Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 6, Issue 04, pp. 1289-1292, April, 2015

RESEARCH ARTICLE

CHANGES IN SOME ANTIOXIDANTS IN HIV POSITIVE PATIENTS ON HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (CASE STUDY OF NSUKKA SOUTH EAST NIGERIA)

[1]Aniagolu, M. O, [2]Ezeanyika, L. U. S, [3]Parker, J., [4]Shu, N., [*,5]Ngwu, A. M, and [6]Ikegwuonu

[1]Department of Clinical Chemistry, Enugu state University of Science and Technology
[2,3]Department of Biochemistry, University of Nigeria Nsukka
[4]Department of Pharmacology, University of Nigeria Nsukka
[5]Department of Haematology, Enugu state University of Science and Technology
[6]Department of Biochemistry, Nnamdi Azikiwe University Awka

ARTICLE INFO

Article History:
Received 17th January, 2015
Received in revised form
13th February, 2015
Accepted 29th March, 2015
Published online 30th April, 2015

Key words:
HIV/AIDS,
HAART,
CD4+,
Antioxidants.

ABSTRACT

Some biochemical changes accompany infection by HIV. Once CD_4+count decrease to 500 cells/mm^3, HAART (Highly Active Antiretroviral Therapy) is initiated. The aim of this work is to elucidate the effect of HAART on some of the biochemical changes caused by HIV/AIDS with reference to some components of the antioxidant system. Seventy subjects comprising twenty apparently healthy control subjects in Nsukka locality and fifty HIV sero positive subjects ready to be placed on HAART were recruited for the study. The concentrations and activities of the following biochemical parameters were estimated by the requisite spectrophotometric methods during three different clinical visits: Catalase, GPX, SOD, +GR and GSH. CD_4+ count was also estimated at each presentation. The results showed that the mean CD4+ counts, the mean activity for glutathione reductase and the mean reduced glutathione concentration were all significantly higher than those of the HIV+ subjects followed by their results 8 months into treatment, 4 months into treatment and finally treatment naïve ($p<0.05$). The mean activity for glutathione peroxidase was highest for the control subjects followed by 4 months into treatment, 8 months into treatment and treatment naïve ($p<0.05$). There was observable improvement in the results which seemed to dull between the 4th and 8th month, revealing either non compliance to treatment by patients, or adverse (toxic) drug effects, which tended towards compromising the positive effects of the drugs.

INTRODUCTION

The HIV/AIDS pandemic has been terrorizing humanity, over the past two decades. It has undermined the health of so many people, all over the world. Some biochemical abnormalities have been shown to accompany infection with HIV (Human Immunodeficiency Syndrome). The illness interferes with the immune system, making people with AIDS much more susceptible to infections, including opportunistic infections and tumors that do not affect people with working immune systems. Human Immuno Deficiency Virus primarily infects vital organs of the human immune system such as CD4+Tcells (a subset of T lymphocytes), macrophages and dentritic cells. It mostly infects and destroys CD4 cells (Alimonti *et al*., 2003). HIV induces the lysis of infected CD4 T cells, also Cytotoxic T cells, kill infected cells. In addition generalized immune activation, coupled with the gradual loss of the ability of the immune system to generate new T cells, all appear to account for the slow decline in CD4 T cell numbers (Hel *et al*., 2006). Once the number of CD4 T cells, drops below 200 cells/mm3 of blood, cellular immunity is lost (Alimonti *et al*., 2003). The reference range for CD4 T cells is 500 -1,500/mm3 of blood. CD4+cell counts are the best guide for when to start antiretroviral therapy. Antiretroviral therapy is generally recommended when CD4+ cell counts are in the range 200 to 350 cells/mm3 of blood (Siegfried *et al*., 2010). This range was recently raised to 350 to 500 cells/mm3 (Rathbun, 2013).
Highly active antiretroviral treatment, slows the course of the disease hence reduces deaths and new infections, but does not cure the disease. Highly active antiretroviral therapy came into existence during the 11th international conference on AIDS in Vancouver, British Columbia, July 7-16 1996 (Barlett, 2006). HAART is actually the use of multiple drugs that act on different viral targets. These drugs act on different stages of the HIV life cycle. Based on the retrovirus life cycle that these drugs inhibits they are broadly classified as entry inhibitors, Nucleoside and Nucleotide reverse transcriptase inhibitors (NRTI), Non-nucleoside reverse transcriptase inhibitors (NNRTI), integrase inhibitors, protease inhibitors

Corresponding author: Ngwu Amauche Martina
Department of Haematology, Enugu state University of Science and Technology, Nigeria.

(Kalyan, 2013; Quashie, 2013; Wensing, 2010). Typical combinations include 2 NRTI¬ + 1PI or 2NRTIs and INNRTI. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers elections or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals which can start chain reactions; these reactions can damage or cause the death of a cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibiting other oxidation reactions. They do these by being oxidized themselves, so they are often reducing agents (Helmut, 1997). Studies have shown that the antioxidant system of the body is adversely affected in HIV infection and changes in its components have been documented by several workers (Pasupathi *et al*., 2009; Sharma, 2014; Suresh *et al*., 2009). The current treatment for HIV infection consists of highly active antiretroviral therapy. This is usually introduced as soon as patient CD4+ count reduces to 500 cells/mm3 of blood. This study was designed to look at the effect of HAART on some antioxidant activities and concentrations in HIV positive patients.

MATERIALS AND METHODS

50 HIV positive HAART naïve individuals attending the AIDS clinic of Bishop Shanahan Hospital Nsukka South East Nigeria. and 20 apparently healthy (HIV negative) individuals (control subjects) were recruited for the study. Informed consent was obtained from the participants and ethical clearance was sought for and obtained from Annunciation Hospital ethical clearance committee, Emene Enugu Nigeria.

Inclusion criteria

HIV positive patients not yet on antiretroviral therapy, but are due to be placed on it by virtue of their CD_4^+ Counts (Patients with CD_4^+ Count below 500 cells/mm^3 of blood are placed on HAART)

Design of the experiment

All subjects were tested at presentation (after confirming their retroviral status), 4 and 8 months into treatment with HAART. Their CD_4^+ counts were also estimated at each presentation.
NB: The combination in use in Bishop Shanahan Hospital is Combivir N (it contains Zidovudine, Lamivudine and Nevirapine).

Specimen Collection and Processing

5mls of venous blood was aseptically collected from each subject, after an overnight fast and emptied into potassium Ethylene diamine tetracetic acid tubes (4mls) and lithium heparin tubes (4mls). Retroviral screening and confirmation were done using the requisite methods. CD_4^+ enumeration was done by the principle of flow cytometry, using the Partec cyflow machine. The antioxidants catalase, glutathione, glutathione peroxidase glutathione reductase and superoxide dismutase were all assay using Randox kits that utilize spectrophotometric methods.

Statistical Analysis

The data were presented as mean ± SEM. The results were analyzed using analysis of variance (ANOVA) and separate comprisons were done, using post HOC Tests. Pearson Correlation was employed in analyzing the relationship between CD4+ count and the antioxidants.

RESULTS

Group I- represents apparently healthy individuals made up of (20 subjects – 11 males and 9 females).

Group II – represents HIV positive individuals before treatment, made up of (43 subjects -34 females and 9 males).

Group III – represents the same HIV positive individuals 4 months into treatment.

Group IV – represents the same subjects 8 months into treatment.

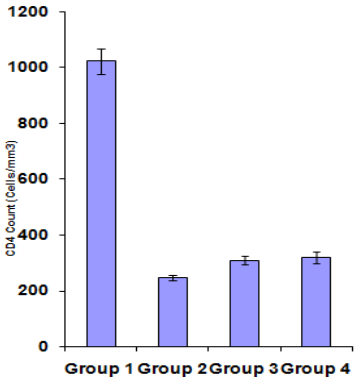

Fig 1. CD4+ count of HIV positive and negative subjects in Nsukka locality

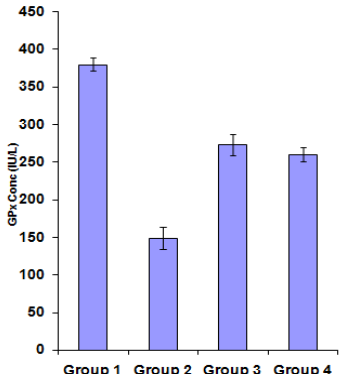

Fig 2. Glutathione peroxidase activity of HIV positive and negative subjects in Nsukka locality

Figures 1-6 are pictorial representations of the effect of highly active anti-retroviral therapy on different Biochemical parameters as seen in Nsukka Locality, Situated in Enugu State South-East Nigeria. In figure 1, mean $CD4^+$ count for apparently healthy subjects (control) (group I) was significantly greater than those of the experimental subjects before and after treatment ($p< 0.05$). The mean count for group IV was greater than that of group III, but the difference did not achieve statistical significance ($p>0.05$). However both results were significantly greater than the mean count for the patients when they were treatment naïve (group II) ($p<0.05$). Figure 2 shows that the mean activity of glutathione peroxidase was significantly greater for group I than for groups III, IV and II ($p<0.05$).

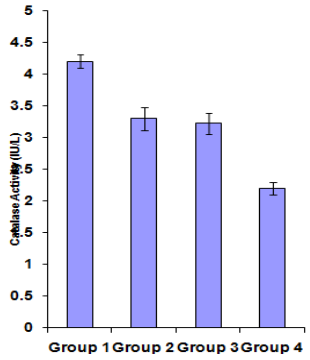

Fig 3. Catalase activity of HIV positive and negative subjects in Nsukka locality

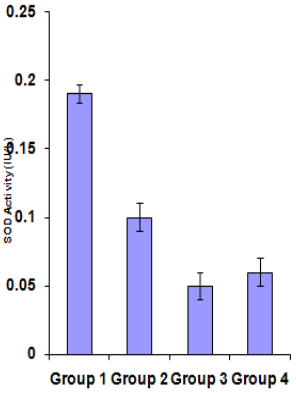

Fig 4. Superoxide dismutase activity of HIV positive and negative subjects in Nsukka locality

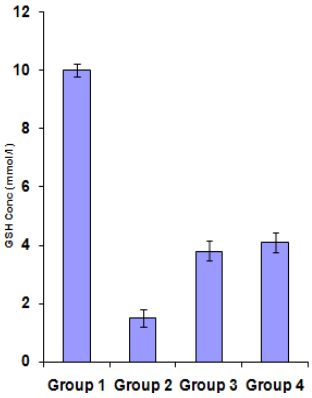

Fig 5. Glutathione concentration of HIV positive and negative subjects in Nsukka locality

Separate comparisons revealed that the difference between the means for group II and IV was not statistically significant ($p>0.05$). Figure 3 shows that the serum catalase activity was greater for group I than for groups II, III and IV ($p<0.05$) in that order. When compared separately the difference between groups II and III, did not achieve statistical significance ($p>0.05$). Figure 4 shows that the mean superoxide dismutase activity was greater for group I than for groups II, IV and III in that order ($p<0.05$). However the differences between groups II, IV and III, did not achieve statistical significance ($p>0.05$). Figure 5 shows that mean concentration of reduced glutathione, was greater for group I than for groups IV, III and II in that order ($p<0.05$). When compared separately the differences between the means of groups IV and III did not achieve statistical significance ($p>0.05$). Figure 6 shows that the mean glutathione reductase activity was greater for group I than for groups IV, III and II ($p<0.05$) in that order. When separately compared, the difference between groups IV and III did not achieve statistical significance. Pearson correlations showed that CD_4 count correlated positively with the activities of, glutathione peroxidase, catalase, superoxide dismutase, glutathione reductase and with serum concentrations of reduced glutathione.

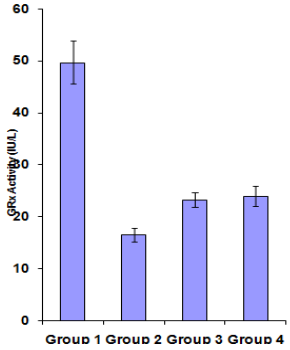

Fig 6. Glutathione reductase activity of HIV positive and negative subjects in Nsukka locality

DISCUSSION

It is a well known fact that the antioxidant system of the body which contains these enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase) and glutathione among others, is important in counteracting the effect of reactive oxygen species (oxidative free radicals) produced during metabolic processes or produced as a result of increased activation of polymorphor nuclear leucocytes during HIV or other infections. In this study, an improvement in the activities of glutathione peroxidase, glutathione reductase and in the concentration of reduced glutathione, was observed in the subjects from HAART naivety to eight months into HAART but the control subjects still showed much higher activities and concentration for these parameters. It was also observed that the activities of catalase and superoxide dismutase did not show any observable improvement even eight months into treatment with HAART. These findings, agreed with the work of, Sharma, 2014 which claims that treatment with HAART have been to cause antioxidant enzyme dysfunction.

However the work disagrees with that of Ibe *et al*., 2013 which showed increases in SOD and catalase activity on of commencement of HAART. CD4+ count itself though observably increased from the basal stage to the eight month, did not really show remarkable increase, when compared to the healthy control group. The question now becomes, what exactly are patients battling with, after the commencement of HAART? Is it the illness that is being treated or the effect of the drugs that are being employed in treating the illness? The antioxidant system by virtue of the reactive oxygen species being produced as a result of infection with HIV due to increased immune metabolism is already under stress, hence placing the immune system under additional stress, due to the increased rate of apoptosis that leads to CD4+ depletion (Ibe *et al*., 2013). NB: reactive oxygen species lead to increased apoptosis. In addition to this, HAART is a combination of drugs that must be metabolized. This process of metabolism apparently generated oxidative free radicals that compromised

the activities of some of the antioxidant enzymes (precisely catalase and SOD) and made it impossible for the concentration of glutathione to increase as should have been expected, after commencement of treatment. NB: the effect of reactive oxygen species (ROS that is oxidative free radicals) is a cyclic reaction. This is because ROS stimulate the production of transcription promoter nuclear factor, (TPNF) which in turn leads to increased viral gene expression, which also leads to increased HIV infection. Increased HIV infection, invariably leads to increase production of reactive oxygen species.

Conclusion

This whole analysis may mean then that increased production of ROS by the consumption of HAART may indirectly encourage the perpetuation of the disease, while trying to ameliorate it. We then recommend that in the treatment of HIV/AIDS with HAART, antioxidant preparations, for example N-acetyl cysteine (NAC), a prodrug of GSH (reduced glutathione), should be included, to help replenish antioxidants and improve survival of HIV patients (Herzenberg *et al*.,1997).

Conflict of interest

Authors declare no conflict of interest.

Authors' contributions

Aniagolu Miriam Obiageli

- Conception and design, Acquisition of data, Analysis and interpretation of data
- Drafting the article, Critical revision of the article
- Final approval of the version to be published

Ezeanyika, Lus

- Drafting the article, Critical revision of the article
- Final approval of the version to be published

Parker Joshua

- Analysis and interpretation of data
- Drafting the article, Critical revision of the article
- Final approval of the version to be published

Shu N

- Final approval of the version to be published

Ngwu, Amauche Martina

- Critical revision of the article
- Final approval of the version to be published

Ikegwuonu

- Final approval of the version to be published

Acknowledgements

We acknowledge the assistance of Dr Simeon Egba and Mrs Beatrice Okoro of Bishop Shanahan Hospital Nsukka. The authors sincerely appreciate the assistance of the Rev. Sisters of Daughters of Divine love congregation, their laboratory staffs both at Annunciation Hospital Enugu.

REFERENCES

Alimonti, J.B., Ball, T.B., Fowks, K.R. 2003. Mechanisms of CD4+T lymphocyte cell death in human immunodeficiency virus infection and AIDS. *Journal of Genetic Virology*. 84 (7): 1649-1661.

Barlett, J.D. 2006. Ten years of HAART. Medscape.

Hel, Z., McGhee J.R. and Mestecky J. 2006. HIV infection: first battle, decides the war. *Trends in Immunology.* 27 (6):274-81.

Helmut, S. 1997. Oxidative stress: Oxidants and antioxidants. *Experimental physiology* 82 (2):291-5.

Herzenberg, L.A., De Rosa, S.C., Dubs, J.G., Roederer, M., Anderson, M.T., Ela, S.W. and Deresinski, S.C. 1997. Glutathione deficiency is associated with impaired survival in HIV disease. *Proceedings of the National Academy of Sciences* 94 (5): 1967-72.

Ibe, B.0., Habu, J.B. and Eze, S.C. 2013. Discordant Levels of SOD and Catalase, Observed in ART Naïve and experienced HIV Patients in South East Nigeria. *Journal of Infectious diseases and Therapeutics*. 1:8-16.

Kalyan Das. 2013. HIV-1 reverse transcriptase and antiviral drug resistance part 1. *Current opinion in virology* 3 (2):111-118.

Pasupathi, P., Ramchandran, T., Sindhu, P.J., Saranavan, G. and Bakthavathsalam, G. 2009. Enhanced oxidative stress markers and antioxidant imbalance in HIV infection and AIDS patients. *Journal of Scientific Research* 1:2.

Quashie, P. K. 2013. HIV Drug Resistance and the Advent of Integrase inhibitors. *Current Infectious Disease Reports* 15 (1): 85-100.

Rathbun, R.C. 2013. Antiretiretrovial therapy, for HIV infection Medscape Reference articles. *HIV Disease* and *Pediatrics HIV*. Updated August 15, 2013.

Sharma, B. 2014. Oxidative Stress in HIV patients receiving Antiretroviral Therapy. *Current HIV Research, 2014*. 12(1): 1-9.

Siegfried, N., Uthman, O. and Rutherford, G. 2010. Siegfried, Nandi ed. "Optimal time for initiation of antiretroviral therapy in asymptomatic, HIV-infected, treatment-naïve adults". Cochrane Database of systematic Reviews (3).

Suresh, D. R, Annam, V., Pratibha, K., and Marutiprasad, B.V. 2009. Total antioxidant Capacity, a novel early biochemical Marker of Oxidative Stress in HIV infected individuals. *Jounal of Biomedical Science* 16; 61.

Wensing, A.M. 2010. Fifteen years of HIV protease inhibitors: raising the barrier to resistance. *Antiviral Research.* 85 (1):59-74.

****