



Antioxidant Status in HIV Positive Pregnant Women

Authors

Rajesh Bhanudas Rajekar¹, Hemakant Deshmukh², Vinayak Patil³

¹Assistant Professor Dept. of Biochemistry Grant Government Medical College and JJ Group of Hospital Byculla Mumbai (MS)

²Assistant Professor Dept. of Biochemistry Terna Medical College Nerul (MS)

³Professor and HOD Dept. of Biochemistry Grant Government Medical College and JJ Group of Hospital Byculla Mumbai (MS)

Corresponding Author

Rajesh Bhanudas Rajekar

Assistant Professor Dept. of Biochemistry

Grant Government Medical College and JJ Group of Hospital Byculla Mumbai (MS)

Email Id: ambad.sawan@gmail.com Mob no. 07999678840.

Abstract

Aims and Objectives: Compare the level antioxidant in HIV positive pregnant, normal pregnant and non pregnant women, socioeconomic status and to determine oxidative stress levels in all study groups

- Glutathione (GSH) concentration
- Malondialdehyde (MDA) concentration
- Total Antioxidant Status (TAS)
- Glutathione Peroxidase (GPx)
- Super Oxide Dismutase (SOD)

Result and Conclusion: The activity of GR, GPx, SOD, MDA and total antioxidant in non pregnant, normal pregnant and HIV positive pregnant women. The activity in non pregnant women and normal pregnant women were 9.27 ± 1.74 , 47.30 ± 10.27 , 1329.7 ± 111.8 , 3.03 ± 0.49 , 1.47 ± 0.18 and 4.37 ± 0.79 , 27.17 ± 2.76 , 944.9 ± 193.1 , 4.50 ± 0.32 , 1.19 ± 0.20 respectively. And the activity was 3.51 ± 1.15 , 19.54 ± 2.51 , 675.3 ± 125.9 , 5.46 ± 0.55 , and 0.88 ± 0.15 found in HIV pregnant women. It shows that the level of antioxidant in HIV pregnant women was significantly decreased but MDA level was slightly increased found compare to non pregnant and normal pregnant women.

On the basis of our result we concluded that the HIV positive pregnant women experience more free radical injury than those with HIV negative pregnant women. Due to oxidative stress, when highly induce it is involved in tissue damage. These mechanisms lead to the decrease of the antioxidant capacity of the body in HIV positive pregnant women.

Keywords: GR, GPx, SOD, MDA, HIV, AIDS, CD4, ROS, GSH and SES.

INTRODUCTION

In 1981, the first cases of atypical skin lesions and an aggressive disease of Kaposi's sarcoma appeared in homosexual men^[1]. These accounts

marked the initial onset of a condition that is called as Acquired Immune Deficiency Syndrome (AIDS). Within twenty years, AIDS became a global epidemic; an estimated 20 million people

died, and an additional 36 million people were living with its causative agent—human immunodeficiency virus (HIV) [2]. India has the third largest HIV epidemic in the world. In 2015, HIV prevalence in India was an estimated 0.26%. 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^[3]. Overall, India's HIV epidemic is slowing down, with a 32% decline in new HIV infections 86,000 in 2015, and a 54% decline in AIDS-related deaths between 2007 and 2015^[4]. India has the third largest HIV epidemic in the world. The HIV epidemic in India is driven by heterosexual sex, which accounted for 87% of new infections in 2015. However, the epidemic is concentrated among key affected populations such as sex workers. The vulnerabilities that drive the epidemic are different in different parts of the country. The five states with the highest HIV prevalence^[5] are Manipur, Mizoram, Nagaland, Andhra Pradesh and Karnataka. All these five states are in the south or east of the India. Some states in the north and northeast of the country have also reported rising HIV prevalence^[5]. A large majority of those infected lived in non-industrialized countries with inadequate financial support to handle the pandemic.

HIV or AIDS pregnant women suffer from several opportunistic infections that occur because of poor immunity. The HIV infection is cellular CD4 immunodeficiency. Different agents appear may trigger apoptosis in CD4+ T cell, including viral protein, inappropriate secretion of inflammatory cytokines by activated macrophages and toxins produced by opportunistic microorganism. Since oxidative stress can also induce apoptosis, it can be hypothesized that such a mechanism could participate in CD4+ T cell apoptosis found in AIDS. Oxidative stress (OS) results from the imbalance between reactive oxygen species (ROS) production and inactivation^[6]. The generation of free radicals is a normal physiological process, but

increased production of free radicals can act on lipids causing lipid peroxidation. The cells have evolved a number of counter acting antioxidant defenses. Free radical scavenging mechanisms includes enzymatic like GR, GPx, SOD, MDA and total antioxidant and nonenzymatic antioxidants i.e. vitamins, minerals which limit the cellular concentration of free radical and prevent excessive oxidative stress.

Some people have no symptoms when they first become infected with HIV. Others develop temporary flu-like symptoms in the first few weeks after being exposed to the virus. These include fever, headache, sore throat, achiness, fatigue, and swollen glands. These symptoms may not seem significant at first because they're similar to flu and generally get better without treatment. It can take as long as 10 years after infection to develop more severe symptoms. During this time, most people experience a gradual reduction in the number of CD4 cells in their blood. Healthy adults have between 500 and 1,200 CD4 cells in every cubic millimeter (mm³) of blood. A person with fewer than 200 cells per mm³ starts to develop serious infections called opportunistic illnesses and has progressed to AIDS.

Human immunodeficiency virus (HIV) infection is a worldwide problem and HIV/AIDS patients suffer from several opportunistic infections that occur because of poor immune system function. Oxidative stress results from the imbalance between reactive oxygen species (ROS) production and inactivation^[7]. HIV weakens the defensive role of the human immune system. Once HIV infects a person, the body attempts to overcome the virus by generating antibodies to fight it off. However, in a rate-limiting process, progression of the disease weakens the body's immune system to thwart off and even defend from infection. As the immune system becomes compromised, several opportunistic infections develop that give rise to AIDS. Under most circumstances, oxidative stress is deleterious to normal cell functions. An emerging view, however, is that, within certain limits, cellular

redox status is a normal physiological variable that may elicit cellular response such as transcriptional activation, proliferation or apoptosis. Exposure to oxidants challenges cellular systems and their responses may create conditions that are favorable for the replication of viruses such as HIV. A variety of enzymatic (superoxide dismutase, catalase, glutathione peroxidase etc) and non enzymatic antioxidants present in human serum become insufficient to circumvent the HIV-1 replication secondary to cellular ROS production (superoxide anion, hydroxyl radical, hydrogen peroxide) by the pro-oxidant effect of inflammatory cytokines or polymorph nuclear leukocyte activation^[8].

In HIV-infected patients increased oxidative stress has been implicated in increased HIV transcription through the activation of nuclear factor κ B (NF- κ B). NF- κ B is bound to factor I κ B in the cytoplasm in its active form, but various factors, such as TNF- α and ROS can cause the release of NF- κ B from factor I κ B, and NF- κ B translocates to the nucleus and binds to DNA. Glutathione (GSH) is one of the major intracellular thiol, which acts as a free radical scavenger and also is thought to inhibit activation of NF- κ B^[9]. NF- κ B is involved in the transcription of HIV-1. Thus, this shows ROS may potentially be involved in the pathogenesis of HIV infection through direct effects of cells and through interactions with NF- κ B and activation of HIV replication^[10]. The present study was aimed to assess oxidative stress markers in HIV/AIDS patients.

Several studies have attributed oxidants as critical role players in the genesis of AIDS. Many have suggested that the mechanisms responsible for the progression of AIDS could be reversed through administration of antioxidant reducing agents. The discovery of HIV led to a broadening of the view that oxidative stress (OS) may have a principal contribution to both the expression of HIV and development of AIDS^[11]. Around the same time, empirical observations from unrelated areas of AIDS research appeared to confirm this postulate

of an oxidative role. Studies even began to report the potential use of reducing agents to suppress HIV expression^[12-13].

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent. Consequently remarkable and dramatic events occur during this period for sustaining mother and fostering the growth and maintenance of fetus^[14]. Pregnancy is a physiological state, which is accompanied by a high-energy demand and an increased oxygen requirement. Both of these may lead to increased oxidative stress. Oxidative stress may be defined as a condition where there is disturbance in the pro-oxidant antioxidant balance, which favors the former^[15]. Lipid peroxidation is an oxidative process which occurs at low levels in all cells and tissues. Under normal conditions a variety of antioxidant mechanisms serve to control this peroxidative process^[16]. The generation of free radicals is a normal physiological process but increased production of free radicals can act on lipids to causes lipid peroxidation. The cells have evolved a number of counter acting antioxidant defenses. Free radical scavenging mechanisms includes enzymatic and non-enzymatic antioxidants which limit the cellular concentration of free radical and prevent excessive oxidative stress. The aim of the present study was to assess the markers of oxidative stress and antioxidative enzymes in pregnant anemic women.

Socioeconomic status (SES) encompasses not just income but also educational attainment, financial security, and subjective perceptions of social status and social class. Socioeconomic status can encompass quality of life attributes as well as the opportunities and privileges afforded to people within society. Poverty, specifically, is not a single factor but rather is characterized by multiple physical and psychosocial stressors. Further, SES is a consistent and reliable predictor of a vast array of outcomes across the life span, including physical and psychological health. Thus, SES is relevant to all realms of behavioral and

social science, including research, practice, education and advocacy.

As India is the country with cultural and geographical variation and also have diverse lifestyle of the people in terms of socioeconomic status. Occupation of the individual also played an important role, around 50-90% seroprevalence rates of HIV is seen in the sex workers living in metro cities of India like Mumbai, Delhi and Chennai. Despite of use of the condoms this rate is increasing among sex workers^[17]. Researches have shown that the socioeconomic status of the person plays important role in spread of HIV^[18]. Below poverty line peoples won't have access to the methods used for safe practices^[19]. Education and awareness about the HIV also plays an important role in spread of HIV. Rural population in India is still neglected in terms of awareness and also in terms of access to the various methods used for safe practices^[20]. In contrary, higher socioeconomic status that is wealthier peoples are now a day's prone to the drug usage, sex with multiple partners which increasing the risk of HIV in these particular population^[21].

Several studies were carried out to know the association between the socioeconomic status of an individual and its relationship with AIDS. HIV have been seen in both upper and lower socioeconomic status. It is necessary to evaluate and update the individual's knowledge about the same and to spread awareness.

In the present study, our aim was to investigate oxidative stress as an indicator of oxygen radical activity and antioxidant defenses in HIV or AIDS pregnant women and compare with normal healthy pregnant women at Grant Government Medical College and JJ group of hospital byculla Mumbai.

MATERIAL AND METHODS

I. Selection of Patients

The study was conducted in the Dept. of Biochemistry and in collaboration with Obstetrics and Gynecology Dept. at Grant Government Medical College and JJ group of hospital byculla

Mumbai. (MS). Investigation was carried out in 100 pregnant women suffering from HIV or AIDS and compared with 100 normal control group composed of age matched healthy pregnant women.

II. Collection of blood samples

Overnight fasting venous 5ml blood samples were collected from HIV or AIDS and normal healthy pregnant women in plain bulb and EDTA bulb. The plasma was separated from plain vacuum tube, aliquoted and stored at -20°C and used for the estimate glutathione reductase (GR), glutathione peroxidase (GPx), Superoxide dismutase (SOD), Malondialdehyde (MDA), and total antioxidant. Serum GR, GPx, SOD and total antioxidant activity were measured by using ELISA and reagents kits will purchased from RANDOX Laboratories Ltd.^[22-25].

III. Data Analysis

Data were expressed as mean \pm SD. Mean values were assessed for significance by paired student -t test. A statistical analysis was performed using the Stastical Package for the Social Science program (SPSS, 23.0). Frequencies and percentages were used for the categorical measures. Probability values $p < 0.05$ were considered statistically significant.

IV. Ethical committee Clearance

All studies on Human Volunteers were approved by the Institutional Ethics Committee of Grant Government Medical College and JJ group of byculla Mumbai vide latter no Sept 2008.

Table no 1:- Shows Age wise distribution of Control group and HIV pregnant women.

Age Group	Non pregnant women (n=2)	Pregnant Women (n=100)	HIV Pregnant Women (n=100)
23-24 yrs	34	12	8
25-26 yrs	23	34	23
27-28 yrs	19	29	27
29-30 yrs	16	14	31
Above 31	8	9	11

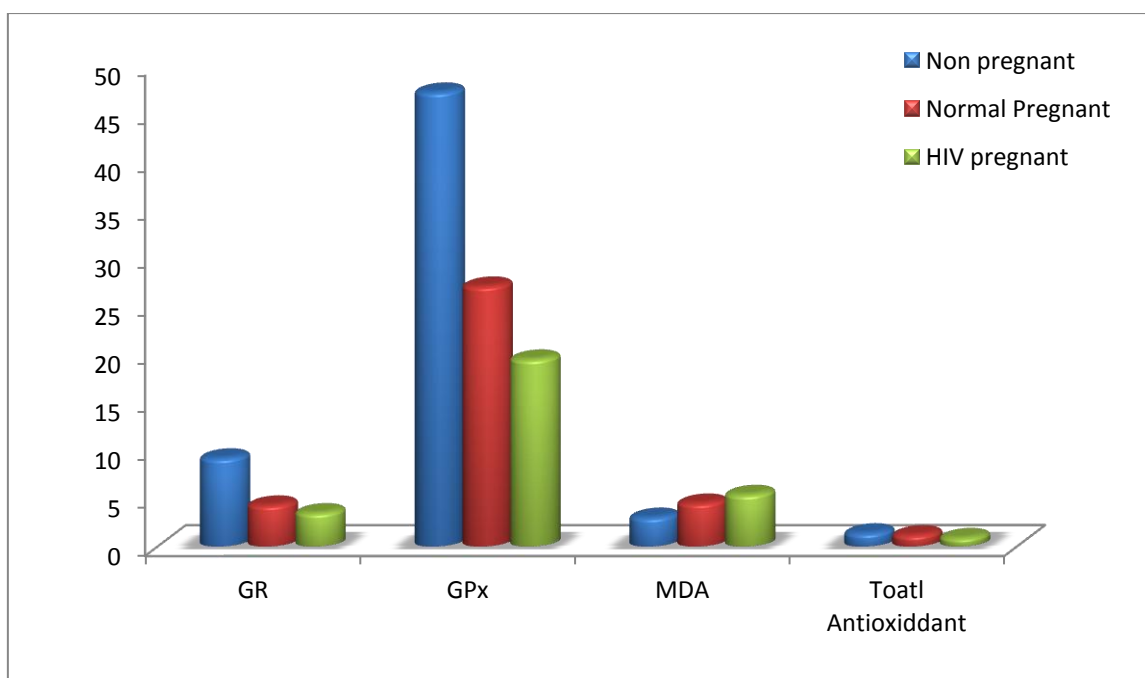
OBSERVATIONS AND RESULTS

Table 2 shows the antioxidant activity in non pregnant, normal pregnant and HIV positive pregnant women

Antioxidants	Non Pregnant Women	Normal Pregnant women	HIV Pregnant women
GR (µg/HB)	9.27 ± 1.74	4.37 ± 0.79	3.51 ± 1.15
GPx (µg/HB)	47.30 ± 10.27	27.17 ± 2.76	19.54 ± 2.51
SOD (µg/HB)	1329.7 ± 111.8	944.9 ± 193.1	675.3 ± 125.9
MDA (nmol/L)	3.03 ± 0.49	4.50 ± 0.32	5.46 ± 0.55
Total Antioxidant (mmol/L)	1.47 ± 0.18	1.19 ± 0.20	0.88 ± 0.15

Table 2 shows that the activity of GR, GPx, SOD, MDA and total antioxidant in non pregnant, normal pregnant and HIV positive pregnant women. The activity in non pregnant women and normal pregnant women were 9.27 ± 1.74, 47.30 ± 10.27, 1329.7 ± 111.8, 3.03 ± 0.49, 1.47 ± 0.18 and 4.37 ± 0.79, 27.17 ± 2.76, 944.9 ± 193.1, 4.50 ± 0.32, 1.19 ± 0.20 respectively. And the

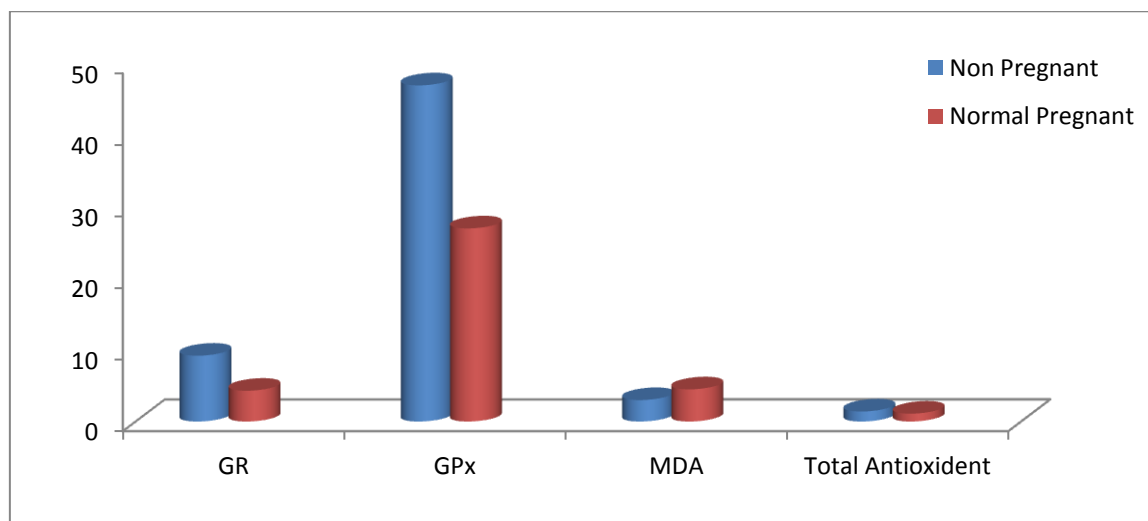
activity was 3.51 ± 1.15, 19.54 ± 2.51, 675.3 ± 125.9, 5.46 ± 0.55, and 0.88 ± 0.15 found in HIV pregnant women. It shows that the level of antioxidant in HIV pregnant women was significantly decreased but MDA level was slightly increased found compare to non pregnant and normal pregnant women.



Graph 1 shows the antioxidant activity in non pregnant, normal pregnant and HIV positive pregnant women

Table 3: comparison antioxidant activity in non pregnant and normal pregnant.

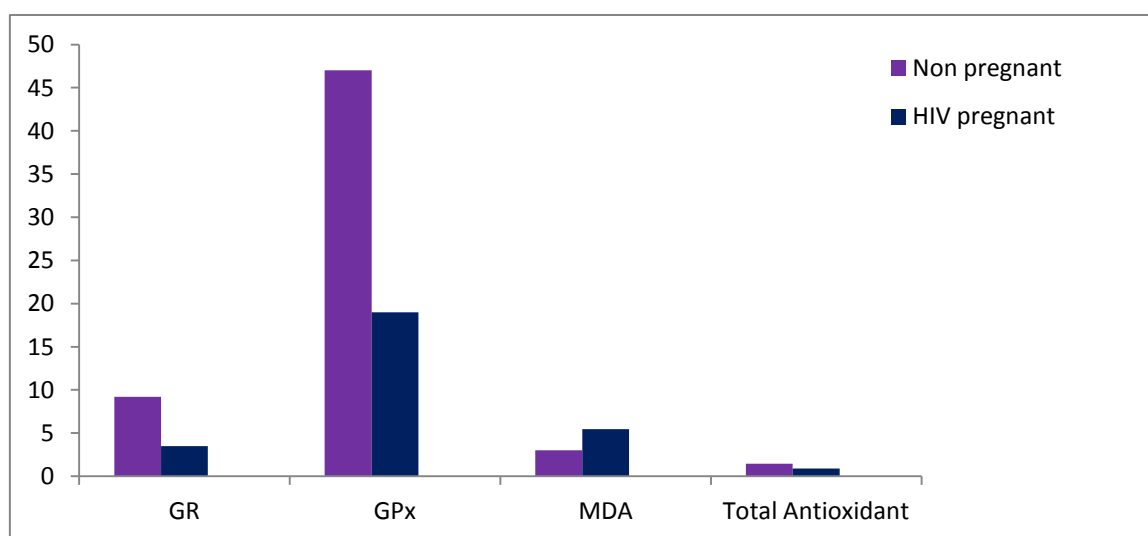
Antioxidants	Non Pregnant Women	Normal Pregnant women
GR ($\mu\text{g}/\text{HB}$)	9.27 ± 1.74	4.37 ± 0.79
GPx ($\mu\text{g}/\text{HB}$)	47.30 ± 10.27	27.17 ± 2.76
SOD ($\mu\text{g}/\text{HB}$)	1329.7 ± 111.8	944.9 ± 193.1
MDA (nmol/L)	3.03 ± 0.49	4.50 ± 0.32
Total Antioxidant (mmol/L)	1.47 ± 0.18	1.19 ± 0.20



Graph 2 comparison antioxidant activity in non pregnant and normal pregnant

Table 4: comparison antioxidant activity in non pregnant and HIV pregnant women

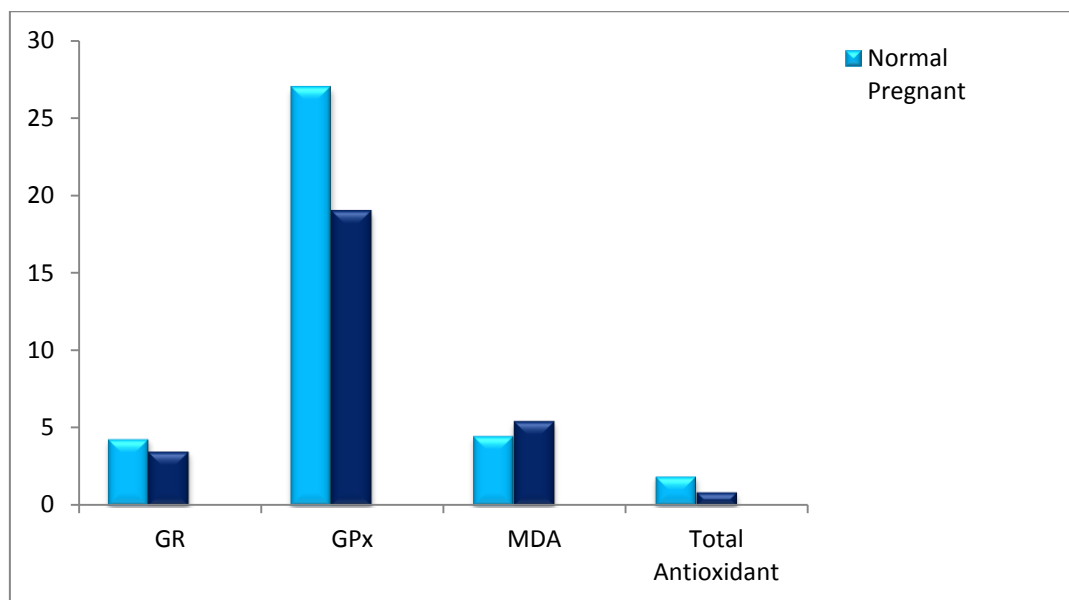
Antioxidants	Non Pregnant Women	HIV Pregnant women
GR ($\mu\text{g}/\text{HB}$)	9.27 ± 1.74	3.51 ± 1.15
GPx ($\mu\text{g}/\text{HB}$)	47.30 ± 10.27	19.54 ± 2.51
SOD ($\mu\text{g}/\text{HB}$)	1329.7 ± 111.8	675.3 ± 125.9
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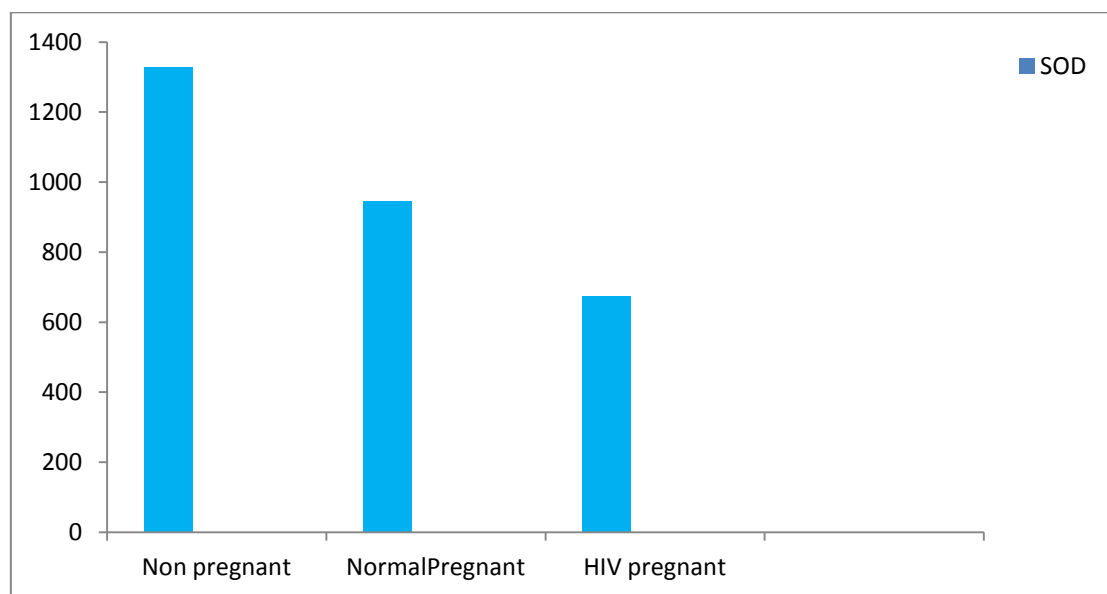
Graph 3 comparison antioxidant activity in non pregnant and HIV pregnant women

Table 5: comparison antioxidant activity in normal pregnant women and HIV pregnant women

Antioxidants	Normal Pregnant women	HIV Pregnant women
GR (µg/HB)	4.37 ± 0.79	3.51 ± 1.15
GPx (µg/HB)	27.17 ± 2.76	19.54 ± 2.51
SOD (µg/HB)	944.9 ± 193.1	675.3 ± 125.9
MDA (nmol/L)	4.50 ± 0.32	5.46 ± 0.55
Total Antioxidant (mmol/L)	1.19 ± 0.20	0.88 ± 0.15



Graph 4 comparison antioxidant activities in normal pregnant women and HIV pregnant women



Graph 5 shows activity of SOD in all study groups

Table no 4, 5 and 6 shows compare activity of antioxidant between non pregnant, normal pregnant and HIV pregnant women. Shows the activity of GR, GPx, SOD and total antioxidant in HIV pregnant women were significantly decreased but the activity of MDA was slightly

increased compare to normal pregnant and non pregnant women. In between non pregnant women and normal pregnant women the activity of GR, GPx, SOD and total antioxidant slightly decreased and MDA level was increased.

DISCUSSION

Oxidative stress results from an imbalance between the generation of reactive oxygen and protective mechanisms. Free radicals, the main causes of oxidative stress, may react with variety of biomolecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue. The oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases.

Antioxidant enzymes such as SOD, MDA, GPx, GR and total antioxidant can directly counter-balance the oxidant attack and protect the cells against DNA damage. SOD and MDA is a decisive antioxidant enzyme in aerobic cells, which is responsible for the elimination of superoxide radicals and it converts two toxic species: Superoxide and hydrogen peroxide (H_2O_2) into water. This diminishes the toxic effects of superoxide radical and other radicals formed by secondary reactions. GR and GPx is a selenocysteine – dependent enzyme. GPx in cells is the most important hydrogen peroxide (H_2O_2) scavenging enzyme^[26].

In the present study, mean SOD levels were found to be 1329.7 ± 111.8 , 944.9 ± 193.1 , 675.3 ± 125.9 in non pregnant, normal pregnant and HIV/AIDS infected pregnant women patients respectively. The present study revealed a decrease in the level of SOD in serum of HIV/AIDS patients than control group but this decrease was statistically insignificant^[27,28]. Such decrease may be due to its detoxification of released ROS (superoxide anion). In HIV/AIDS infected patients, the autoxidation results in the formation of hydrogen peroxide, which inactivates SOD. Therefore, the accumulation of hydrogen peroxide may be one of the explanations for decreased activity of SOD in these patients. The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn-SOD. As in the present study the total thiol level were increased which might leads to its auto-

oxidation leading to more production of superoxide anion because of which SOD utilized in its detoxification leading decreased level of it. Catalase is an enzyme present in most of the aerobic cells, it protects them from oxidative stress by exerting a dual function; it catalyzes the decomposition of hydrogen peroxide to produce water and oxygen catalytic function or oxidation of H donors i.e. peroxidase function.

In present study MDA level was found to be significantly increased in HIV pregnant women and normal pregnant women as compared to control. This increase was gradual with the progression of pregnancy, while antioxidants SOD, GPx, GR and total antioxidant were found to be decreased in normal pregnancy and HIV patient's pregnancy. Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Markers of lipid peroxidation i.e. MDA have been increased during the progression of normal pregnancy^[29]. In our study we found that the MDA activity also increased in with normal and HIV pregnancy. Ishihara Similar observation was made by Kodliwadmath et al^[30]. In the present study, it was found that there is significant increase of lipid peroxides in all the three trimesters. Since RBC has no nucleus, increased oxidative stress causes induction of antioxidant enzyme activities and this increase suggests a role of superoxide dismutase in the protection of embryonic development against free radical damage, which was observed by Carone et al^[31]. But, Stephen Wisdom et al^[32] and Davidge et al^[33] found that there is reduced superoxide dismutase activity in the third trimester of normal pregnancy as compared to non-pregnant women. Behne^[34] and Pathak et al^[35] have shown that there is a progressive fall in the activity of plasma Glutathione peroxidase and superoxide dismutase as pregnancy advanced. Our study reveals similar findings, but the decrease of both superoxide dismutase and Glutathione peroxidase were statistically significant. Yu^[36] suggested that

reduced glutathione is an effective reductant and plays an important role in a variety of detoxification processes. The enzyme Glutathione reductase plays a pivotal role in replenishing and maintaining optimum concentrations of reduced glutathione in biological systems. A gradual decrease in the activities of glutathione reductase and catalase throughout the three trimesters of pregnancy were observed in our study.

HIV/AIDS remains a significant development problem in India, and understanding the factors that can halt the spread of the disease is both an economic and a public health priority.

In this study found that higher socio-economic status was associated with the likelihood of HIV testing through VCT; that lower socio-economic status was associated with the likelihood of testing at integrated facilities; and that PMTCT and integrated testers were similar to non-testers and had lower levels of educational attainment compared with VCT testers. These results have implications for the implementation of programmes designed to ensure access to testing in low-resource settings^[37]. They suggest that provider-initiated modes of testing can increase uptake among socio-economically disadvantaged strata to a greater extent than traditional VCT at stand-alone facilities. Secondly, the lack of socio-economic differentials for PMTCT is consistent with the notion that expanding testing through PMTCT has reduced socio-economic obstacles for women^[38]. It is important to develop comparable ways to reach men and address the gender dimension of HIV testing, which has been recognized in global documents. Thirdly, given low levels of testing worldwide and the persistence of socio-economic obstacles to the uptake of testing, continued efforts are needed to encourage testing among the less affluent through multiple means.

CONCLUSION

On the basis of our result we concluded that the HIV positive pregnant women experience more free radical injury than those with HIV negative

pregnant women. Due to oxidative stress, when highly induce it is involved in tissue damage. These mechanisms lead to the decrease of the antioxidant capacity of the body in HIV positive pregnant women.

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