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Original Article

CORRELATION OF TOTAL ANTIOXIDANT STATUS (TAS) WITH DNA DAMAGE IN HIV/AIDS PATIENTS

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ABSTRACT

Objective: The search of a suitable biomarker for an impact of antiretroviral therapy (ART) on oxidative stress and resultant deoxynucleic acid (DNA) damage in human immunodeficiency virus and acquired immune deficiency syndrome (HIV/AIDS) patients is ongoing. There is urgent need of such biomarker that could alert the clinician to investigate further non-AIDS-related diseases in HIV/AIDS patients taking antiretroviral therapy. Such a tool should be inexpensive and facilities for its determination easily available. The objective of this study was to explore total antioxidant status (TAS) as a biomarker for oxidative DNA damage in HIV/AIDS patients with ART.

Methods: This was a cross-sectional study involving 300 HIV-positive and 100 HIV-negative subjects have aged 20–60 y. We used plasma levels of the oxidized base, 8-hydroxy-2-deoxyguanosine (8-OHdG), as our biomarker of oxidative DNA damage. 8-OHdG was measured with the highly sensitive 8-OHdG check enzyme-linked immunosorbent assay (ELISA) kit.

Results: The varying ART has not had much effect on TAS levels, but there were different levels of DNA damage in ART first line, ART second line and ART not yet started patients. There is a negative correlation between TAS & DNA damage.

Conclusion: In this study, we observed that ART plays a significant role in the oxidative DNA damage. Decreased TAS is associated with increased DNA damage.

Keywords: Human Immunodeficiency virus (HIV) infection, Insulin resistance, DNA damage, 8-OH-dG.

INTRODUCTION

Antiretroviral therapy has been a spectacular success. For those who are motivated to take therapy and who have access to lifelong treatment, AIDS-related illnesses are no longer the primary threat, but a new set of HIV-associated complications have emerged, resulting in a novel chronic disease that for many will span several decades of life. Treatment does not fully restore immune health; as a consequence, the number of inflammation-associated and/or immunodeficiency complications such as cardiovascular disease and cancer are increasing in importance. Cumulative toxicities from exposure to antiretroviral drugs for decades cause clinically relevant metabolic disturbances and end-organ damage [2].

After ART although there is some improvement in CD4 count, disturbed glutathione redox status and decreased antioxidant levels seem to persist even during HAART, and these effects not only may contribute to persistent immunodeficiency in these patients but also may potentially enhance the toxicity of such therapy. The antioxidant deficiency in HIV-positive populations is probably due to increased utilization of antioxidant micronutrients because of increased oxidative stress rather than to inadequate dietary intake [1]. A rapidly growing and remarkably consistent evidence base indicates that many markers of inflammation are higher in antiretroviral-treated adults than in age-matched uninfected individuals [3, 4]. However, a great deal of work remains to be completed in defining the exact roles of oxidative DNA damage in the pathogenesis of diseases.

Oxidative damage results from biochemical interactions between reactive oxygen species (ROS) and target bio-molecules. ROS can damage nucleic acids, lipids, and proteins; this damage fig. prominently in the etiology and progression of numerous cancers as well as coronary and carotid atherosclerosis. Oxidative stress is central to the pathogenesis of HIV while excessive chronic immune activation from the viral infection has a pro-oxidant effect leading to consumption of antioxidants [5]. Although many damaged DNA lesions have been identified, we have chosen 8-hydroxy-2-deoxyguanosine (8-OHdG) as our biomarker of oxidative damage. The importance of this lesion stems from the fact that it is both abundant in DNA and it is mutagenic. Current evidence suggests that 8-OHdG lesions present in DNA during cellular replication results in somatic mutation, the driving force behind carcinogenesis [8].

The search of a suitable biomarker for an impact of antiretroviral therapy on oxidative stress and resultant DNA damage in HIV/AIDS patients is ongoing. There is urgent need of such biomarker that could alert the clinician to investigate further non-AIDS-related diseases in HIV/AIDS patients taking antiretroviral therapy. Such a tool should be inexpensive and facilities for its determination easily available.

In this study, we determined one of the oxidative stress marker total antioxidant status and correlated it with DNA damage marker 8-OHdG in HIV-positive patients, not on ART,HIV-positive patients on ART first line, HIV-positive patients on ART second line and HIV-negative controls.

The objective of this study was to explore TAS as a biomarker for oxidative DNA damage in HIV/AIDS patients with ART.

MATERIALS AND METHODS

Subject selection

A cross-sectional study was carried out on HIV-1 infected patients at the outpatient infectious disease unit and ART centre of the Sir J J Hospital &Grant Government Medical College, Mumbai, over a period of one year, from February 2014 to March 2015.

Ethical approval

The protocol study was approved by the institutional ethics committee (No. IEC/Pharm/902/2013) and National AIDS Control Organisation Delhi, India (T-11020/67/2011-NACO).

Inclusion criteria

All participants were 20 y of age or older HIV-positive patients detected by serial ELISA/Western blot method and were included in this study after giving their informed consent. Normal control HIV-negative subjects (*n* = 100), were selected from the outpatient department of Sir J. J. Group of Hospitals, Mumbai Maharashtra, India.

Exclusion criteria

Exclusion criteria included pregnant women, patients with chronic diseases like hepatitis, diabetes, renal impairment, cardiovascular co-morbidities, neurological, psychiatric disorders, various malignancies, as well as heavy smokers, alcoholics, and tobacco-chewers and HIV patients with the withdrawal of combination ART. The demographic details were collected from each patient and entered into the proforma. Subsequent to this, a detailed history was taken.

Sample collection

Venous blood samples were collected in plain and lithium heparin vacutainers as an anticoagulant. Blood was centrifuged (4000 g, 10 min, 4 °C) to separate the plasma. The collected plasma was stored at–70 °C with aseptic precautions. Plain blood samples 2 h after collection were centrifuged at 3000 rpm for 5 min; serum was separated and collected in sterile tubes.

Characteristics of the antiretroviral therapy administered to patients

The list of antiretroviral therapy administered to Indian HIV-1 patients is as follows:

Biochemical methods

Determination of total antioxidant status (TAS)

Serum total antioxidant status was evaluated using ferric reducing antioxidant power (FRAP) assay [9]. The FRAP assay used antioxidants as reductants in a redox-linked colorimetric method. In this assay, at low pH a ferric-tripyridyl triazine (Fe 3++-TPTZ) complex was reduced to the ferrous form, which was blue colored and monitored by measuring the change in absorption at 593 nm.

Acetate buffer (300 mmol/l, pH 3.6); 10 mmol/l 2,4,6-tri-pyridyl-s-triazine (TPTZ) in 40 mmol/l HCl and 20 mmol/l $FeCl_3\_6H_2O$ in the ratio of 10:1:1 giving the working FRAP reagent. FRAP reagent (750µl) was mixed with 25µl serum or standard in a test tube. After exactly 10 min at 25 °C, the absorbance at 593 nm was read against reagent blank. Fe (II) standards were used. The change in absorbance was directly proportional to the reducing power of the electron-donating antioxidants present in the serum. The absorbance change was translated into a FRAP value (in µmol/lit) by relating the change in absorbance at 593 nm of the test sample to that of a standard solution of known FRAP value. The concentrations in mmol/l were calculated.

Determination of DNA damage marker 8-hydroxy-2-deoxyguanosine (8-OHdG)

We used plasma levels of the oxidized base, 8-hydroxy-2-deoxyguanosine (8-OHdG), as our biomarker of oxidative damage. 8-OHdG was measured with the highly sensitive 8-OHdG check enzyme-linked immunosorbent assay (ELISA) kit (Stress Xpress ELA Kit). Stress Marq's 8-OH-d G ELA is a competitive assay that can be used for the quantification of 8-OH-dG in urine, cell culture, plasma, and saliva. The ELA utilizes an anti-mouse IgG-coated plate and tracers consisting of an 8-OH-dG enzyme conjugate. Procedures were followed as manufacturer's instructions.

Samples and standards (50 uL) were added to microtiter plates precoated with 8-OHdG. This was followed by the addition of 8-OHdG monoclonal antibodies (50 uL). Plates were then sealed and incubated at 4 °C overnight. In this primary reaction, the 8-OHdG in the serum competes with the 8-OHdG already bound to the plate for the monoclonal antibody. Higher levels of 8-OHdG in the sample will lead to lower levels of antibody binding to the plate. To remove the antibodies bound to 8-OHdG in the serum, the plates were washed with 250 ml diluted washing buffer and a second enzyme-labelled antibody (100 uL) was added to each well.

This second antibody will bind to the 8-OHdG monoclonal antibody already attached to the plate. After repeating the plate wash, 100 uL of chromogen was added to each well and incubated in the dark for 15 min. The development of colour is proportional to the amount of antibody in the plate, which in turn is inversely related to the amount of 8-OHdG in the serum sample. Lower colour means higher amounts of 8-OHdG. Results are expressed in nanograms per millilitre.

First line therapy	Second-line therapy
Tenofovir	Tenofovir
Lamivudine	Lamivudine
Ritonavir	Ritonavir
	Atazanavir

Preparation of data

Average absorbance reading of the NSB well and average absorbance reading of B_0 wells were determined. Then we subtracted the average NSB readings from average B_0 readings &calculated $\%B/B_0$ (% of Sample or Standard Bound/Maximum Bound). Then we obtained a Standard curve plot $\%B/B_0$ for Standards using 4 parameter logistic equations. The sample concentration was determined using above equation.

Statistical methods

Student's *t*-test was performed to assess differences between two means. Statistical analysis was performed using EPI-INFO 7 statistical software for medical research studies. Differences in means between groups were tested using independent-sample t-tests. We calculated the 95% confidence interval for the difference between the population means. Mann–Whitney test for non-parametric continuous variables, Chi-square or Fisher's exact test for categorical variables were used to test for statistical significance.

RESULTS

A total of 400 (300 HIV-1 Positive and 100 HIV-Negative) subjects were included in this study. All the subjects were divided into two age groups (20-40yrs) and (40-60 *y*).

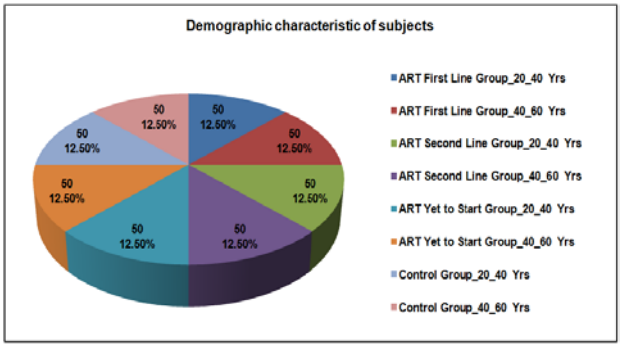

Fig. 1: Pie diagram showing demographic characteristic of subjects

Total antioxidant status (TAS)

Total antioxidant status was significantly lower in HIV-positive patients with antiretroviral therapy than HIV-negative subjects. The mean TAS for HIV-positive patients was lower than that for HIV-negative subjects. The mean TAS for HIV positive and ART are not started in age group (20-40yr) was 0.86 mmol/l and in

age group (40-60yrs) was 0.84 mmol/l. The mean HOMA for HIV positive in both age group (20-40yrs) and (40-60 yrs) with ART (First line) were 0.81 and 0.74 mmol/l & (Second line) 0.79 and 0.74 mmol/l.

Table 1: It shows the clinical characteristics (male/female ratio & age), CD4 (cells/ul), plasma DNA damage marker 8-OHdG (ng/ml) and serum total antioxidant status (mmol/l) of subjects

Group	Subgroup	Male/Female count	Age years	CD4 cells/ul	8-OHdG ng/ml	TAS mmol/l
HIV-negative	20-40 y	37/13	34.8	NA	1.07±0.25	1.04±0.22
control	40-60 y	32/18	47.3	NA	1.30±0.32	0.98±0.11
HIV-positive	20-40 y	28/22	31.6	624	1.81±0.66**	0.86±0.09*
without ART	40-60 y	33/17	45.2	602	1.95±0.56**	0.84±0.08*
HIV-positive ART first line	20-40 y	30/20	34.3	502	2.50±0.91**	0.81±0.11*
	40-60 y	41/9	48.3	465	3.13±0.92**	0.74±0.1*
HIV-positive ART second line	20-40 y	36/14	35.6	400	2.79±0.95**	0.79±0.09*
	40-60 y	43/7	47.5	376	3.27±0.99**	0.74±0.11*

Values are mean±SEM of subjects. The data were analyzed by one-way ANOVA. * P<0.05 and **p<0.01 significant when compared to control

ART-naive HIV-positive patients with age group (20-40yr) with abnormal TAS were 28 (56%) and in age group (40-60yrs) were 36 (74%). The abnormal values of TAS were significant in HIV-positive patients in both age groups (20-40yrs) and (40-60yrs) with ART (First line) were 27 (54%) and 34 (68%) & (Second line) 31(62%) and 35(70%). These results show decreased TAS in HIV-positive patients with Antiretroviral Therapy.

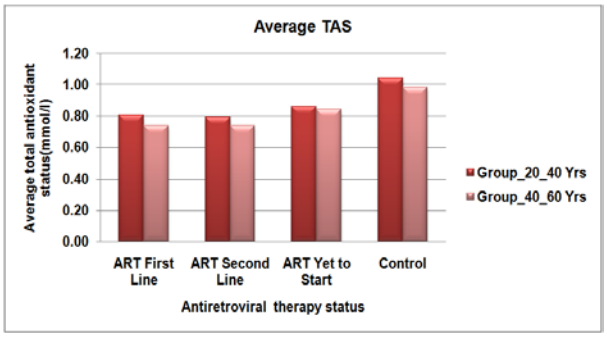

Fig. 2: Bar diagram showing average serum total antioxidant status with different antiretroviral therapy status among subjects

DNA damage marker: 8-OH-dG

Under normal physiological conditions in all aerobic organisms, there is a balance maintained between endogenous oxidants and numerous enzymatic and non-enzymatic antioxidant defences. When an imbalance occurs, oxidants produce extensive oxidative damage to DNA, which, in turn, contributes to aging, malignant tumours, and other degenerative diseases.

DNA damage was significantly higher in HIV-1 positive patients with antiretroviral therapy than HIV-negative subjects. In this study DNA damage marker, 8-OH-dG was measured by ELISA. The mean 8-OH-dG for HIV-positive patients was higher than that for HIV-negative subjects. The mean 8-OH-dG for HIV positive and ART is not started in age group (20-40yr) was 1.81 ng/ml and in age group (40-60yrs) was 1.95 ng/ml. The mean 8-OH-dG for HIV positive in both age group (20-40yrs) and (40-60yrs) with ART (First line) were 2.50 ng/ml and 3.13 ng/ml & (Second line) 2.79 ng/ml and 3.27 ng/ml. Antiretroviral therapy accelerates DNA damage in HIV-positive patients. These results show there was the increase in DNA damage in HIV-positive patients with Antiretroviral Therapy.

Co-relation of TAS and DNA damage marker

In this study, we observed that there was a negative co-relation of total antioxidant status and DNA damage. The lower mean TAS values were observed in HIV-positive patients with ART first line and second line in both age groups (20-40 y &40-60 y) than ART-naive & controls, apposite pattern that is higher DNA damage marker 8-OH-dG was observed in HIV-positive patients with ART first line &second line in both age groups (20-40 y &40-60 y) than HIV-positive without ART and controls.

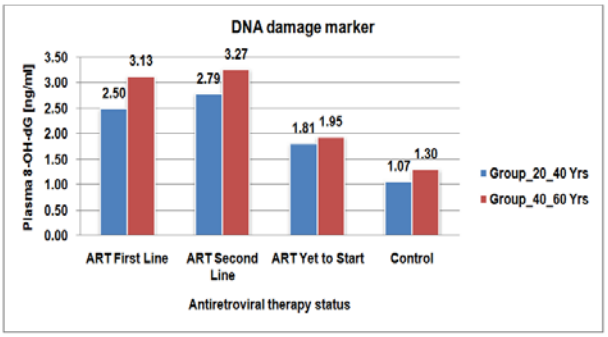

Fig. 3: Bar diagram showing average plasma DNA damage marker with antiretroviral therapy status among subjects

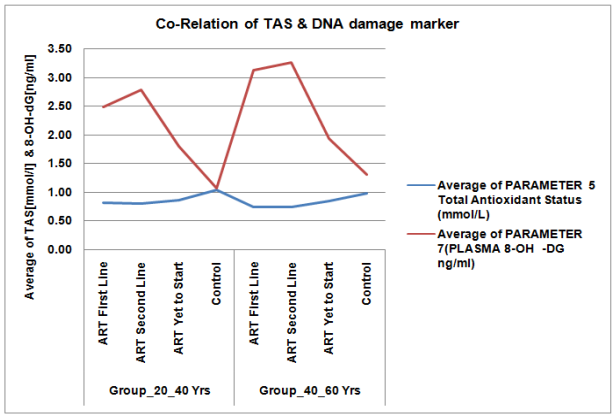

Fig. 4: Graph showing correlation of average serum total antioxidant status with average plasma DNA damage marker

DISCUSSION

Several studies report that enhanced oxidative stress in HIV infection may have a pathogenic role in this disorder. The decrease in antioxidants that accompanies HIV infection suggests a potentially important role of nutritional supplementation and good nutrition in general in the proper management of HIV/AIDS. The inclusion of antioxidants in the therapeutic approach in managing HIV-1 seropositive patients will prevent the additional damage that free radicals could do to such patients [7].

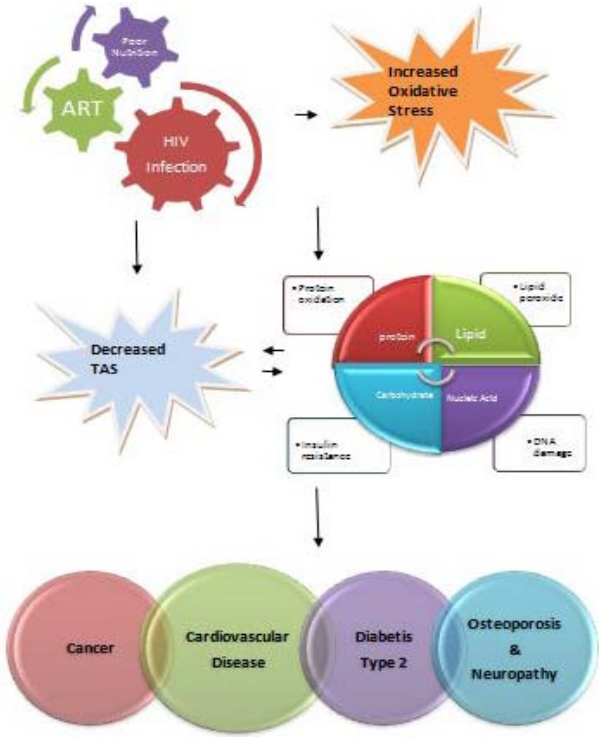

Fig. 5: Correlation of HIV, ART, Lifestyle with decreased TAS, increased oxidative stress and resultant non ART diseases

HAART may increase chemically reactive species in circulation, possibly by producing more oxidized metabolites deriving from the interaction between ROS and infected cell biomolecules. This is supported by several biochemical mechanisms, such as mitochondrial interference, following treatment with HAART-NRTI (Nucleoside Reverse Transcriptase Inhibitors), and activation of the P450 hepatic system by HAART, when comprising Protease Inhibitors (PI) [6].

In the present study, we show that HIV-infected patients, particularly those with antiretroviral therapy, have enhanced oxidative DNA damage, as assessed by increased 8-oxodG accumulation. Notably, this increase in oxidative DNA damage was accompanied by a marked decline in Total Antioxidant Status. Under normal physiological conditions in all aerobic organisms, there is a balance maintained between endogenous oxidants and numerous enzymatic and non-enzymatic antioxidant defences. When an imbalance occurs, oxidants produce extensive oxidative damage to DNA, which, in turn, contributes to aging, malignant tumours, and other degenerative diseases. In all living cells, damaged DNA is repaired enzymatically so that they regain their normal function, whereas mis-repaired DNA can result in mutations (base substitution, deletions, and strand fragmentation) leading to carcinogenesis. Although a broad range of DNA products are produced during oxidative damage to DNA (bases and sugar modifications, covalent crosslink's, single-and double-stranded breaks), most interest focused on nucleobase modifications and especially on the abundant lesion of 8-oxo-2-deoxyguanosine because it is formed *in vivo* and can be measured quantitatively in cells following hydrolysis of the DNA to component bases [15, 16]. Our study data suggests that ART may have anticipated effects on antioxidant defences and resultant DNA damage that should be further explored.

The follow-up study might have given the exact role of ART impact on TAS and resultant DNA damage in HIV/AIDS patients. Due to lack of time and funding, we could not do the follow-up study.

CONCLUSION

Inflammation may prove to be the key that unlocks some of the mysteries of HIV disease, and advance in the HIV/AIDS field can contribute to the development of anti-inflammatory therapies that will also benefit people with a host of other diseases. The state of knowledge about the role of inflammation in the pathogenesis of HIV disease and non-AIDS conditions has advanced remarkably in just a few years, but much remains to be learned.

However, a great deal of work remains to be completed in defining the exact roles of oxidative DNA damage in the pathogenesis of diseases. In this study, we explored the negative correlation of total antioxidant status and oxidative DNA damage marker 8-OHdG in HIV/AIDS patients with and without antiretroviral therapy. With this established study, TAS might be a possible biomarker to determine oxidative DNA damage in HIV/AIDS patients. In future modulation of DNA repair and management of oxidative stress might be useful in disease prevention and improving HIV/AIDS therapy.

ABBREVIATION

8-OHdG: 8-hydroxydeoxyguanosine, ART: Antiretroviral Therapy, HIV: Human Immunodeficiency Viru, IR: Insulin Resistance, HOMA: Homeostasis Model Assessment, ELISA: Enzyme Linked Immunosorbent Assay

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

All authors declare that there are no conflicts of interests

REFERENCES

1. Johane P Allard, Elaheh Aghdassi, Jenny Chau. Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am J Clin Nutr 1998;67:143–7.
2. Steven G Deeks, Sharon R Lewin, Diane V Havlir. The End of AIDS: HIV infection as a chronic disease. Lancet 2013;382:1525-33.
3. Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, *et al*. T cell activation is associated with lower CD4+T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. J Infect Dis 2003;187:1534–43.
4. Neuhaus J, Jacobs DR Jr, Baker JV, Calmy A, Duprez D, La Rosa A, *et al*. Markers of inflammation, coagulation, and renal function are elevated in adults with HIV infection. J Infect Dis 2010;201:1788–95.
5. JA Olaniyi, OG Arinola. Essential trace elements and antioxidant status in relation to severity of HIV in Nigerian patients. Med Principles Practice 2007;16:420–5.
6. Antonella Mandas, Eugenio Luigi Iorio, Maria Gabriella Congiu. Oxidative imbalance in HIV-1 infected patients treated with antiretroviral therapy. J Biomed Biotechnol 2009. Doi.org/10.1155/2009/749575. [Article in Press]
7. Ogunro PS, Ogungbamigbe TO, Ajala MO, Egbewale BE. Total antioxidant status and lipid peroxidation in HIV-1 infected patients in a rural area of southwestern Nigeria. Afr J Med Med Sci 2005;34:221-5.
8. Raynoo Thanan, Shinji Oikawa, Yusuke Hiraku, Shiho Ohnishi, Ning Ma. Oxidative stress and its significant roles in neurodegenerative diseases and cancer. Int J Mol Sci 2015;16:193-217.
9. Benzie IFF, Strain JJ. The ferric reducing ability of olasma. (FRAP) as a measure of "Antioxidant Power": The FRAP assay. Anal Biochem 1996;239:70–6.
10. Saba khan, Roshan Alam, Moinuddin, Asif Ali. Oxygen free radical modified DNA: implications in the etiopathogenesis of systemic lupus erythematosus. Indian J Clin Biochem 2009;24:123-30.
11. Patrick WG Mallon, Patrick Unemori, Rebecca Sedwell, Adrienne Morey. *In vivo*, nucleoside reverse-transcriptase inhibitors alter expression of both mitochondrial and lipid

metabolism genes in the absence of depletion of mitochondrial DNA. J Infect Dis 2005;191:1686–96.

12. Gibson B Kagaruki, Mary T Mayige, Ester S Ngadaya, Kagaruki, *et al.* Magnitude and risk factors of non-communicable diseases among people living with HIV in Tanzania: a cross-sectional study from Mbeya and Dar es salaam regions. BMC Public Health 2014;14:904.
13. Surendra S. Ambegaokar and dennis L. Kolson. heme oxygenase-1 dysregulation in the brain: implications for HIV-associated neurocognitive disorders. Curr HIV Res 2014;12:174-88.
14. Steven G Deeks, Andrew N Phillips. HIV infection, antiretroviral treatment, ageing, and non-AIDS related morbidity. Br Med J 2009;338. Doi:10.1136/bmj.a3172.
15. Olinski, Rozalski, Gackowski. Urinary measurement of 8-oxodg, 8-oxogua, and 5HMUra: A noninvasive assessment of oxidative damage to DNA. Antioxid Redox Signal 2006;8:1011-9.
16. Melis, van Steeg, Luijten M. Oxidative DNA damage, and nucleotide excision repair. Antioxid Redox Signal 2013;18:2409–19.
17. Jean-Claude Dusingize, Donald R Hoover, Qiuhu Shi. Associations of HIV infection with insulin and glucose levels in antiretroviral-naïve Rwandan women: a cross-sectional analysis. Br Med J Open 2013;3:e003879. Doi:10.1136/ bmjopen-2013-003879. [Article in Press]
18. Surapon Tangvarasittichai. Oxidative stress, insulin resistance, dyslipidemia, and type 2 diabetes mellitus. World J Diabetes 2015;6:456-80.
19. Yermilov V, Rubio, Becchi, Friesen, Pignatelli. Formation of 8-nitroguanine by the reaction of guanine with peroxynitrite *in vitro*. Carcinogenesis 1995;16:2045–50.
20. Rodriguez, Song, Crouse. *In vivo* bypass of 8-oxodG. PLoS Genet 2013;9:e1003682. Doi:10.1371/journal.pgen.1003682. [Article in Press]
21. Suzuki, Yasui, Geacintov. Miscoding events during DNA synthesis past the nitration-damaged base 8-nitroguanine. Biochemistry 2005;44:9238–45.
22. Peter Macpherson, Flavia Barone, Giovanni Maga, Filomena Mazzei. 8-Oxoguanine incorporation into DNA repeats *in vitro* and mismatch recognition by MutSa. Nucleic Acids Res 2005;33:5094-105.
23. Ivan Rusyn, Shoji Asakura, Brian Pachkowski, Blair U. Bradford expression of base excision DNA repair genes is a sensitive biomarker for *in vivo* detection of chemical-induced chronic oxidative stress. Cancer Res 2004;64:1054-7.
24. Miriam Casula, Irene Bosboom-Dobbelaer, Karlijn Smolders. Infection with HIV-1 induces a decrease in mtDNA. J Infect Dis 2005;191:1468–71.