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*RESEARCH ARTICLE*

# ANTIOXIDANT STATUS IN HIV/AIDS ADOLESCENT PATIENTS OF MANIPUR.

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## *Abstract*

**Background:** Reactive oxygen and nitrogen species are considered as two of the prime suspects for oxidative stress in HIV disease, which in turn stimulate HIV provirus replication and the development of AIDS.
**Objective:** The aim of the present study was to assess early markers of oxidative stress which may diminish the total antioxidant capacity in HIV patients. Pro-oxidant marker Thiobarbituric Acid Reactive species (TBARS) was estimated in serum. Antioxidant parameters including Reduced Glutathione (GSH), Vitamins C and E were also assessed.
**Design:** A cross-sectional study was carried out with 120 HIV-infected adolescents (10-19 years of age) attending out patient department in a tertiary care hospital. Fifty age and sex matched healthy volunteers served as controls. Patients were divided on the basis of their absolute $CD_4^+$ counts into 3 groups – Group 1 (<200 $CD_4^+$ cells/$mm^3$), Group 2 (200-499 $CD_4^+$ cells/$mm^3$), Group 3 (>500 $CD_4^+$ cells/$mm^3$).
**Results:** Mean serum TBARS level were significantly higher in GR-1 (8.54 ± 1.19 nmol/mL), GR-2 (7.69 ± 0.84 nmol/mL) and GR-3 (6.97 ± 0.9 nmol/mL) than in healthy subjects (4.33 ± 0.93 nmol/mL). Also, there was significant ($p < 0.05$) decrease in the levels of vitamin E, vitamin C and GSH among GR-1 and GR-2, though the decrease in levels of vitamin E and vitamin C in GR-3 patients when compared to controls was found to be insignificant ($p > 0.05$). The TBARS exhibited a negative correlation when compared with $CD_4^+$counts whereas antioxidant markers showed a positive correlation.
**Conclusion:** HIV-infected adolescents have an inadequate antioxidant status, which could influence the progression to AIDS. An adequate micronutrient status and reduced glutathione (GSH) could improve the clinical condition in these patients and minimize free radical production and cellular oxidative stress. More detailed studies need to be conducted at this age group patient to correlate the redox imbalance and disease progression with antioxidant supplementation.

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## Introduction:-

The acquired immunodeficiency syndrome (AIDS), is a fatal illness caused by a retrovirus known as the human immunodeficiency virus that breaks down the body's immune system by infecting $CD_4^+$ cells and progressively

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leading to AIDS [1].The hallmark of HIV infection is numerical and functional decline in $CD_4^+$cells, which result in profound immunodeficiency. There are 33.2 million people worldwide living with HIV infection and the number is as high as 2.5 million only in India. Manipur has a prevalence of 1.13% [2].

Oxidative stress, defined as the imbalance between the oxidant and antioxidant systems, is thought to be associated with the progression of human immunodeficiency virus (HIV) infection. It has been observed that perturbations in antioxidant defence system and consequently redox imbalance are present in many tissues of HIV-infected patients. Oxidative stress may contribute to the different stages of viral replication and its consequences such as inflammatory response and decreased immune cell proliferation. Reduced glutathione (GSH) is the predominant endogenous antioxidant in mammalian cells and vitamins C and E are the antioxidant nutrients that protect cells as well as tissues against damage by reactive oxygen and nitrogen species.

The demand of such antioxidant increases during episodes of infectious disease, when the immune system is challenged to eliminate pathogenic organisms. Chronic infections, such as HIV infection place a long-term strain on antioxidant capacity, which can be counterbalanced by increasing the dietary antioxidant intake. The intake of vitamin C and E recommended for healthy subjects may thus be less than adequate to deal with the increased oxidative stress of HIV infection. This stress can damage cells and tissues of the immune system and lead to increased severity of disease [3]. Infections may also affect the absorption, tissue distribution and excretion of nutrients [4]. It is thus not surprising that a low intake of vitamin C, low plasma concentrations of vitamin E and reduced serum glutathione level have been associated with a greater risk of progression into AIDS in HIV infected subjects and can also be correlated with a poor survival in AIDS patients [5,6,7].

Only few studies of antioxidant status in HIV infected adolescents are reported from India. However, because adolescents are at high risk of HIV infection in India particularly in Manipur, the current study provides a novel opportunity to examine the association of antioxidant status with HIV infection in this age group.

In the current observational study, we tried to build-up the relationship among $CD_4^+$cell count, total antioxidant capacity and lipid peroxidation in HIV/AIDS adolescent patients.

## Materials and Methods:-

This study was conducted at the department of Biochemistry in collaboration with ART centre, department of Microbiology, Regional Institute of Medical Sciences, Manipur from September 2008 to April 2010. All human volunteers participated in the study were enrolled in the study only after getting the informed consent.the study was approved by the Institutional Ethical Committee of Regional Institute of Medical Sciences, Manipur

**Study population:-**

The study population included 120 HIV seropositive patients, belonging to both sex i.e. male and female in the age group of 10–19 years who had acquired HIV infection through sexual activity or intravenous drug use were recruited. For the diagnosis and confirmation of HIV infection, we followed the National AIDS Control Organization (NACO) recommendations for HIV testing [8]. All the patients were subjected to detailed history taking and clinical examinations. Informed consent of the patients was taken before testing. Absolute $CD_4^+$T lymphocyte counts for HIV-positive participants were stratified on the basis of Centres for Disease Control and Prevention criteria for HIV/AIDS classification: Group 1: <200 cells/$mm^3$; Group 2: 200–499 cells/$mm^3$; and Group 3:≥500 cells/ $mm^3$ [9].

50 age and sex matched healthy HIV seronegative individuals without the history of high risk behaviour were taken as controls.

**Exclusion criteria:-**

Any ongoing infection decompensated liver disease of any etiology, individuals below 10 years and above 19 years old was excluded from the study.

**Sample collection and erythrocyte lysate preparation:-**
Ten millilitres of whole blood from HIV seropositive patients and healthy volunteers were collected by venous puncture in EDTA and plain vacuum tubes. The serum was separated and stored at -20°C and used for the following assays within 2 days of sample collection.

**Biochemical investigation:-**
The$CD_4^+$ lymphocyte count was estimated by Fluorescence Activated Cell Sorter (FACS) count System (Becton Dickinson).(10)

**Estimation of MDA (Malondialdehyde):-**
Thiobarbituric acid reactive substances (TBARS) in plasma were estimated by measuring the pink chromogen developed by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation. The absorbance of clear supernatant was measured against reference blank at 535 nm[11].

**Estimation of Vitamin E and C:-**
Plasma vitamin E was measured by the reduction of ferric ions to ferrous ions by tocopherols after xylene extraction and subsequent reaction of ferrous ions with bipyridyl to give red colour which could be measured at 520 nm [12].Plasma ascorbic acid was estimated by the 2, 4 – DNPH method [13].

**Estimation of Reduced Glutathione (GSH):-**
Serum reduced glutathione (GSH) was estimated colorimetrically by Quantichrom TM Glutathione Assay kit (DIGIT-250) through 96 well method [14].

**Statistical analysis:**
All the results were expressed as mean ± S.D in each group. The differences between the variables were assessed by unpaired T- test. Statistical analysis was done using SPSS software version 16.0. A p value of < 0.05 was considered significant.

## Results:-

| Parameters | Control (n=50) | Study groups | | |
|---|---|---|---|---|
| | | Group I (n=40) | Group II (n=40) | Group III (n=40) |
| **Age (mean ± SD)** | 15 ± 3 | 16 ± 2 | 16 ± 1 | 14 ± 2 |
| **Sex (Males)** | 50% | 67.5% | 65% | 75% |
| **Body mass index ($kg/m^2$)** | 21 ± 4.7 | 16 ± 4.1* | 19 ± 5.8 | 19 ± 2.3 |

**Table 1:-** Age, sex and BMI in different study groups. Values were expressed as mean ± SD. *p< 0.05 versus controls.

It is evident from Table 1 that there is no significant difference in age and sex among the groups (P>0.05). The mean body mass index(BMI) in GR-1, GR-2, GR-3 groups of HIV/AIDS patients were 16 ± 4.1, 19 ± 5.8 and 19 ± 2.3 respectively. The BMI in GR-1 group showed a significant decrease while compared to healthy controls (21 ± 4.7)

| Parameters | Control (n=50) | Study groups | | |
|---|---|---|---|---|
| | | Group I (n=40) | Group II (n=40) | Group III (n=40) |
| TBARS(nmol/mL) (m±SD) | 4.33 ± 0.93 | 8.54 ± 1.19* | 7.69 ± 0.84* | 6.97 ± 0.9* |
| Vitamin E( mg/dl) (m± SD) | 1.12 ± 0.23 | 0.28 ± 0.07* | 0.33 ± 0.56* | 0.56 ± 0.22 |
| Vitamin C( mg/dl) (m± SD) | 6.5 ± 0.71 | 3.7 ± 0.47* | 4.1 ± 0.95* | 5.8 ± 0.2 |
| GSH(µmol/L) (m± SD) | 5.21 ± 1.03 | 1.87 ± 0.79* | 1.95 ± 0.74* | 2.05 ± 0.83* |

**Table 2:-** Levels of plasma TBARS and vitamin C, vitamin E and GSH in different study groups. Values were expressed as means ± SD. *p<0.05 versus control group. The mean TBARS level in control, GR-1, GR-2 and GR-3 are 4.33 ± 0.93 nmol/mL, 8.54 ± 1.19 nmol/mL, 7.69 ± 0.84 nmol/mL and 6.97 ± 0.9nmol/mL respectively. The lowest vitamin E level is found in GR-1 group (0.28 ± 0.07) and highest in control group (1.12 ± 0.23). The

difference is statistically significant as evident from P values (<0.05). Similar trend was also seen in vitamin C with lowest level found in the case of GR-1 group (3.7 ± 0.47) and highest level in control group (6.5 ± 0.71). As expected, the highest value of GSH (5.21 ± 1.03) is found in the control group and the lowest value is seen in GR-1(1.87 ± 0.79) (Table 2).

## Discussion:-

In our study there is no significant difference in the age group of HIV seropositive individuals as against controls. Majority of the HIV positive individuals are males. Moreover those with severely depleted $CD_4^+$ counts have lower BMI indices when compared to the controls. These findings are similar to the other previously reported studies [15,16]

An increase in plasma concentration of lipid peroxidation by-product (TBARS) was detected in HIV positive patients in our study. This increase is consistent with the finding of other studies that showed TBARS concentration as a marker of oxidative stress in HIV positive patients [17].This trend suggests the role of lipid peroxidation in compromising the cellular redox status of the HIV patients. Increase in oxidative stress produced as a result of various cytotoxic effects of viral replication will result in further modification of DNA and proteins thus contributing to the disease aetiology.

Vitamin C & E levels were found to be significantly low among the HIV positive group with a $CD_4^+$ count <500 cells/$mm^3$. Though this findings correlate with other studies conducted before, but our findings were extended to the adolescent age group [18,19,20]. Vitamin E, a potent chain breaking lipid soluble antioxidant, reacts with lipid peroxyl radicals eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage. Vitamin C represents the major water-soluble antioxidant in the human body [21]. Increased oxidative stress may be the cause of vitamin C & E depletion. Micronutrient deficiency especially among the adolescent age group can also be a possibility of decreased vitamin C & E.

Plasma glutathione levels (GSH) were significantly decreased in all the HIV infected groups when compared to healthy controls. This finding in the younger age group is in conformity with previous study findings reported on adult age group [22,23,24]. Increased consumption of GSH by the GSH-Px reaction and decreased release into the circulation from the liver, the site of GSH production, might account for the low plasma concentrations. Considering the role of GSH in immune function the loss of thiol compounds, especially of GSH, represents a critical feature of HIV-disease progression. Our study findings suggest the role of increased oxidative stress and decreased antioxidant capacity as evident by reduced Vitamin C & E along with decreased reduced glutathione levels in the pathogenesis of HIV/AIDS patients when compared with healthy controls.

## Conclusion:-

Oxidative stress caused by HIV infection may accelerate progression of HIV disease, which enhanced lipid peroxidation in HIV/AIDS patients with concomitant failure of antioxidant defence mechanisms. This study showed that even in the adolescent age group concomitant increase in lipid peroxidation and breakdown of antioxidant status in HIV/AIDS individuals contribute to the possibility of poor immunity. Although the cause of this altered redox balance which may be due to increased redox imbalance or decreased intake of the micronutrients was beyond the scope of the study. Further study needs to be conducted to review any change in viral replication and disease progression on antioxidant supplementation.

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