EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 10
66-71
Year: 2018

EVALUATION OF RESPONSE OF IMMUNE PARAMETERS IN HIV SUBJECTS ON ANTIRETROVIRAL THERAPY IN PORT HARCOURT

Chinwe Rosemary Oparaodu, Confidence Kinikanwo Wachukwu and Easter Godwin Nwokah*

Department of Medical Laboratory Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

***Corresponding Author: Easter Godwin Nwokah**
Department of Medical Laboratory Science, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Nigeria.

Article Received on 13/08/2018 **Article Revised on 04/09/2018** **Article Accepted on 24/09/2018**

ABSTRACT
This cross sectional study was aimed at evaluating the response of immune parameters in HIV positive subjects on antiretroviral therapy in Port Harcourt, Rivers State, Nigeria. A total of three hundred subjects were involved in this study out of which two hundred were HIV positive subjects and 100 were HIV negative apparently healthy subjects between 20 and 70 years of age who were attending clinic at Braithwaite Memorial Specialist Hospital, Port Harcourt. Subjects who had diabetes, tuberculosis, severe malaria as well as pregnant women were excluded from this study. Blood samples were collected for the analysis of immune and haematological parameters. CD4 and CD3 counts were carried out using Fluorescent Activated Cell Sorter Count automation (FACSCount). CD8 Counts was derived by the subtraction of CD3 values from CD4 values. Total lymphocyte counts and white blood cell counts were determined using automated haematology analyzer (Sysmex XP-300). The HIV subjects had significant low CD4 counts value of 341.73 ± 84.21 as against the control value of 902.52 ± 33.39 ($p<0.0001$); Total WBC counts of 4.97 ± 1.53 as against control value of 6.34 ± 1.73 ($p<0.0001$) and CD4/CD8 ratio of 0.316 ± 2.02 as against control value of 1.773 ± 0.35 ($p<0.0001$). Conversely, CD8 counts of 1082.05 ± 41.63 of HIV subjects were significantly higher than the control subjects of 509.09 ± 94.16 ($p<0.0001$). Meanwhile, there was no significant difference ($p=0.669$) in CD3 counts between the HIV infected (1366.05 ± 181.23) and the control subjects (1411.43 ± 179.52). Similarly, there was also no significant difference ($p=0.031$) between lymphocyte counts of HIV subjects (44.03 ± 16.05) and the control (34.48 ± 10.92) ($p=0.031$). Also, there were no significant differences between the parameters among the HIV subjects according to gender. The male CD3 levels were 1381.27 ± 198.82/ml while the female were 1354.41 ± 169.55/ml ($p = 0.862$), male CD4 levels were 290.92 ± 90.54/ml, while that of female were 380.59 ± 78.16/ml ($p = 0.209$), male CD8 levels were 1223.58 ± 54.12/ml while the female were 973.82 ± 55.74/ml ($p = 0.061$), TWBC for male were 5.07 ± 1.60 x 10^9/ml and that of the female were 4.89 ± 1.50 x 10^9/ml ($p = 0.656$) and Lymphocytes in male HIV subjects were 44.59 ± 18.28 x 10^9/ml while the levels for female HIV subjects were 43.60 ± 14.38 x 10^9/ml ($p = 0.820$). However, the mean CD4/CD8 of the male HIV subjects were 0.237±1.67 while the female HIV subjects were 0.391±1.40 ($p=0.699$) respectively. The results from this study indicate that there is incomplete immune reconstitution among HIV subjects on antiretroviral therapy. It is recommended that more studies be conducted on the factors that are responsible for this phenomenon in HIV subjects on antiretroviral therapy.

KEYWORDS: HIV, Immune Response, ART.

INTRODUCTION
Human immunodeficiency virus (HIV) is a retrovirus which specifically targets the cluster of differentiation 4 (CD4) T-lymphocytes in humans, leading to impairment in immune responses.[1] There are two types of HIV; HIV-1 and HIV-2, with HIV-1 being the more virulent, easily transmitted etiologic agent of major HIV infections world over.[2] HIV pandemic is the most persistent medical challenge so far known to humanity in recent time.[3] HIV has killed over 25 million persons in the last 30 years.[4] By the end of 2014, about 36.9 million people were living with HIV globally, and sub-Saharan Africa was the region most affected having about 25.8 million persons living with HIV (WHO, 2014). About 9% of the global figure above live in Nigeria.[5] The prevalence of HIV in Nigeria is 3.4% and 15.2% in Rivers State, yet the country has a high number of people living with HIV infection.[6] This makes Nigeria the second largest HIV burden in the world.[7] The infection with HIV and subsequent depletion of CD4 T-cells leads to Acquired Immunodeficiency Syndrome (AIDS). AIDS has no cure yet, but the introduction of ART has arrested the rate of CD4 T-cell depletion.

The immune system is usually compromised in HIV infection, principally resulting from the depletion of CD4

T-cells. The other cells of the immune system such as monocytes, macrophages and dendritic cells are also infected by HIV. The macrophages eventually become reservoir of HIV infection.[8] HIV attaches to dendritic cells through several molecular structures,[9] and they concentrate the viral particles before transmitting them to CD4 cells. Consequently, there is a reduction in CD4 cells and eventually reduction in B-cell function in synthesis of immunoglobulins. There is also a decrease in the number of natural killer cells because the depleted CD4 cells cannot produce enough cytokines for their activation[8] Also, there is impairment in CD8 T-cells function, though the infected by HIV, is impairment contributes to the scope of the virus from immune control and eventual collapse of the human immune system.[10]

Antiretroviral treatment brings about a number of benefits to HIV-infected individuals including improvement in CD4 counts, viral load suppression and reduction in opportunistic infections.[11] However, ART has been reported not being able to prevent persistent T-cell activation,[12] although there was reduction in the generation of latently infected cells[13,14] have reported a 30-35% new cases of infection in high-income countries. Diagnosis and treatment of acute and recent HIV infection have been noted to be the driver of the HIV epidemic.[13] Despite obvious increase in CD4 counts there is persistence of immunodeficiency due to HIV.[15] Antiretroviral therapy helps to boost the CD4 T-cell counts in HIV subjects, by preventing the replication of the virus. It is believed that when the CD4 T-cell count has been normalized the individual's immune system becomes stronger and consequently becomes reconstituted and able to ward off other opportunistic infections. However, ART is not able to prevent persistent T-cell activation;[12] this has caused therapeutic switch as well as abuses. Consequently, there is a failure of the immune system, and the individual may eventually die.

There is paucity of data within the scope of this study especially within the study location as infected HIV subjects are burden with drugs resistance, lack of immune reconstitution even when they stick adherence thus, the study was intended to evaluate the immune status of HIV infected subjects on drugs. Also, a therapeutic switch should be secondary to an underlying factor therefore, the study tried to investigate whether the first line ART is no longer potent to warrant a switch to second line as assumed and seen in most cases. Furthermore, CD4 is the most common routine immune parameter assayed in the area of this present study hence, the study tried to investigate other immune parameters such as CD3, CD8, TWBC and TLC.

The aim of this study was to evaluate the immune reconstitution status of HIV subjects who are receiving antiretroviral therapy.

MATERIALS AND METHODS

This cross sectional study was carried out among subjects who were attending clinics at Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria. Following ethical clearance obtained from BMSH ethical committee and the Rivers State Hospitals Management Board Research and Ethics Committee and informed consent, a total of three hundred subjects (aged between 20 and 70 years) and who met inclusion criteria were recruited for the study. Two hundred were HIV-positive subjects, who had been on ART for at least one year, while one hundred were HIV-negative apparently healthy subjects. Subjects who had diabetes, tuberculosis, severe malaria as well as pregnant women were excluded from this study. A well-structured questionnaire was the study instrument and the subject's record book was used as well as secondary data source which some of the information were subjected to verification. Five milliliters (5ml) of blood were collected from each subject using standard technique. The blood samples were dispensed into Ethylene diethyl tetracetic acid (EDTA) container for CD4 count as well as the other immune and haematological parameters.

CD4 and CD3 counts were carried out using Fluorescent Activated Cell Sorter Count automation (FACSCount) (Lewis, 2014). CD8 Counts was derived by the subtraction of CD3 values from CD4 values. Total lymphocyte counts and white blood cell counts were determined using automated haematology analyzer (Sysmex XP-300).

Statistical Analysis: Data generated from the research were analyzed using Excel 2007 programme and SPSS (Version 20). Students' independent t-test was used to compare means of parameters between HIV patients and the control subjects. P-values less than 0.05 were considered statistically significant at 95% confidence level.

RESULT

Table 1 shows Comparison of immunological parameters in HIV and control subjects. The HIV subjects had significant low CD4 counts value of 341.73 ± 84.21 while the control value is 902.52 ± 33.39 ($p<0.0001$), TWBC counts of 4.97 ± 1.53 and the control value is 6.34 ± 1.73 ($p<0.0001$) and CD4/CD8 ratio of 0.316 ± 2.02 with a control value of 1.773 ± 0.35 ($p<0.0001$). Conversely, CD8 counts of 1082.05 ± 41.63 of HIV subjects were significantly higher than the control subjects of 509.09 ± 94.16 ($p<0.0001$). Meanwhile, there were no significant differences in CD3 counts of the HIV and control subjects 1366.05 ± 181.23 and1411.43 ± 179.52 ($p=0.669$) and lymphocyte counts of HIV subjects were 44.03 ± 16.05 and the control were 34.48 ± 10.92 ($p=0.031$) respectively.

Table 2 shows Comparison of Immunological Parameters for HIV-Infected Patients according to Gender. There were no significant differences between

the parameters among the HIV subjects according to gender. The male CD3 levels were 1381.27 ± 198.82/ml while the female were 1354.41 ± 169.55/ml (p = 0.862), male CD4 levels were 290.92 ± 90.54/ml, while that of female were 380.59 ± 78.16/ml (p = 0.209), male CD8 levels were 1223.58 ± 54.12/ml while the female were 973.82 ± 55.74/ml (p = 0.061), TWBC for male were 5.07 ± 1.60 x 10^9/ml and that of the female were 4.89 ± 1.50 x 10^9/ml (p = 0.656) and Lymphocytes in male HIV subjects were 44.59 ± 18.28 x 10^9/ml while the levels for female HIV subjects were 43.60 ± 14.38 x 10^9/ml (p = 0.820). However, the mean CD4/CD8 of the male HIV subjects were 0.237±1.67 while the female HIV subjects were 0.391±1.40 (p=0.699) respectively.

Table 1: Comparison of immunological parameters in HIV and control subjects.

Variables	CD3/ml	CD4/ml	CD8/ml	TWBC ($x10^9$)	LYM ($x10^9$)	CD4/CD8
HIV Subjects n = 200	1366.05±181.23	341.73±84.21	1082.05±41.63	4.97±1.53	44.03±16.05	0.316±2.02
Control Subjects n = 100	1411.43±179.52	902.52±33.39	509.09±94.16	6.34±1.73	34.48±10.92	1.773 ±0.35
p-value	0.669	<0.0001	<0.0001	<0.0001	0.031	0.0001
Remark	N/S	Sig	Sig	Sig	Sig	Sig

Sig=Significant; N/S=Non Significant

Table 2: Comparison of Immunological Parameters for HIV-Infected Patients According to Gender.

Variables	CD3/ml	CD4/ml	CD8/ml	TWBC ($x10^9$)	LYM ($x10^9$)	CD4/CD8
Males n = 86	1381.27±198.82	290.92±90.54	1223.58±54.12	5.07±1.60	44.59±18.28	0.237±1.67
Females n =114	1354.41±169.55	380.59±78.16	973.82±55.74	4.89±1.50	43.60±14.38	0.391±1.40
p-value	0.862	0.209	0.061	0.656	0.820	0.699
Remark	N/S	N/S	N/S	N/S	N/S	N/S

N/S=Non Significant

DISCUSSION

This study was carried out to assess immune reconstitution parameters in HIV subjects on antiretroviral therapy. In all intracellular infections in humans, the response of the T-cells is very important.[16] HIV subjects had lower levels of CD3 than control subjects, although this was not statistically significant. Antiretroviral therapy enhances immunity by reducing immune activation and CD3 levels in HIV subjects are indicative of the level of cellular immune response[17] in the infection. Thus, with the content of the subjects with antiretroviral therapy cellular immune responses are activated in the subjects. The CD4 level in the HIV subjects on HAART was significantly lower than the level for control subjects. The observation could be attributed to the apoptosis of CD4 T-cells in HIV infection.[18] CD4 levels have been used as a marker for the staging of HIV, making decisions for the therapy and also assessing how effective the treatment has been.[19] The significantly lower level of CD4 T-cells in HIV subjects in this study could also be due to antiretroviral failure that may result from viral mutation and toxicity of HIV drugs.[20] This finding agrees with the work of Gandhi and Walker (2002)[15], who reported that HIV-associated immunodeficiency, persists in the subjects. In this present study, the CD8 levels of HIV subjects were significantly higher than the control subjects. One of the features of HIV infection is expansion of the CD8 T cells.[21] The proliferation of CD8 cells in HIV infection is usually driven by the levels of the viral RNA in the body.[22] Therefore, the significantly higher level of CD8 counts in the HIV subjects may be due to the expansion of CD8 cells that accompanies HIV infection.

The CD4/CD8 ratio in this study was significantly lower in the HIV subjects than in control subjects. Caby *et al.*, (2016)[23] had reported that there is still immune activation in HIV infection, even in those receiving antiretroviral therapy and that this can lead to the depletion of CD4 cells, as well as expansion of CD8 cells. The depletion in CD4 and expansion in CD8, results in the characteristic low CD4/CD8 ratio seen in HIV infected subjects.[24] Healthy individuals usually have low-grade immune activation but this is accelerated in HIV infection therapy causing a deterioration of the immune system.[25] HIV therapy reduces immune activation.[26] However, individuals treated with HIV therapy do not verily achieve similar levels of immune activation as healthy individuals.[25]

Furthermore, data from this study also indicate that HIV subjects, despite being on therapy, have significantly (p <0.0001) lower total white blood cell counts compared to the control subjects. This is likely due to the immune deficiency that occurs in HIV infection. There is apoptosis of CD4 cells in HIV infection.[18] Since CD4 T-cells are a subset of body's white blood cells, a decline in their population would affect the total white blood cell counts. This finding agrees with the work of Chukwuezi *et al.*, (2013)[27] who have reported a similar finding. The

significantly low level of total white blood cells can also be due to the myelosuppressive effects of the HIV therapy.[28] Obeagu *et al.*, (2014)[29] have reported a similar finding from their work. The lymphocyte count for the HIV subjects was significantly higher than that of control subjects ($p = 0.031$). It has been reported that viral infections result in lymphocytosis.[30] The HIV infection elicits an immune reaction in which the lymphocytic cells are mobilized to defend the body against the infection.

Based on gender, there were no significant differences in the levels of all the parameters between male HIV subjects and female HIV subjects. The CD3 counts between male and female HIV infected subjects did not differ significantly. Since CD3 levels reflect cellular immune response in HIV subjects,[17] this finding indicates that HIV subjects from both genders have similar immune response in antiretroviral therapy.

The CD4 level for the female HIV subjects was higher than the level for male subjects, although the difference was not statistically significant ($p=0.209$). Menolesau and Marinescu, (2013)[31] reported that women tended to have a higher CD4 count than male subjects, probably because of the immune-modulatory effect sex hormones have on viral replication.[32] Generally, women are reported to have higher CD4 counts than men, whether healthy or infected with HIV, and in cases of HIV infection, repopulate their peripheral CD4 T-cells more quickly than men when viral replication is suppressed.[33]

Male HIV subjects had higher CD8 levels than the female subjects. However, the difference was not statistically significant ($p=0.061$).

Generally, male subjects seem to have a higher risk of poor CD4 T-cell response than female subjects, under antiretroviral therapy.[34] Several reasons have been adduced for this: from higher thymic output in women than that of men[35] to the anti-apoptotic effects of sex hormones in women.[36] It could also be as a result of latter expression of CCR5 on the surface of CD4 cells in women than in men.[37]

However, the female HIV subjects had significantly higher CD4/CD8 ratio than male subjects. In healthy subjects, the ratio is usually above 1.0 but can fall below 1.0 in HIV-infected subjects.[38] It will fall further in the absence of HIV therapy[39] but normally rises above 1.0 with antiretroviral therapy.[40]

The results from this study indicate that there is incomplete immune reconstitution among HIV subjects on antiretroviral therapy. It is recommended that more studies need to be conducted on the factors that are responsible for this phenomenon in HIV subjects on antiretroviral therapy.

CONCLUSION

This study was carried out to determine immune reconstitution parameters in HIV positive subjects on antiretroviral therapy. The immune reconstitution parameters that were determined were significantly lower in the HIV positive subjects than in the control subjects. In other words, the quality and quantity of CD4 cells and other immune cells are impaired in the HIV subjects, despite their being on HIV therapy. However, based on gender, female HIV subjects had higher CD4/CD8 ratio, with other parameters being non-significantly different. This finding indicates that female subjects have higher chances of immune reconstitution under antiretroviral therapy, than male HIV positive subjects.

REFERENCES

1. Gwarzo, M. Y & Muhamad, S. A. Setracellular SOD activity and plasma malondialdehyde in HIV subjects in Kano as surrogate markers of CD4 status. International Journal of Biomedical Science, 2010; 6(4): 294-300.
2. Reeves, J. D. & Doms, R. W. Human immunodeficiency virus type 2. Journal of Gerogal Virology, 2002; 83: 1253-1265.
3. Motoyo, B. O., Usen, U., Folarin, B. O, Okerentugha, P. O., Innocent-Adiele, H. C. & Okonko, I. O. Prevalence and seasonal variations of HIV 1 and 2 infections among children in Abeokuta, South West Nigeria. Journal of Microbiology Research, 2013; 3(3): 107–110.
4. World Health Organizaton (WHO) (2014). Global update on the health sector response to HIV. WHO, Geneva.
5. UNAIDS (2014). The Gap report: Children and pregnant women living with HIV. Joint United Nations Programme on HIV and AIDS. Geneva.
6. Awofala, A. A. & Ogundele, O. E. HIV epidemiology in Nigeria. Saudi Journal of Biological Sciences, 2016; 6: 1-7.
7. Isichei, C., Brown, P., Isichei, M., Njab, J., Oyebode, T. & Okonkwo, P. HIV prevalence and associated risk factors among rural pregnant women in north Central Nigeria. American Journal of Health Research, 2015; 3(1): 18-23.
8. Mohan, H. (2010). Textbook of pathology (6th ed). USA Jypee Brothers Ltd.
9. Nasreddine, N., Borde, C., Gozlan, J., Belecc, L., Marechal, V. & Hocini, H. Advanced glycation and products inhibit both HIV infection a transmission in trans of HIV-1 from monocyte-derived dendritic cells to autologies T-cells. Journal of Immunology, 2011; 186: 5687-5695.
10. McMichael, A. J. & Rowland-Jones, S. L. Cellular immune responses to HIV. Nature, 2001; 410(6831): 980-987.
11. Dagogo-Jack, S. HIV therapy and diabetes risk. Diabetes Care, 2008; 31(6): 1267-1268.
12. Vinikor, M. J., Cope, A. & Gay, C. L. Antiretroviral therapy initiated during acute HIV infection fails to

prevent persistent T-cell activation. Journal of Acquired Immune Deficiency Syndrome, 2013; 62(5): 505-508.
13. Archin, N. M., Vaidya, N. K. & Kuru, W. L. Swiss HIV cohort study: Treatment-naïve individuals are the major source of transmitted HIV-1 drug resistance in men who have sex with men in Swiss HIV cohort study. Clinical Infectious Diseases, 2014; 58(2): 285-294.
14. Gunthard, H. F., Aberg, J. A., Eron, J. J., Hoy, J. F., Telenti, A., Benson, C. A., Burger, D. M., Cahn, P., Gallant, J. E., Glesby, J., Reiss, P., Saag, M. S., Thomas, D. L., Jacobsen, D. M. & Voldberding, P. A. Antiretroviral treatment of adult HIV infection. Journal of American Medical Association, 2014; 312(4): 410-425.
15. Gandhi, R. T. & Walker, B. D. Immunologic control of HIV-1. Annual Review of Medicine, 2002; 53: 149-182.
16. Namu, S. J., Gicheru, M. M. & Nganga, Z. W. Antiretroviral drugs toxicity and immune status of HIV patients under comprehensive care in Embu, Kenya. European International Journal of Science and Technology, 2013; 2(1): 55-68.
17. Roitt, N. (2006). Immunology. London: Oxford Press.
18. Kashou, A. H. & Agrawal, A. Oxidants and antioxidants in the pathogenesis of HIV/AIDs. The Open Reproductive Science Journal, 2011; 3: 154-161.
19. Chukwuma, G. O., Ezeifeka, G. O., Ahaneku, G. I., Chukwuma, D. M., Nwaokorie, F. O., Kalu, S. O., Ndembi, N. & Manaffa, P. O. Total lymphocyte counts in resource limited settings. European Scientific Journal, 2013; 9(27): 63-73.
20. Sharma, S. K. & Soneja, M. HIV and immune reconstitution inflammatory syndrome. Indian Journal of Medical Research, 2011; 134: 866-877.
21. Catlfamo, M., Wilhem, C., Tcheung, L., Proschan, M., Friesan, T., Park, J. H., Adelsberger, J., Baseler, M., Makarelli, F., Davey, R., Roby, G., Rehm, C. & Lane, C. CD4 and CD8 cell immune activation during chronic HIV infection: Roles of homeostasis, HIV type 1 IFN and IL-7. The Journal of Immunology, 2011; 186(4): 2106-2116.
22. Catalfamo, M., Mascio, M. D., Hu, Z., Srnivasula, S., Thaker, V., Adelsbergr, J., Rupert, A., baseler, M/, Tagaya, Y. & Roby, G. HIV infection-associated immune activation occurs by two distinct pathways that differentially affect CD4 and CD8 T-cells. Proceedings of the National Academy of Science, USA, 2008; 105: 19851-19856.
23. Caby, F., Guihot, A., Lambert-Niclot, S., Guiguet, M., Boutollaeu, D., Agher, R., Valentina, M. A., Tubiana, R., Calvez, V., Marcellin, A. G., Carcelin, G., Autran, B., Costaglioja, D. & Katlama, C. Determinants of a low CD4/CD8 ratio in HIV-1 infected individuals despite long term viral suppression. Clinical Infectious Diseases, 2016; 12(19): 1297-1303.
24. Sauce, D., Larsen, M. & Fastenackels, S. HIV disease progression despite suppression of viral replication is assassinated with exhaustion of lymphopoiesis. Blood, 2011; 117: 5142-5151.
25. Buggert, M., Frederiksen, J., Noyan, K., Svard, J., Barqasho, B., Sonnerborg, A., Lund, O., Nowak, P. & Karlsson, A. C. Multiparametric bioinformatics distinguish the Cd4/CD8 ratio as a suitable laboratory predictor of combined T-cell pathogenesis in HIV infection. Journal of Immunology, 2014; 192: 2099-2108.
26. Tilling, R., Kinlock, S., Goh, L. E., Cooper, D., Perrin, L., Lampe, F., Zaunders, J., Hben, B., Tsoukas, C., Anderson, J. & Jannosy, G. Paralled decline of CD4/CD38 T-cells and viraemia in response to quadruple highly antiretroviral therapy in primary HIV infection. AIDS, 2002; 16: 589-596.
27. Chukwuezi, O., Chukwuezi, V. I. & Ezeifeka, G. O. Relationship between haematological values white blood cell, CD4 and HIV infection in individuals not yet on antiretroviral in Onitsha metropolis, Anambra State, Nigeria. The Experiment, 2013; 13(2): 843-846.
28. Owirendu, W. K. B. A., Quaye, L., Amidu, N. & Addai-Mensoh, O. Prevalence of anaemia and immunocological markers among Ghanian HAART-naïve HIV-patients and those on HAART. African health Sciences, 2011; 11(1): 2-15.
29. Obeagu, E. I., Obarezi, T. N., Oghuabor, B. N., Anaebo, O. V. N. & Eze, G. C. Pattern of total white blood cell and differential count values in HIV positive patients receiving treatment in Federal Teaching Hospital, Abakiliki, Ebonyi State, Nigeria. International Journal of Life Science, Biotechnology and Pharama Research, 391), 2014; 186-189.
30. Okeke, C. O., Amilo, G. I., Ifeanyichukwu, M. O., Obi, E. O., Okeke, A. C. & Ubeh, N. C. Effect of HIV infection and antiretroviral therapy differential white cell count. International STD Research and Reviews, 2015; 3(1): 14-22.
31. Menolesau, L. & Marinescu, P. Sex differences in HIV-1 viral load and absolute CD4 cell count in long term survivors of HIV-1 infected patients from Giurgiu, Somania. Revista Romania de Medicinal Laborater, 2013; 21: 217-224.
32. Fish, E. N. Sex-based differences predispose immune response. Nature Reviewed immunology, 2008; 8: 737-344.
33. Giordano, T. P., Wright, J. A., Hassan, M. O., White, A. C., Graviss, E. A. & Visnegarwala, F. Do sex and raced ethnicity influence CD4 cell response in patients who achieve virology suppression during antiretroviral therapy? Clinical Infectious Diseases, 2003; 37: 433-437.
34. Gandhi, R. T., Spritzler, J. & Chan, E. Effects of baseline and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in HIV-1 positive subjects: Results from ACTG 394. Journal of Acquired

Immune Deficiency Syndrome, 2006; 42(4): 426-434.
35. Pido-Lopez, J., Imani, N. & Aspinall, R. Both age and gender affect thmic suspect: More recent tymic migrants in females and males as they age. Clinical and Experimental Immunology, 2001; 125(3): 409-413.
36. Molly, E. J., O'Neill, A. J. & Grantham, J. J. Sex-specific alterations in neutrophil apoptosis: The role of estradiol and progesterone. Blood, 2003; 102(7): 2653-2650.
37. Portales, P., Clot, J. & Corbeau, P. Sex difference in HIV-1 viral load due to sex difference in CCR5 expression. Annals of Internal medicine, 2001; 134(1): 81-82.
38. Li, G., Verheyen, J., Rhea, S. Y., Voet, A., Vandanime, A. M. & Theys, K. Functional conservation of HIV-1 gag: Implications for rational drug design. Retrovirology, 2013; 10(126): 127-132.
39. Bonnet, F., Thiebaut, R., Chene, G., Neau, D., Pellegrin, J. L., Mercie, P., Beylot, J., Dabis, F., Salamon, R. & Malat, P. Determinant of clinical progression in antiretroviral naïve HIV-infected patients starting wighly active antiretroviral therapy. HIV Medicine, 2005; 6: 198-205.
40. Normann, A., Jung, C., Vallbracht, A. & Flehmia, B. Time course of hepatitis a viraemia and viral lead in the blood of human hepatitis in patients. Journal of Medical Virology, 2004; 72: 10-16.