Association of age related severity in oxidative stress and blood urea nitrogen levels in patients with dementia: A coastal Karnataka study

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ABSTRACT :

Introduction: It has been shown that occurrence of dementia increases with age. Oxidative stress has been implicated in the development of dementia. In the present work, we measured malondialdehyde (MDA), total thiols and other routine biochemical parameters in dementia patients to establish a relationship between them.

Method: Study was carried out in 51 dementia patients and 30 healthy controls. The serum from patients and controls were analyzed for MDA, protein thiols, lipid profile, RBS and BUN by spectrophotometric methods.

Results: There was a significant increase in MDA level ($p<0.001$) and blood urea nitrogen (BUN) level ($p<0.001$) and decrease in thiols levels ($p<0.001$) in dementia patients compared to healthy controls. There was a positive correlation between levels of MDA and age of patients ($r=0.542$, $p<0.001$). Negative correlation was observed between total thiols and age of the patients. ($r= - 0.420$, $p<0.05$) and total thiols and blood urea nitrogen ($r= -0.343$, $p<0.01$).

Conclusion: In line with previous studies, our study has shown increased oxidative damage in dementia patients irrespective of their etiology. This study also explores the relationship between increased BUN concentration and dementia.

Key words: Dementia, oxidative stress, MDA, Thiols, BUN.

INTRODUCTION:

Dementia is referred to as a group of chronic disorders which is characterized by memory loss, development of multiple cognitive defects seen in cases of altered physiological conditions. These altered conditions are marked by changes in the personality of an individual accompanied by loss of intellectual function. The alteration of the physiological conditions leading to dementia could be due to any medication or multiple etiology leading to social and occupational dysfunction.(1) It has been studied that dementia becomes more frequent in occurrence with age. It has got a prevalence of 5%-10% in those above 65 years of age and 20% in those over 80 years of age.(2) There are several factors and conditions that can lead to dementia. These can be, Alzheimer's disease (AD), vascular disease, dementia with Lewy bodies, alcoholic dementia, Creutzfeldt–Jakob disease, Parkinson's disease, genetic or a metabolic disease and toxic or a traumatic disease.

Our study was conducted to show that oxidative stress was a predisposition factor of dementia. Oxidative stress helps in promoting the pathogenesis of dementia. (3) Oxidative stress will occur in a condition where there is an imbalance in the oxidant and anti-oxi-

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dant level. Oxidants produced as a result of various bio-chemical reactions in our body can damage all biological molecules like DNA, RNA, cholesterol, lipids, carbohydrates, proteins and anti-oxidants.[4] The central nervous system is specifically vulnerable to damage caused by free radicals and reactive oxygen species (ROS). Brain is known to consume high amount of oxygen and has abundant lipid stores. So, when there is scarcity of anti-oxidant enzymes in the brain tissue, oxidative stress is seen. [5,6] Brain is an ideal target and also has abundant iron sources which catalyse free radical reactions, leading to high metabolic turnover and oxidative reactions in the mitochondria of brain cells. Electrons can leak from the mitochondrial respiratory chain by attaching themselves to molecules and form free radicals. Here, free radicals and catalysts are brought together in close proximity. [7-9]

The main type of dementia and prototype for study of dementia is Alzheimer's disease (AD). AD is known to be the most common degenerative disorder of the brain. [10] There have been many etiological hypotheses for AD. They are performed for neuron degeneration induced by the ROS and free radicals in oxidative stress.[11, 12] Free radical injury can lead to both AD and Parkinson's disease.[7-9] In 1956, Harman proposed the free radical theory which attributed to the ageing process by non- specific damage to macromolecules by these free radicals.[13,14] AD in advanced age is characterized by poor plasma antioxidant status and increased plasma lipid peroxidation, as a well as low resistance to peronyl radical exposure.[15] Oxidative stress is a major cause leading to protein oxidation [16] and ageing.[17,18] Studies on AD showed that the levels of anti-oxidants were decreased with an associated increase in protein oxidation, lipid peroxidation, DNA oxidation, advanced glycation end product and ROS formation responsible for its pathogenesis. [3, 19, 20] Along with age, metabolic factors can also cause dementia. [21] Blood urea nitrogen reveals the levels of urea nitrogen in the body and also suggests the proper functioning of kidney and liver. The increase in BUN suggests acute kidney injury. This can be due to the increase in oxidative stress as age advances and deterioration of antioxidant levels. Based on these observations made so far, the protein thiols and lipid peroxidation end product, malondialdehyde, lipid profile, random blood sugar (RBS) and blood urea nitrogen (BUN) were estimated in the blood of patients suffering with dementia. This could suggest an association of oxidative stress in dementia with aging and could also pave a way for a potent anti-oxidant therapy.[22]

MATERIALS AND METHODS

Subjects and Samples

The study was carried out on 51 dementia patients being treated at A.V Baliga Hospital, Udupi and 30 healthy controls. The mean age of patients with dementia was 68 ± 9 years and that of healthy controls was 67 ± 8 years. There were 21 males and 30 females in the patient group. The healthy controls were not on any kind of prescribed medication or dietary restrictions. Other demographic and clinical characteristics are reported in table 1. Informed consent was taken from all subjects involved in the study and was approved by institutional ethical committee. Blood samples (5 mL) were drawn into plain vacutainers from the antecubital veins of healthy controls and dementia patients. The blood was allowed to clot for 30 min and centrifuged at 2000g for 15 min for clear separation of serum.

Biochemical determinations:

Special chemicals like 5' 5' dithio-bis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), and standard MDA were obtained from sigma chemicals, St Louis, MO, USA. All other reagents were of chemical grade.

Thiol assay: Reaction mixture contained 900 µL 2 mM Na2 EDTA in 0.2 M Na2HPO4, 20 µL 10 mM DTNB in 0.2 M Na2HPO4 and 100 µL serum. Reaction mixture was incubated at room temperature for 5 min; absorbance read at 412nm. Appropriate sample and reagent blanks were prepared simultaneously and the respective absorbance was noted. Corrected absorbance values were used to calculate serum protein thiols using the molar extinction coefficient 1600 M^{-1} cm^{-1} and values expressed as µM. The calibration curve was produced using GSH dissolved in Phosphate buffered saline (PBS).

MDA assay: Reaction mixture contained 1 mL 0.67% thiobarbituric acid (TBA), 500 µL 20% Tri carboxylic acid (TCA) and 100 µL serum. Incubated at 100°C for 20 minutes; centrifuged at 12,000rpm for 5 minutes. Absorbance of supernatant read at 532 nm. MDA was determined by using molar extinction coefficient 1.56 x 105 M^{-1} cm^{-1} and values were expressed as µM.

Other biochemical parameters such as random plasma glucose (RPG) , serum levels of urea (converted to BUN), cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG) were determined by automated analyzer Hitachi 912O.

Parameters	Controls (N = 30)	Cases (N = 51)
MDA (µM/L)	0.19 ± 0.04	0.38 ± 0.13*
Thiols (µM/L)	362.54 ± 59.96	244.63 ± 74.92*
Random Plasma Glucose (mg/dL)	110.10 ± 11.55	131.35 ± 29.87*
Blood urea nitrogen (mg/dL)	27.96 ± 5.29	37.74 ± 12.13*
Serum Creatinine (mg/dL)	0.82 ± 0.20	1.02 ± 0.27
Cholesterol (mg/dL)	157.76 ± 28.64	159.92 ± 30.25
Triglycerides (mg/dL)	187.00 ± 17.93	186.47 ± 26.63
High density lipoprotein (mg/dL)	46.70 ± 7.62	48.39 ± 7.98
Low density lipoprotein (mg/dL)	105.13 ± 6.94	108.05 ± 17.21

*p <0.001, compared to controls (Values are expressed in mean±SD)
Table 1: Serum levels of different parameters in patients with dementia compared to healthy controls.

Statistical Analysis

The results were expressed as mean ± standard error of mean (SEM). A p<0.05 was considered statistically significant. Statistical analysis was performed using the statistical package for social sciences (SPSS-16, Chicago, USA). Independent sample t-test was used to compare mean values. Pearson correlation was applied to correlate between the parameters.

RESULTS:

A significant increase in MDA level (p<0.001) and decrease in thiols levels (p<0.001) was observed in dementia patients compared to healthy controls. A significant increase (p<0.001) was also observed in the levels of blood urea nitrogen (BUN) and random blood sugar (RBS) in dementia patients when compared to controls. There was a positive correlation between levels of MDA and age of patients (figure 1) (r=0.542, p<0.01) and MDA and random blood sugar (r=0.247, p<0.05). Negative correlation was observed between MDA and total thiols (r =-0.489, p<0.001), total thiols and age of the patients (figure 2)(r=- 0.420, p<0.01), total thiols and random blood sugar (r=-0.390, p<0.01), total thiols and blood urea nitrogen (r=-0.343, p<0.01). The other parameters did not show any significant difference. The Pearson correlation between the different serum estimates are shown in table 2.

Serum Estimates	Age (years)	Random blood sugar (mg/dL	Blood urea nitrogen (mg/dL)	Serum thiols concentration (µM/L)
Serum MDA concentration (µM/L)	0.542†	0.247‡		-0.489*
Serum thiols concentration (µM/L)	- 0.420 †	- 0.390†	-0.343†	

Table 2: Pearson correlation between serum estimates
*p<0.001, †p<0.01, ‡p<0.05

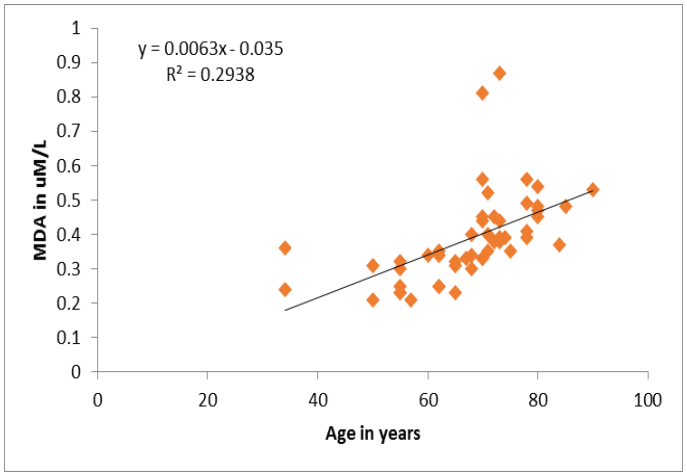

Figure.1 showing correlation between age and MDA

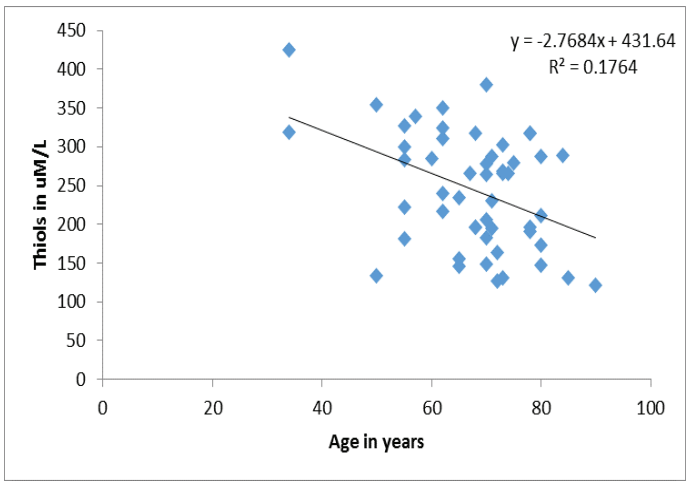

Figure.2 Showing correlation between age and thiols

DISCUSSION

In line with previous studies, our study has shown increased oxidative damage in dementia patients irrespective of their etiology. This indicates the possible role of antioxidants in prevention and progression of dementia. After conducting the study on 50 patients we found that lipid peroxidation increased in patients with oxidative stress. The marker we used to measure lipid peroxidation was malondialdehyde (MDA) in plasma. This increase has already been reported in cases of Alzheimer's disease (AD) [23] and Mild cognitive Impairment (MCI). [24] These free radicals are associated with oxidative stress which is involved in the pathophysiology of aging and various age -related diseases, including cataract, atherosclerosis, diabetes, diabetic retinopathy, chronic inflammatory disease of the GIT, aging of skin, diseases associated with cartilage, Alzheimer's disease AD) and other neurological disorders. AD has been reported as the most common form of dementia among the elderly. [18]

Oxidative stress plays a major role in most of the neurological disorders. Lipids in neuronal tissues in the form of unsaturated fatty acids are the major sites for free radical injury. Double bonds present in these fatty

acids are the prime targets of oxidants which results in peroxidation of neuronal membrane and this oxidative insult over a period of time results in cognitive decline as observed in dementia, bipolar disorder and schizophrenia. [25] One of the main peroxidation products is malondialdehyde (MDA) which increases in the cognitive deficit observed in Dementia. In our study we have observed significant increase in MDA in dementia patients compared to healthy controls.

In the present study, there was an increase in random blood glucose levels in dementia patients when compared to controls. Although the increase was within the normal range of random blood glucose, we can state that hyperglycemia itself is one of the predisposing factors in dementia. [26, 27] The oxidative stress generated due to hyperglycemic condition can further add up to the total oxidant load and worsen the condition in dementia. Therefore management of blood glucose levels in neurological disorders should be considered as important as other neuropsychological assessment.

The significant increase in the blood urea nitrogen level was also reported in our study. This shows that the dementia might be due to a metabolic cause. The increase in blood urea nitrogen signifies acute kidney injury. This increase in BUN can also be due to the heightened oxidative stress and free radical damage observed in old age [28, 29] which can lead to necrosis of the glomerulus. This necrosis over a period of time affects the filtering capacity of the kidney and can result in accumulation of numerous organic substances that possibly act as uremic neurotoxins. Thus increase in uremic neurotoxins can result in encephalopathy. [30] Therefore monitoring these metabolites is very crucial in the management of dementia.

The positive correlation between MDA and age of the patient in dementia indicates that as age progresses there will be increase in oxidative stress and depletion of antioxidant stores which results in increase in MDA. [31, 32] The positive correlation between random blood sugar and MDA indicates the severity of oxidative stress in hyperglycemic state. In accordance with previous studies, our study shows increase in blood glucose levels resulting in severe oxidative stress which causes membrane peroxidation and release of membrane peroxidation product such as MDA. [33]

In the present study, we observed a decrease in total thiols levels in dementia patients compared to healthy controls. These proteins bound thiols (-SH) groups are present abundantly in plasma and act as antioxidant in the body. These thiol groups have been shown to participate in various reductive reactions. The decrease in thiols indicates the heightened oxidative stress and depletion of antioxidant stores. The negative correlation between total thiols and age of the patient and total thiols and MDA shows the role of thiols in combating the reactive oxygen species and free radicals, thereby preventing oxidants induced membrane damage. The negative correlation is also observed between total thiols and blood urea nitrogen levels. This clearly signifies that presence of high amount of urea nitrogen is an indication of oxidative stress. Thus to neutralise these oxidants, thiols are consumed and hence their levels are decreased in the blood.

In conclusion, there is increased oxidative stress and free radical toxicity in dementia patients that causes membrane damage. This is further enhanced in dementia with high blood glucose and blood urea nitrogen levels. This may be due to the accumulation of uremic neurotoxins which increases the load of oxidative stress in metabolic dementia.

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