Original Article

Alteration in Oxidative Stress Markers in Blood of Patients with Dementia

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Abstract

Background: Dementia is a neuro-degenerative disease characterized by a decrease in memory, attention and cognitive functions. The oxidant-antioxidant mechanism in the body plays an important role in aging and pathogenesis of various diseases including dementia.

Objective: The present study assessed the levels of antioxidants (Superoxide dismutase and catalase) and Malondialdehyde (indicator of lipid peroxidation) in blood of patients with dementia.

Methods: Blood levels of oxidative markers (superoxide dismutase, catalase and malondialdehyde) were measured using standard methods in 30 patients of Alzheimer's dementia and 30 non-demented age and sex matched subjects.

Results: There was decrease in anti-oxidant levels with an increase in serum malondialdehyde levels in patients with dementia.

Conclusion: Alteration in oxidative stress markers may play an important role in pathogenesis of neurological diseases like dementia.

Keywords: Superoxide dismutase, catalase, malondialdehyde, oxidative damage

Introduction

The term dementia refers to a group of disorders characterized by development of multiple cognitive defects including memory loss, that occur due to altered physiological conditions, effects of medications or other multiple etiologies leading to social and occupational dysfunction. Clinically the diagnosis of dementia is made by Diagnostic and statistical manual of mental disorders, 4th edition (DSM IV) [1]. According to DSM IV, dementia can be of various types like Alzheimer's disease, vascular dementia, dementia due to head trauma, HIV, Parkinson's disease etc. The prevalence of dementia in persons more than 65 years of age has been reported to be between 3.6% - 10.3% in Western countries and 1.8 - 10.8% in Asian countries [2].

Free radicals are important biochemical intermediates of metabolism associated with cellular homeostasis. A free radical is a highly reactive chemical species possessing an unpaired electron [3]. They attack all classes of biomolecules with the lipids being most susceptible. In the presence of oxygen they cause lipid peroxidation. The balance between free radicals and the antioxidant defenses is a key factor for preventing the development of noxious processes at the cellular and tissue level. The excessive production of free radicals and depletion of antioxidants is related to aging and disease processes [4].

The brain is more susceptible to oxidative brain damage as it utilizes about 20% of oxygen consumed by the body and has high iron and polyunsaturated fatty acids [5-7]. Changes in the oxidative stress markers in brain play an important role in pathogenesis of neurological and psychiatric diseases [8]. Studies evaluating oxidative status in brain tissue have conflicting reports [9-12]. Few studies have assessed the blood oxidative markers in dementia. This study evaluated the oxidative stress markers in blood of patients with dementia.

Materials and Methods

The study was conducted on 30 patients of Alzheimer's type of dementia (as diagnosed by DSM-

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IV) and 30 age and sex matched controls. Informed written consent was taken from the subjects. The study was approved by the Institute Ethical Committee.

Inclusion criteria for patients:

- Newly diagnosed cases, not on treatment
- Male subjects, above 65 years of age
- Patients fulfilling the diagnostic criteria of dementia as per DSM–IV classification
- MMSE score of less than 26

Exclusion criteria:

- Patients addicted to alcohol or drug abuse
- Patients suffering from major psychiatric disorder, chronic illness
- Any other concurrent drug intake

Estimation of enzymes:

5 mL of blood was collected from antecubital vein after 12 hours fasting in EDTA vials. Plasma was separated by centrifuging the samples at 3000 rpm for 10 minutes. Packed cells were used for estimation of catalase and superoxide dismutase (SOD). Plasma was used for assay of TBARS levels.

Malondialdehyde (MDA) was assayed using Thiobarbituric acid method [13]. 2.5 mL of 20% trichloroacetic acid was added to 0.5 mL of plasma in a test tube and allowed to stand for 10 minutes at room temperature. After centrifugation at 3500 rpm for 10 minutes, the supernatant was decanted and the precipitate was washed once with 2mL of 0.5M sulphuric acid. 2mL of 0.5M sulphuric acid and 3mL of TBA in 2M sodium sulfate were added to this precipitate and the coupling of lipid peroxide with TBA was carried out by heating in a boiling water bath for 30 minutes. After cooling in cold water, the resulting chromogen was extracted with 4mL of n-butanol by vigorous shaking. Separation of the organic phase was facilitated by centrifugation at 3000rpm for 10 minutes and its absorbance was measured at 530nm. The values were expressed in terms of malondialdehyde (nmol/mL)

The activity of SOD in erythrocytes was determined by the method described by Marklund and Marklund with some modifications as described by Nandi and Chatterjee[14,15]. The erythrocytes were washed twice with normal saline and hemolysed with 3 volumes of cold distilled water. 0.5mL of erythrocyte hemolysate was mixed with 3.5mL ice cold water, 1.0mL of ethanol and 0.6mL of chloroform. The hemolysate was mixed properly after each dilution and centrifuged for 10 minutes at 3000rpm. The supernatant was used for assay of SOD by taking different aliquots of 20, 50, 100, 150, 200 and 500µL of supernatant. The assay system contained 50mM air equilibrated Tris-buffer (pH 8.5), freshly prepared 2.6mM pyrogallol solution in 10mM HCl and different concentrations of hemolysate. The reaction was started by addition of 100 µL of freshly prepared pyrogallol to the cuvette containing tris-buffer, EDTA and hemolysate. The rate of increase in the absorbance at 420nm was recorded for 2 minutes, from 1 minute 30 sec to 3 minute 30 sec in a spectrophotometer. The lag of initial 1 min 30 sec was allowed for steady state of auto-oxidation of pyrogallol to be attained. The 50% inhibition of pyrogallol by SOD was measured at 420 nm and the activity expressed as U/gHb.

Measurement of catalase was done by the method described by Sinha [16]. Different amounts of hydrogen peroxide, ranging from 10 to 160 µmoles, were taken in small test tubes and 2mL of dichromate acetic acid was added to each. This led to production of unstable blue precipitate of perchromic acid, the color of which changes to green on heating for 10 minutes in water bath, due to formation of chromic acetate. The mixture was cooled at room temperature and optical density measured at 570 nm in spectrophotometer. The results were expressed as U/g Hb. The results were analyzed by SPSS 17, using unpaired t-test.

Results

The study was done on male subjects with mean age of cases being 72.69 ± 5.73 and that of controls 73.67 ± 4.76 years. Catalase levels in controls were higher (6.24 ± 2.93 U/g Hb) compared to 3.85 ± 1.83 U/g Hb in patients of dementia ($p<0.05$). Similarly, the SOD levels were higher ($p< 0.05$) in controls (2302 ± 866 U/gHb) compared to cases (2053 ± 600 U/g Hb). The levels of MDA were higher in cases at 2.47 ± 0.88 nmol/mL as compared to controls who had 2.25 ± 0.79 nmol/mL. ($p<0.05$)

Discussion

The present study evaluated the oxidative stress in

patients of dementia in comparison to age and sex matched controls. The results revealed a decrease in blood antioxidant levels (catalase and SOD) in patients with dementia, with higher levels of MDA. Most of the studies on dementia have evaluated the oxidative stress in regions of brain. This is in contrast to the present study, wherein the levels in blood have been examined and not in the brain tissue.

The evidence from tissue studies is however, conflicting. Gsell et al in their study, evaluated the levels of catalase and SOD in patients with dementia [9]. They found a reduced activity of catalase in parieto-temporal cortex, basal ganglia and amygdale. There was however no significant changes in level of SOD. Marcus et al demonstrated a significant decrease in SOD in frontal and temporal region [10]. There was also a decrease in catalase in temporal region.

Cantuti-Castelvetri et al found a significant increase in SOD activity in calcarine cortex and other areas like caudate nucleus, subthalamic nucleus and globus pallidus [11]. Aksenov et al reported elevated levels of catalase in hippocampus and inferior parietal cortex in Alzheimer patients [12]. All these studies have used brain tissues, from dead patients which was not possible in the present study setup to procure. Hence the blood levels were examined for evidence of oxidative stress.

In a recent study, Padurarui et al demonstrated a decrease in SOD with an increase in MDA in serum of patients with Alzheimer's disease [17]. Similar results of decrease in SOD and catalase and changes in MDA in blood samples have been reported by Casado et al [18].

Perrin et al showed that Cu/Zn superoxide dismutase, glutathione peroxidase and catalase were the main enzymes involved in cellular protection against free radical induced damage [19]. Serum MDA levels are taken as surrogate indicators of lipid peroxidation. The decrease in the levels of antioxidant enzymes namely SOD and catalase with an increase in MDA in our study, suggests a shift in the oxidant-antioxidant balance. The blood levels of antioxidants and MDA reflect the global status of oxidative stress in the body in contrast to the changes that occur at specific tissues. It can be deduced that there is increased lipid peroxidation in patients with dementia, leading to cellular dysfunction. The altered oxidative status is known to lead to increased permeability of blood brain barrier, altered tubulin formation, inhibition of mitochondrial respiration and changes in neurotransmitter and ion levels [20], thereby affecting neuronal functioning. The changes in oxidant-antioxidant balance occur in normal aging process, but are pronounced in dementia. It is hypothesised that the shift in the oxidant-antioxidant balance plays an important role in pathophysiology of dementia.

Key Points

There is an increase in oxidative stress markers with a decrease in antioxidant levels as represented by elevated MDA levels and reduced catalase and SOD levels in blood of patients with dementia.

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