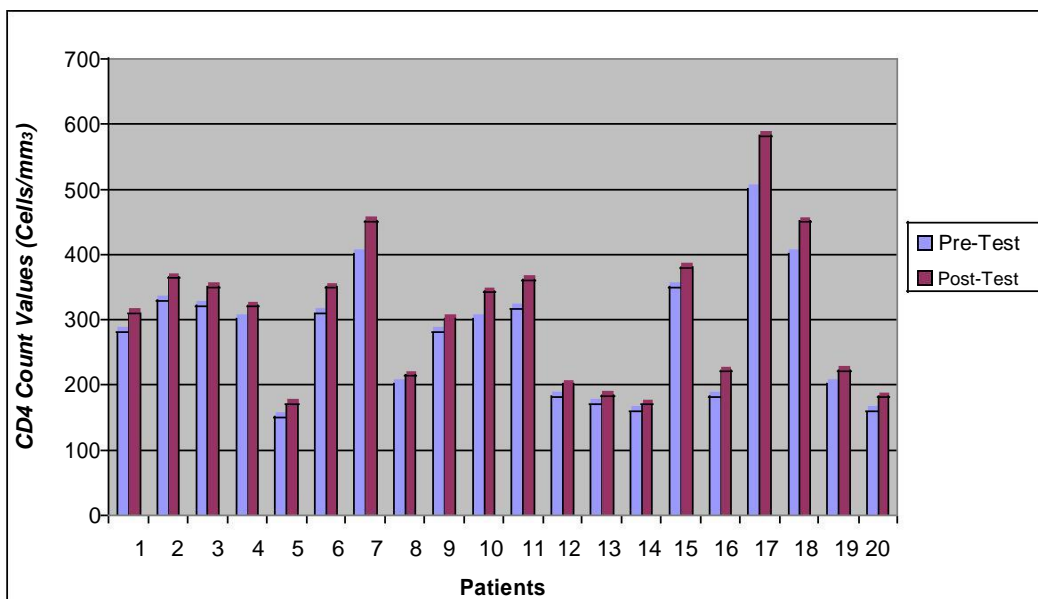
**Figure 1.** Pre and post CD4 count values (experimental groups).
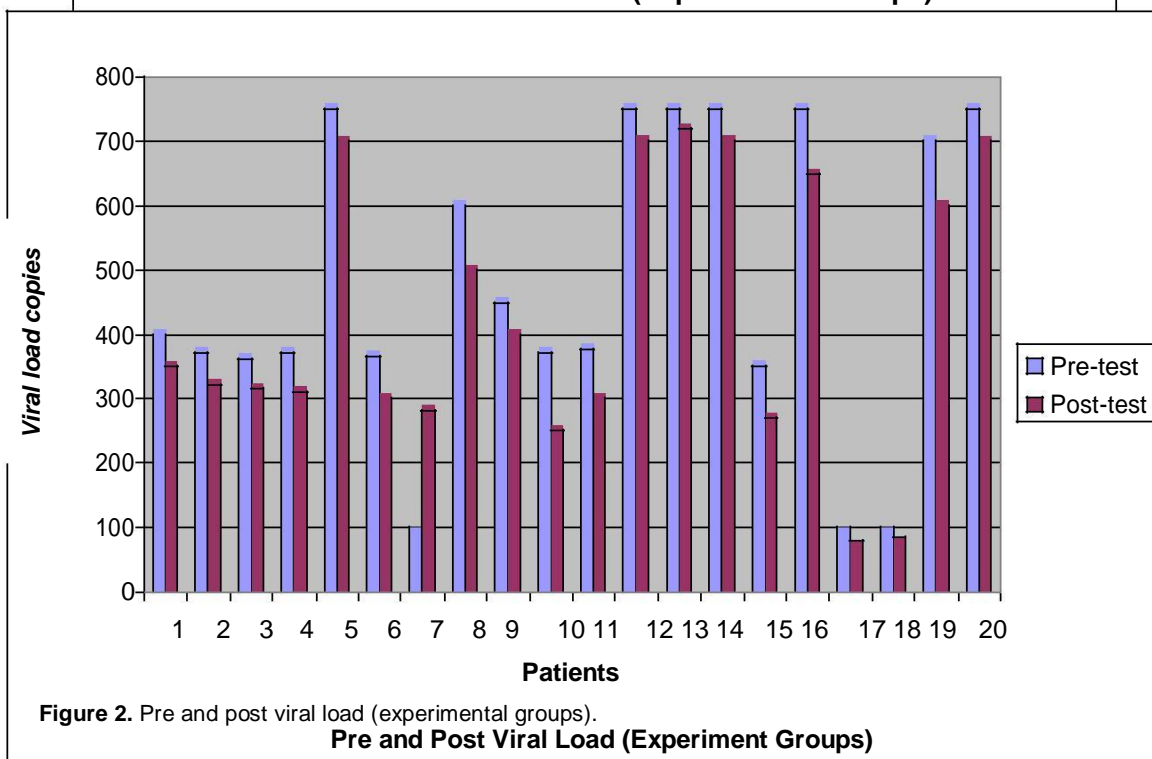**Pre and Post CD4 Count Values (Experimental Groups)**



**Figure 2.** Pre and post viral load (experimental groups).
**Pre and Post Viral Load (Experiment Groups)**

**Table 6.** Student t-test for post-treatment viral load (N = 40).

| Group | Mean | SD | df | t- value | %Δ |
|---|---|---|---|---|---|
| Experimental | 439666.5 | 2051 | 40 | 11.5 | 26 |
| Control | 470000 | 2158 | | | |

Critical value = 2.04. SD = Standard deviation; df= degree of freedom; %Δ= percentage change; p≤ 0.05.

weak system due to long term HIV infection and opportunistic infections.

### Limitations and implications for further research

This study did not take into consideration the fact that some of the patients were already on antiretroviral medi-cation and there were no restrictions on alcohol, smoking and patients' lifestyles. Further studies should take these short comings into account. Furthermore, it will be important in future studies to include health and fitness activities as an essential component of programme objectives for HIV management and to quantify the amount of exercise which will lead to beneficial increase

in CD4 count and decrease viral loads leading to overall health improvement and physical condition of HIV positive patients.

## Conclusion

In view of the marked changes found in the participants'
CD4 count and viral loads, it is concluded that regular participation in structured exercise programme could lead to beneficial changes in CD4 count and decrease viral loads in seropositive individuals.

## ACKNOWLEDGEMENT

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