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LIPID PEROXIDATION AND ANTIOXIDANTS IN ALZHEIMER'S DISEASE

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ABSTRACT

The concepts of oxidative stress and antioxidants have been implicated in neurodegenerative disorders. Oxidative stress was assessed by estimating lipid peroxidation product in the form of thiobarbituric acid reactive substances (TBARS). Enzymatic antioxidants like superoxide dismutase(SOD), glutathione peroxidase(GPx), catalase, ceruloplasmin and non enzymatic antioxidant vitamins like vitamin-E, vitamin-C were estimated in either serum or plasma or erythrocyte in 25 patients of Alzheimer's disease and control subjects in the age group 60-85 years. The trace elements copper, zinc and selenium were also estimated. Plasma lipid peroxidation levels were significantly raised but activity of superoxide dismutase, glutathione peroxidase, catalase, ceruloplasmin and levels of vitamin–E, vitamin-C, copper; zinc and selenium were significantly reduced in Alzheimer's disease in comparison with control subjects. Study indicates that elevated oxidative stress and reduced antioxidant status may be playing role in cholinergic, noradrenergic and dopaminergic cell loss and involved in pathogenesis of Alzheimer's disease.

KEY WORDS

Lipid peroxidation, Antioxidants, Alzheimer's disease

INTRODUCTION

Alzheimer's disease [AD] is the most common and progressive neurodegenerative disorder. It is most common form of dementia in the elderly. Alzheimer's disease affects nearly 20 million people worldwide, incidence rate from 0.5% per year at 65 to 8-10% at 85 years [1, 2]. Recent advances in molecular genetics and neurochemistry has been involvement of excitotoxicity and oxidative stress in cell death [3]. Pathological hallmark of Alzheimer's disease is multiple distinct and overlapping pathway of neuronal damage, which includes senile plaques and neurofibrillary tangles [4].There is prominent loss of cholinergic, noradrenergic and dopaminergic neurons [5] and increase in deposition or production of the amyloid beta protein in the brain. Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease by finding of enhanced lipid peroxidation in specific area of brain in post-mortem studies [6]. Antioxidants have role to

protect cell from pathogenic oxidation. These are glutathione peroxidase, superoxide dismutase, catalase, vitamin-E and vitamin-C. Trace elements are required in a small concentration for activation of antioxidant enzymes. The study planed to investigate lipid peroxidation and certain antioxidants for understanding the pathogenesis of Alzheimer's disease.

MATERIALS AND METHODS

The study was carried out June 2004 to December 2007 with 25 clinically examined Alzheimer's disease patients ranging in age group 60-85 years admitted in government medical college hospital, Miraj. Diagnosis of Alzheimer's disease was done by physicians and conformed by senior neurologist of government medical college hospital, Miraj. Age and sex matched 25 normal healthy persons as per IFCC guidelines were included as control subjects. The patients with associated renal disease, liver disease, lung disease, thyroid disease, gastrointestinal disease, alcoholic, tobacco chewers and smokers etc. that could alter the required parameters were excluded from the study. Also the patients having obvious malignancy, hepatic, renal, or cardiac disease were excluded. The study was approved from the ethical committee of the institute and written consent was obtained from patients as well as controls.

The fasting venous blood samples obtained under sterile condition from Alzheimer's disease patients and healthy controls just before starting any drug treatment. The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of Dodge et al [7], modified by Quist [8]. The packed cells were used for the analysis of MDA, SOD, catalase and GPx. MDA was determined as the measure of thiobarbituric acid reactive substances (TBARS) [9]. SOD activity was determined in the hemolysate by the method of Mishra and Fridovich based on the inhibition of autoxidation of epinephrine to adenochrome at pH 10.2 [10]. Catalase activity was measured by the method of Beer and Seazer [11] and GPx activity by Paglia and Valentine in erythrocytes [12]. Levels of ceruloplasmin were estimated by colorimetric method by Karl [13]. Concentration of vitamin-E was estimated in plasma according to the method of Baker and Frank [14] and vitamin-C by Natelson method [15]. Serum copper, zinc and selenium levels were estimated by Atomic Absorption Spectrophotometer (AAS), Parkin Elmer model-3030. All the measurements were carried out by using Microsoft office 'Excel' with Windows 2003 operating system and all the data were analyzed using the statistical package SPSS-10.0.

Table 1
Comparative account of Lipid peroxidation and antioxidants in Alzheimer's disease.

S. No.	Parameters	Healthy Controls (n=25) Mean ± SD	Alzheimer's disease (AD) (n=25) Mean ± SD
1	MDA n moles/gm of Hb.	3.20± 0.68	5.57 ± 0.88*
2	SOD IU/gm of Hb.	1866.60± 522.65	1450.20 ± 325.50*
3	Catalase nmole/H_2O_2 decomposed/mg protein/min.	645±110.10	428 ±95.88*
4	GPx IU/gm of Hb.	55.55± 5.58	38.40 ± 3.99*
5	Ceruloplasmin IU/L	18.20 ±6.87	65.7 ±4.4*
6	Vitamin-E mg/ dl	380 ±20.07	644.5 ±55.47*
7	Vitamin-C mg/ dl	20.25 ±8.32	56.9 ±10.0*
8	Copper µgm/dl	108 ± 14.48	92 ± 3.22
9	Zinc µgm/dl	98.50 ± 7.27	74.85 ± 6.24
10	Selenium µgm/dl	18.18 ± 1.52	16.10 ± 1.00

*Values are expressed in mean ± SD. (n) = Number of observations. *indicates p < 0.001 compared with control subjects.*

RESULTS AND DISCUSSION

Thiobarbituric acid reactive substances (TBARS), the indicator of lipid peroxidation were significantly elevated in Alzheimer's disease. In Alzheimer's disease increased production or deposition (or both) of the amyloid beta protein with senile plaques in the brain. Beta amyloid proteins interact with vascular endothelial cells, produce free radicals. In AD the reactive microglia cells present at surrounding of plaques, on activation of microglia cells can generate ROM [16]. In AD increased N-methyl-D aspartate receptors trigger an influx of calcium that binds to calmodulin to activate nitric oxide synthase, enhanced activity of nitric oxide synthase (NOS) may increase nitric oxide[17,18]. Nitric oxide combines with superoxide radical to form peroxynitrate. Thus these free radicals may elevate membrane lipid peroxidation in the brain of AD and this might be causing loss of cholinergic, noradrenergic and dopaminergic neurons in AD.

Antioxidant enzymes status was studied by investigating erythrocyte SOD, GPx and catalase activity and serum ceruloplasmin levels. Activity of SOD and serum zinc levels was decreased in AD. (Table 1) Zinc stabilizers the structure of superoxide dismutase and when zinc ions removed, result in loss of SOD activity [19]. Thus low zinc levels might be responsible for reduced SOD activity and increase concentration of superoxide radicals. This suggests that increased formation of superoxide radicals and decrease activity of SOD. These superoxide radicals combine with nitric oxide and elevate oxidative stress.

Levels of selenium and activity of GPx were decreased in AD. (Table 1) GPx contains selenium in the form of single selenocysteine residue. [19] This decreased selenium concentration decrease formation of reduced glutathione. This may cause inhibition of glutathione peroxidase. The elevated oxidative stress and decreased selenium can cause dramatic reduction in glutathione peroxidase activity.

Increased oxidative stress in AD may oxidize hemoprotein subunit of catalase. Due to oxidation there may be dissociation of tetrameric hemoprotein molecule and results in loss of catalase activity. Elevated oxidative stress may inhibit synthesis of ceruloplasmin which might be responsible for reduced levels of ceruloplasmin in AD. The increased oxidative stress in AD may increase consumption of vitamin E and vitamin C, which may be responsible for decreased vitamin-E & vitamin-C in AD. This decreased antioxidant vitamins might be causing oxiradical inedited injury in AD.

The study indicates that elevated oxidative stress and reduced activity of antioxidant enzymes (SOD, GPx, and Catalase) and levels of antioxidant vitamins (vitamin-E & C) may cause loss of cholinergic, noradrenergic and dopaminergic neurons and play a role in pathogenesis of Alzheimer's disease.

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