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Analysis of Oxidative DNA Damage in HIV- Positive Pregnant Women

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Abstract

Objective: *Oxidative stress is central to Human Immunodeficiency Virus (HIV) pathogenesis. Increased oxidative stress leads to increased oxidative DNA damage in HIV infected patients. The objective of this study was to analyse oxidative DNA damage in HIV- positive pregnant women.*

Methods: *This was a case-control study involving 100 HIV-positive women as cases and 100 HIV-negative women as controls. 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: *Increased oxidative DNA damage was observed in HIV-positive pregnant women than HIV-positive non pregnant women and controls.*

Conclusion: *Oxidative stress-induced DNA lesions may contribute to carcinogenesis. Hence management of oxidative stress induced DNA damage is very important in HIV-positive mothers and their newborns.*

Keywords: ***DNA damage, Human Immunodeficiency virus (HIV) infection, 8-OH-dG, oxidative stress, pregnancy.***

Introduction

India has the third largest HIV epidemic in the world. In 2015, HIV prevalence in India was an estimated 0.26%.[1] This figure is small compared to most other middle-income countries but because of India's huge population (1.2 billion) this equates to 2.1 million people living with HIV. In the same year, an estimated 68,000 people died from AIDS-related illnesses.[2] The epidemic disproportionately affects women, who account for 40 per cent of the total infections in the country.It is estimated that out of 27 million pregnancies every year, nearly 49,000 occur in HIV-positive mothers. The number of women infected with Human Immunodeficiency Virus (HIV) is still increasing worldwide3. Clinical status of pregnant HIV-infected women has improved considerably with the introduction of ART. This resulted in decreased rates of HIV MTCT.[3] Nonetheless, studies have reported that despite the benefits of ART during pregnancy, some women exposed to ARV showed anemia, nausea, vomiting, hyperglycemia and elevated aminotransferase.[4] The dramatic success of antiretroviral therapy has allowed people with HIV to live longer, but their chances of getting cancer are also rising. The previous studies have shown that exposure to oxidative stress is greater in HIV/AIDS patients. Oxidative stress-induced DNA lesions may contribute to carcinogenesis. In these patients, cancer induction may be a pathological consequence of elevated ROS levels, which lead to increased steady-state levels of oxidative DNA damage, which in turn leads to a higher risk of mutations that may activate onco-genes or inactivate tumour-suppressor genes.

Oxidative damage results from biochemical interactions between reactive oxygen species (ROS) and target bio-

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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 DNAlesions 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.[6]

Material and Methods

2.1 Subject Selection

A case-control study was carried out on HIV- 1 infected patients at the outpatient infectious disease unit (OPD) and ART centre of the Sir J J Hospital &Grant Government Medical College, Mumbai over a period of one year, from February 2015 to March 2016. We have selected 50 HIV-negative pregnant women and 50 HIV-negative non-pregnant women from OPD after evaluation of their medical records (negative serial ELISA/Western blot for HIV before three months of sample collection) as HIV-negative controls and 50 HIV- positive pregnant women and 50 HIV-positive non-pregnant women detected by serial ELISA/Western blot method from ART centre. We used power analysis method for sample selection at 5% significance level for 95% confidence.

2.2 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-11020/74/2014-NACO).

2.3 Inclusion criteria

All participants were 20 years of age or older HIV- positive women detected by serial ELISA/Western blot method (n = 100 out of which 50 are pregnant) were included in this study after getting their informed consent.In this study, we included HIV-positive women with first-line antiretroviral therapy only. The family history of all subjects was recorded and the subjects without any diabetes history were chosen.

Normal control HIV- negative women (n = 100out of which 50 are pregnant) who are negative to ELISA/Western blot test for HIV before three months of sample collection, were selected from outpatient department of Sir J.J. Group of Hospitals, Mumbai Maharashtra, India. From 100 HIV-negative women 50 women are pregnant.

2.4 Exclusion criteria

Women with multiple pregnancies and those who defaulted ART and/or antenatal clinic visits were excluded from the analysis.

We collected the demographic details from each patient and entered into the pro-forma. Subsequent to this, we have taken detailed history of each patient.

2.5 Sample collection

We collected venous blood samples in plain and lithium heparin vaccutainers 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. We centrifuged plain blood samples 2 h after collection at 3000 rpm for 5 min; then we separated the serum and collected it in sterile tubes.

2.6 Different treatment regimens as per NACO guidelines

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

The first-line therapy includes Tenofovir, Lamivudine and Efavirenz.

The second-line therapy includes Tenofovir, Lamivudine, Ritonavir and Atazanavir.

In this study, we included HIV-positive women with first-line antiretroviral therapy only.

2.7 Biochemical methods

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.[11] 8-OHdG was measured with the highly sensitive 8-OHdG check enzyme-linked immunosorbent assay (ELISA) kit (StressXpress ELA Kit). StressMarq's 8-OH-d G ELA is a competitive assay that can be used for the quantification of 8-OH-d G in urine, cell culture, plasma, and saliva. The ELA utilizes an anti-mouse IgG-coated plate and tracer consisting of an 8-OH-d G enzyme conjugate. It is important to note that the OH-d G antibody used in this assay recognizes both free 8-OH-d G and DNA-incorporated 8-OH-d G .Since complex samples such as plasma, cell lysates, and tissues are comprised of mixtures of DNA fragments and free 8-OH-d G. The assay is based on the competition between 8-hydroxy-2-deoxyguanosine (8-OHdG) and a 8-OHdG –acetylcholine-esterase (AChE) conjugate (8-OHdG Tracer) for a limited amount of 8-OH-d G Monoclonal Antibody. Because the concentration of 8-OH-d G Tracer is held constant while the concentration of 8-OH-d G varies, the amount of 8-OH-d G Tracer that is able to bind to the 8-OH-d G Monoclonal Antibody will be inversely proportional to the concentration of 8-OH-d G in the well. This antibody-8-OH-d G complex binds to goat

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polyclonal anti-mouse IgG that was previously attached to the well. The plate was washed to remove any unbound reagents and then Ell man's Reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow colour and absorbs strongly at 412 nm. The intensity of this colour, determined spectrophotometrically, is proportional to the amount of 8-OH-d G Tracer bound to the well, which is inversely proportional to the amount of free 8-OH-d G present in the well during the incubation. We followed all the procedures as manufacturer's instructions.

Preparation of Data

Average absorbance reading of the NSB well and average absorbance reading of B0 wells were determined. Then we subtracted the average NSB readings from average B0 readings &calculated %B/B0 (% of Sample or Standard Bound/Maximum Bound).Then we obtained a Standard curve plot %B/B0 for Standards using 4 parameter logistic equations. The sample concentration was determined using above equation.

2.8 Statistical methods

We performed Student's t test to assess differences between two means. We used EPI-INFO 07 statistical software for statistical analysis for medical research studies. We used Microsoft® Excel 2007 for production of charts. We compared group means of all parameters using the ANOVA test. We compared categorical data in HIV-infected patients using the Pearson's Chi-Square test. We considered a p-value< 0.05 statistically significant. We used logistic regression method for correlation of DNA damage marker 8-OH-dG in HIV-positive pregnant women, HIV-positive non-pregnant women with controls.

Results and Discussion

Table 1: It shows the clinical characteristics (age), CD4 (cells/ul), plasma DNA damage marker 8-OHdG (ng/ml) of subjects

Group	No of subjects	Age (mean) years	CD4count (mean) Cells/ul	8-OHdG (mean) ng/ml
HIV-positive pregnant women	5	031 ± 6	458 ± 223	3.20±0.83**
HIV-positive non-pregnant women	5	031 ± 7	543 ± 198	2.50±0.91**
HIV-negative pregnant women	5	031 ± 6	Not Applicable	1.35±0.42
HIV-negative non-pregnant women	5	031 ± 4	Not Applicable	1.30±0.32

*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*

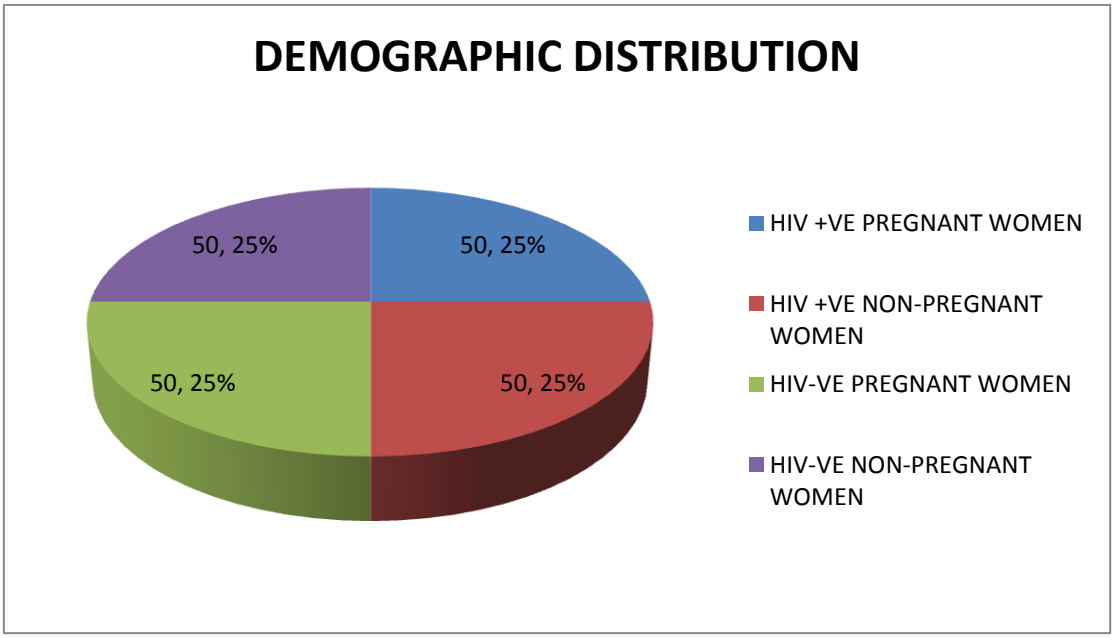

Figure 1: Shows the demographic distribution of subjects

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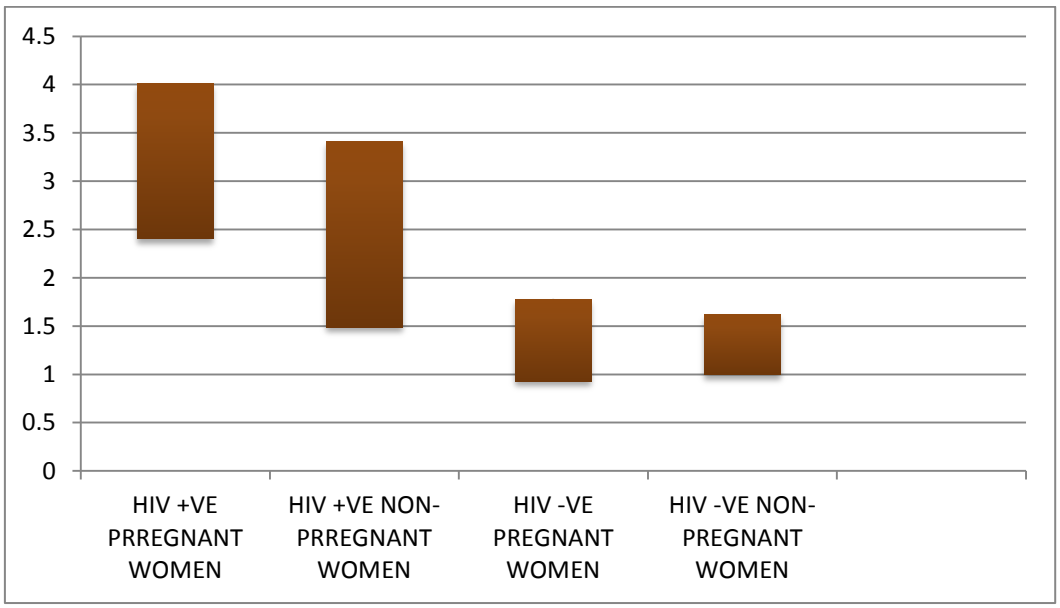

Figure 2: Shows DNA damage marker 8OH-Dg in different groups

A total of 200 (100 HIV-1 Positive and 100 HIV-Negative) subjects were included in this study. All the subjects were divided into four groups. First group consist of HIV-positive pregnant women (n=50), second group consist of HIV-positive non-pregnant women (n=50), third group consist of HIV-negative pregnant women (n=50) and fourth group consist of HIV-negative non-pregnant women (n=50).

Under normal physiological conditions in all aerobic organisms, there is a balance maintained between endogenous oxidants and numerous enzymatic and non-enzymatic antioxidant defenses. 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- positive pregnant women than HIV-positive non-pregnant women and control groups. In this study DNA damage marker, 8-OH-dG was measured by ELISA. The mean 8-OH-dG for HIV-positive pregnant women was higher than that for HIV-positive non-pregnant women. The mean 8-OH-dG for HIV-positive pregnant women was3.20±0.83 ng/ml. The mean 8-OH-dG for HIV-positive non-pregnant women was 2.50±0.91 ng/ml. The mean 8-OH-dG for HIV-negative pregnant women was 1.35±0.42ng/ml. The mean 8-OH-dG for HIV-negative non-pregnant women was 1.30±0.32ng/ml. These results show there was the increase in DNA damage in HIV-positive pregnant women.

Several studies report that enhanced oxidative stress in HIV infection may have a pathogenic role in this disorder.[7,8] 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.[9]

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 non-AIDS related cancers in HIV/AIDS patients. In this study, we determined the oxidative DNA damage marker 8-OHdG in HIV/AIDS pregnant women. Increased DNA damage was observed in HIV-positive pregnant women than HIV-positive non-pregnant women and controls. This damaged DNA might be passed to the newborns and in future they are having the risk of cancer because of muted DNA. Hence the management of oxidative DNA damage must be the priority in future research of HIV/AIDS.

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Conflict of Interests

All authors declare that there are no conflicts of interests

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