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**ORIGINAL ARTICLE**

# Markers of Oxidative Stress in Generalized Anxiety Psychiatric Disorder: Therapeutic Implications

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There is growing evidence that oxidative stress contributes to the pathogenesis of anxiety disorders. Our aim was to measure oxidative stress in anxiety disorders subjects, and assesses the potential confounding influences of anti anxiety therapy. Serum malondialdehyde and antioxidant levels were estimated in patients at the time of presentation and also after anti- anxiety therapy for 3 months. During the period of study no antioxidant/s was given to the patients and control subjects. Serum malondialdehyde levels were significantly higher in the anxiety disorders patients in comparison to control cases. Also, the antioxidant activity of enzymes super oxide dismutase, glutathione and non enzymatic antioxidant levels of vitamins E and C were significantly lower in patients compared to controls at the initial presentation. After 3 months of anti anxiety treatment all the above parameters showed reversal in the respective levels of serum malondialdehyde and antioxidant activity. Anti anxiety medications results in reduced oxidative stress which indicates that oxidative stress is not the cause, but rather a consequence, of anxiety disorders.

*Key words: Oxidative damage, anti- anxiety therapy, anxiety disorders*

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Oxidative stress has been implicated in the pathogenesis of diverse disease states and may be a common pathogenic mechanism underlying many major psychiatric disorders, as the brain has comparatively greater vulnerability to oxidative damage. The current evidence for the role of oxidative stress in psychiatric disorders, and its academic and clinical implication has been reviewed recently (Ng et al 2008). The broadest data for oxidative stress mechanism have been derived from studies conducted in schizophrenia, where evidence is available from different areas of oxidative research, including oxidative marker assay, psychopharmacology studies and clinical trials of antioxidants. The most common disorder in late life is generalized anxiety disorder (GAD), which is characterized by >6 months of worry about a number of life domains (e.g. relationships,

finances and health), difficulty in controlling the worry, and associated physical symptoms such as restlessness, fatigue, muscle tension and insomnia that interfere with social or occupational functioning. A new onset of GAD among older adults is often related to a depressive disorder. The combination of major depressive disorder and GAD has a worse prognosis overall, requiring 50% more time to respond to treatment and incomplete recovery from the depression (Ng et al 2008).

Evidence show that free radical attacks to the cells in the brain can, over time, impair mental acquity. In fact, the brain may bear the brunt of free radical attacks since it is rich in fatty acids, a favorite free radical target, and it uses large quantities of oxygen- a potential source of free radicals (Knight et al 1988)**.** Keeping these facts in view, the present study was under taken with the objective to find the role of free radicals and endogenous enzymatic antioxidant in patients of anxiety disorders.

We have measured the levels of oxidative stress along with the status of antioxidant enzymes in the serum of the anxiety patients to assess the influence of anti- anxiety medications in the patient of generalized anxiety disorder and also to assess clinico-pathological correlation at the initial presentation and post- treatment.

## MATERIALS AND METHODS

The present study includes 50 cases of generalized anxiety disorder selected from the outpatient Department of Medicine, Sir Sunder Lal Hospital, Banaras Hindu University, Varanasi. 20 healthy individuals matched for age and sex were selected as a control group. Patients were subjected to Hamilton rating score for anxiety and those who were having a >14 score were selected. All the patients were ensured of any previous treatment and only those were included who had not received any anti-anxiety or anti-depressant treatment. The patients were subjected to anti-anxiety or anti-depressant treatment (without antioxidants) for a period of three months. Blood samples were collected from the ante cubital vein of control individuals as well as from the patients before therapy and after treatment for the separation of serum. Informed consent of each participant was obtained for induction into purely scientific enquiry necessitating blood samples.

### Oxidative stress, enzymatic antioxidants and vitamin assay

Serum malondialdehyde levels in the patients and control were assayed by thiobarbituric acid technique of Philpot, 1963. Assay of super oxide dismutase (SOD) was based on the ability of the enzyme to inhibit the auto oxidation of pyrogallol (Marklund and Marklund 1974). The enzyme inhibition caused by the serum was calculated and the enzyme activity was expressed in mg protein/ml of serum. Glutathione peroxidase (GSH) determination was performed using Ellman's reagent (Beutler et al 1963). The value of GSH was expressed in □M of DTNB conjugated/mg of protein. Assay of vitamin E was estimated using the method of Baker and Frank, 1968. Serum ascorbic acid (vitamin C) level was assayed by the technique of Mc Murray and Gowenlock, 1988. Student's t test was employed for the statistical analyses of data to compare each group. The data were presented as mean + SEM.

## RESULTS

The present study was conducted in the Department of Medicine and Biophysics of Institute of Medical Sciences, Banaras Hindu University on the selected patients of generalized anxiety disorder who were subjected to anti-anxiety or anti-depressant treatment for a period of three months and their results were compared with age and sex

matched healthy controls. There were 27 (54%) males and 23 (46%) females in the anxiety group of patients. The general characteristics of the study group are outlined in Table I.

Anxiety and tension was the most common symptom followed by insomnia, depressed mood and gastrointestinal, autonomic cardiovascular, somatic, respiratory and genitourinary symptoms.

Serum MDA levels were measured as the marker of the oxidative stress and were observed to be significantly raised in anxiety patients at the initial presentation in comparison to control cases.

Before the anti- anxiety therapy, elevated levels of serum malondialdehyde level in anxiety patients in comparison to the control cases were accompanied by lower levels of antioxidant enzyme activity of superoxide dismutase, glutathione peroxidase and non-enzymatic antioxidant activity levels of vitamin E and vitamin C (Table III). Anti-anxiety medications for a period of three months to the anxiety patients exhibited a reversal trend with lower value of serum malondialdehyde along with a significant increase in the values antioxidant enzyme activity of superoxide dismutase, glutathione peroxidase, and non-enzymatic antioxidant activity levels of vitamin E and C.

Hamilton anxiety rating score was significantly decreased in cases after 3 month of treatment in comparison to cases without treatment and this difference was statistically highly significant Table IV.

**Table I**. General characteristics of the study group

| **Variables** | **Control** | **Patients** |
|---|---|---|
| Number | 20 | 50 |
| Gender (M/F) | 14/6 | 27/23 |
| Age (years) | 56 ± 9 | 58 ± 6 |

**Table II.** Symptoms in Anxiety patients

| **Symptoms** | **No of patients** | **Percentage** |
|---|---|---|
| Anxious | 39 | 78 |
| Tension | 36 | 72 |
| Fears | 13 | 26 |
| Insomnia | 33 | 66 |
| Intellectual | 9 | 18 |
| Depressed mood | 29 | 58 |
| Somatic (Muscular) | 7 | 14 |
| Somatic (Sensory) | 17 | 34 |
| Cardiovascular | 22 | 44 |
| Respiratory | 15 | 30 |
| Gastrointestinal | 27 | 54 |
| Genitourinary | 11 | 22 |
| Autonomic symptoms | 23 | 46 |
| Behavior at interview | 27 | 54 |

**Table III.** Serum MDA level and antioxidant enzyme activities in control and generalized anxiety disorder cases without and with anti- anxiety therapy

| Variables | Control (n=20) | Generalized anxiety disorder (n=50) | |
|---|---|---|---|
| | | Pre-treatment | Post-treatment (after 3 months) |
| MDA (m Mol/L) | 0.21 ± 0.53 | 0.35±0.11 | 0.23 ± 0.06# |
| SOD (mg protein/ml of serum) | 20.12 ± 3.65 | 6.22±1.27 | 15.34±4.86 |
| GSH (µM of DTNB conjugated/ml of protein) | 0.13 ± 0.03 | 0.05 ± 0.01* | 0.09 ± 0.02# |
| Vitamin E (mg/L) | 11.84 ± 1.21 | 9.58 ± 1.46* | 11.02 ± 1.44# |
| Vitamin C (mg/dl) | 2.12 ± 1.81 | 1.89 ± 0.23* | 1.97 ± 0.24# |

Pre- treatment vs. control * $p<0.01$, Post – treatment vs. pre-treatment # $p<0.01$

**Table IV.** Pre treatment and Post treatment Hamilton anxiety rating score in anxiety disorders cases

| Group | Cases | | Statistical Significance | |
|---|---|---|---|---|
| | Pre treatment | Post treatment after 3 months | 't' value (Paired) | 'p' value |
| Hamilton rating anxiety score | 35.94±6.99 | 11.71±3.62 | 13.56 | <0.001 |

## DISCUSSION

Oxidative stress is a condition which modifies the normal intracellular balance between oxidant substances produced during aerobic metabolism and antioxidant system processes which perform the function of neutralization, putting a series of protective mechanisms, of both an enzymatic and non-enzymatic nature, in action. Enzymatic systems include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). In non-enzymatic systems, the most important molecules are glutathione, alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), flavonoids, the phenol compounds and the minerals zinc, copper and selenium (Halliwell and Gutteridge 2007).

Numerous physiological and pathological processes such as ageing, excessive caloric intake, infections, inflammatory disorders, environmental toxins, pharmacological treatments, emotional or psychological stress, ionizing radiation, cigarette smoke and alcohol increase the bodily concentration of oxidizing substances, known as reactive oxygen species (ROS) or, more commonly, free radicals. These are chemical species which are highly reactive owing to the presence of free unpaired electrons. An increase in free radicals compromises the delicate homeostatic mechanisms which involve neurotransmitters, hormones, oxidizing substances and numerous other mediators.

Owing to their structure, which is rich in double bonds, polyunsaturated fatty acids (PUFAs) render cellular membranes vulnerable to damage from free radicals, causing peroxidation. The damage induced by lipid peroxidation renders the cell unstable, and therefore compromises fluidity, permeability, signal transduction and causes receptor, mitochondrial DNA and nuclear alterations.

Oxidizing stress from free radicals is one of the

factors which contribute to an increase in the speed of the cell cycle and consequent premature cell death, leading to many degenerative illnesses in the central nervous system, as well as psychiatric disturbances. Peripheral systems undergo a process of atherogenesis and can lead to pathologies in the cardiovascular system.

Our study indicates that there is an association between increased oxidative stress and the generalized anxiety psychiatric disorders. Morphological, biochemical and molecular studies both in experimental animals and in human beings also reveal that oxidative stress plays a primary role in the development of degenerative changes in the cells and tissues of our body. The highest degree of oxidative damage usually occurs in organs like brain, heart and skeleton muscle since these organs are composed primarily of post- mitotic cells (Sohal and Weindruch 1996). The central nervous system shows increased susceptibility to oxidative stress because of its high consumption rate (20% of the total oxygen inhaled by the body) that accounts for the increased generation of oxygen free radicals and reactive oxygen substrates. Since all the cells and tissues of our body are also equipped with anti oxidative enzymes like super oxide dismutase (SOD), glutathione peroxidase (GPX),glutathione reductase (GRd) and substances like reduced glutathione (GSH), they dispose the free radicals as and when they are generated thereby protecting the cells and tissues from the oxidative attack. Normally a balance is maintained between the oxidative attack of the free radicals and the antioxidative defense system prevailing in the cells and tissues of body. But when the balance is tilted more towards the generation of free radicals, then degenerative changes cause many degenerative diseases. Brain has a low level of antioxidative defense system as the concentration of various antioxidative enzymes like SOD, GPX, GRd and catalase is low in brain (Savolainen 1978). In addition brain has a high iron and ascorbate content in certain regions, which provide favorable environment for generation of oxygen free radicals. Brain is also enriched with polyunsaturated fatty acids that render neuronal cells easily vulnerable to oxidative attack. The interplay of all these factors contributes to enhance the oxidative stress (Zhang et al 1993)

## CONCLUSION

Patients with generalized anxiety disorder had higher level of MDA and lower levels of SOD, glutathione, Vitamin E and Vitamin C before therapy and showed reversal of values following treatment over a period of three months thereby suggest an association between increased oxidative stress and the generalized anxiety disorders.

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