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Original Research Article

Oxidative stress in HIV positive children

Bina F. Dias*, Aruna Srinivas

Department of Biochemistry, Lokmanya Tilak Municipal Medical College, Sion, Mumbai 400022, India

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***Correspondence:**
Dr. Bina F. Dias,
E-mail: binadias29@yahoo.in

ABSTRACT

Background: The objective is to assess oxidative stress by measuring the concentration of malondialdehyde in HIV positive children and compare it with normal children (not suffering from any disease) of the same age group.
Methods: In this prospective comparative study, we analysed malondialdehyde in 80 HIV positive children in the age group of 6-12 yrs from lower socio-economic strata and compared the values with 85 normal children not infected by any disease, of the same age group and similar socio-economic strata at L.T.M.M. College. Estimation of Malondialdehyde was carried out by using the method of SADASIVUDU by thiobarbituric acid reaction.
Results: The level of Malondialdehyde was significantly higher in HIV positive children than in controls.
Conclusions: The increased levels of Malondialdehyde confirm the involvement of oxidative stress in the pathophysiology of this infection in children.

Keywords: HIV, Malondialdehyde, Oxidative stress

INTRODUCTION

Human immunodeficiency virus infection (HIV), the cause of AIDS (Acquired immunodeficiency syndrome) is a worldwide pandemic with enormous adverse health and economic implications, particularly in the developing world.[1] During the past dozen years, frustration and progress have characterized the evolution of knowledge of HIV infection and AIDS in children.[2] AIDS is the end stage disease representing the irreversible breakdown of immune defence mechanism, leaving the patient susceptible to progressive opportunistic infections and malignancies.[3]

Literature documents that HIV infection is associated with oxidative stress.[4-6] Several lines of evidence suggest that oxidative stress contributes to the pathogenesis of HIV infection.[7,8] Oxidative stress is an abnormal phenomenon occurring inside our cells or tissue caused by excessive production of free radicals. Excess production of free radicals damage essential macromolecules of the cell, leading to abnormal gene expression, disturbance in receptor activity, immunity perturbation, mutagenesis, protein or lipofushin deposition.[9]

A free radical is a molecule that contains an unpaired electron in its outer orbit. The main radicals are superoxide radical, hydroperoxyl radical, hydrogen peroxide and hydroxyl radical which are formed during complete reduction of oxygen to water.

$O_2 + e^- \rightarrow O_2^*$ Superoxide radical

$O_2^* + H_2O \rightarrow HO_2^* + OH^-$ Hyderoperoxyl radical

$HO_2^* + e^- \rightarrow H_2O_2$ Hydrogen peroxide

$H_2O_2 + e^- \rightarrow {}^*OH + OH^-$ Hydroxyl radical

Each of these oxygen derived intermediates is considered highly reactive because its unstable electron

configurations allow for the attraction of electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid peroxidation, DNA damage and protein degradation.[10,11] The most well-described consequence of the generation of free radicals and ROS is lipid peroxidation. Lipid peroxidation is a chain reaction initiated by free radicals attacking fatty acid side chains in the phospholipids of cell membranes.[7,12,14,15.] Malondialdehyde is the end product of lipid peroxidation. A common method to evaluate the extent of oxidative stress in vivo is to measure lipid peroxidation end product such as malondialdehyde.[7]

METHODS

The present study was carried out in Lokmanya Tilak Municipal Medical College. A total of 165 children (80 HIV positive children and 85 normal children not suffering from any disease) under the age group of 6-12 yrs attending routine outpatient department of Lokmanya Tilak Municipal Medical Hospital belonging to lower economic strata were selected for present study. 5.0ml of venous blood was collected by vein puncture in a plain vacutainer. Serum obtained from the sample was separated and the estimation of malondialdehyde was carried out by Sadasivudu method.[16] Lipids were separated from serum proteins using 40% trichloroacetic acid to determine their amount by thio-barbituric acid. Thus thio-barbituric acid reacting substances other than lipid peroxides are easily eliminated in acid solution. 0.5 ml of 40% TCA solution was added to 0.5ml serum and mixed thoroughly. To this, 1.0ml of 0.67% TBA solution was added and mixed thoroughly. The tubes were covered with marbles/aluminium foil and kept in boiling waterbath for 60 minutes. The tubes were cooled immediately in ice cold water and 2.0ml butanol was added. The tubes were mixed on a vortex and then centrifuged at 3000 rpm for 15 minutes. The upper butanol layer was removed and its absorbance was read in a spectrophotometer at 533 nm.

RESULTS

Malondialdehyde was monitored in 80 HIV positive children (males 46 and females 34) in the age group of 6-12 years (mean±SD age 8.12±2.3) and 85 control children (males 53 and females 32) of the same age group (mean±SD age 8.89±2.24). The results were subjected to statistical analysis. Unpaired "t" test (in present study "z" test) was applied and the statistical significance was established. Present study showed a significance level ($p<0.001$). The mean S.D of HIV positive children was 7.06±3.56µmol/L and that of controls was 2.56±1.21µmol/L. These results showed that there was a significant increase of malondialdehyde in HIV positive children as compared to normal children. This finding supports previous observations that HIV infection is associated with oxidative stress. Extensive research carried out by Jareno EJ, et al showed an increasing trend in the concentration of MDA in HIV positive children as compared to controlswhich were similar to present study.[4,7,13,18,19] Lipid peroxidation product (MDA) evaluates the extent of oxidative stress. This explains the increased concentration of malondialdehyde in HIV positive children. It can be seen from present study that the specific assay of serum MDA is useful for the clinical management of these children suffering from HIV infection.

Table 1: Malondialdehyde levels in HIV positive and control children.

Parameter	HIV Positive children n_1=80	Control children n_2=85	'p' value	Significance
MDA (µmol/L)	7.06 ± 3.51	2.56 ± 1.21	< 0.001	Highly significant

Values expressed as mean ± standard deviation.

Table 1 shows a marked increase in the oxidative stress marker malondialdehyde (MDA) in HIV positive children (significance level $p<0.001$) as compared to controls. Mean±SD of HIV positive children: 7.06±3.56µmol/L to mean±SD of controls: 2.56±1.21µmol/L).

DISCUSSION

HIV infection induces a wide array of immunological alterations resulting in the progressive development of opportunistic infections and malignancy, which results in AIDS.

Of the mechanism contributing to this progression, oxidative stress induced by the production of reactive oxygen species (ROS) may play a critical role in the stimulation of HIV replication and the development of immunodeficiency.[17,18] Oxidative stress occurs when free radical production exceeds your body's ability to neutralize them. This imbalance happens when:

- Antioxidant production is diminished.
- When free radicals are produced in excess.

A Free radical is a molecule carrying an unpaired electron. All free radicals are extremely reactive and will seek out and acquire an electron in any way possible.

In the process of acquiring an electron, the free radical will attach itself to another molecule, thereby modifying it biochemically. However, as free radicals steal an electron from the other molecules, they convert these

molecules into free radicals, or break down or alter their structure. Thus free radicals are capable of damaging virtually any biomolecule, including proteins, sugars, fatty acids and nucleic acids. Harman points out that free radical damage occurs to longlived biomolecules, such as collagen, elastin and DNA; mucopolysaccharides; lipids that make up the membranes of cells and organelles such as mitochondria and lysosomes. The main radicals are:

- *Superoxide (O2) ion
- *Hydroxyl group (OH)
- *Alkoxyl radical (AR)
- *Peroxyl radicals (ROO)

Other molecules that are technically not free radicals, but act like them, are:

- *Singlet oxygen (O)
- *Hydrogen peroxide (H_2O_2) and
- *Hypochlorous acid (HOCL)

Collectively, the free radicals and non-free radicals are called "Oxidants" or "reactive oxygen species". Free radicals are short-lived because of their extreme reactivity.[11]

Excessive production of reactive oxygen species such as superoxide anion, hydroxyl radical and hydrogen peroxide may be produced in oxidative stress due to polymorphonuclear leucocyte activity in infectious conditions and/or due to prooxidant effect of tumour necrosis factor α (TNF-α) produced by activated macrophages during the course of HIV infection. These reactive oxygen species (ROS) can attack bases in nucleic acids, amino acid side chain in proteins and the double bonds in polyunsaturated fatty acids, thereby compromising cell integrity and function. Moreover ROS can stimulate HIV replication through the activation of tumour necrosis factor.[17]

Oxidative stress has emerged in recent years as a suspected component in the pathogenesis of HIV disease. When the balance between free radicals and antioxidant supply is tipped, resulting oxidative stress can cause many problems-either of its own, or in the case of HIV causing increased viral replication.[6]

The present study exhibited an increased concentration of malondialdehyde in HIV positive children as compared to controls. This could be explained because of the increased concentration of malondialdehyde produced due to a chain reaction initiated by free radicals. The increase of MDA concentration in HIV positive children confirms the involvement of oxidative stress in the pathophysiology of this infection in children.[4]

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