Original Research Article

Comparative evaluation of enzymatic and non-enzymatic antioxidants in alzheimer dementia

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ARTICLE INFO

Article history:

Received 13-01-2020

Accepted 26-02-2020

Available online 30-06-2020

Keywords:

Superoxide dismutase (SOD)

Glutathione peroxidase (GSH-Px)

MDA (Malondialdehyde)

Ascorbic acid (Vit C)

Alpha-Tocopherol (Vit E)

Amyloid- β (A β)

Reactive oxygen species (ROS)

ABSTRACT

The senile dementia is a neurodegenerative disease characterized by loss of memory, attention and cognitive functions. The oxidants- antioxidant plays important role by several mechanism by reducing toxic Amyloid- β in neurons. The Alzheimer's disease is due to Amyloid- β deposition in the brain and production of neurofibrillary tangles. Amyloid- β induces reactive oxygen species in brain of Alzheimer dementia patients. In the present study we evaluated the levels of Superoxide dismutase, Glutathione peroxidase, Catalase, Vitamin C and Vitamin E and Vitamin B6 in Alzheimer dementia patients. It has been observed lower levels of Enzymatic, Non-Enzymatic Antioxidants and increased serum levels of malondialdehyde significantly in Alzheimer's dementia patients as compared to healthy controls ($p < 0.001$). Thus, altered oxidative stress markers may play important role in neurodegenerative diseases like Senile dementia. Serum levels of Superoxide dismutase, Glutathione peroxidase, Catalase, Vitamin C and Vitamin E and Vitamin B6 and Malondialdehyde (indicator of Lipid peroxidation) were measured using standard methods in 50 healthy controls and 50 Alzheimer's dementia patients.

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1. Introduction

Dementia is a syndrome, chronic or progressive in nature in which there is deterioration in cognitive function it affects memory, thinking, orientation, calculation, learning capacity, language and judgement".¹ The diagnosis of dementia clinically made by Diagnostic and statistical manual of mental disorders.² The most common type of dementia are Alzheimer's disease, Vascular dementia, Lewy body dementia, Frontotemporal dementia and HIV associated dementia.³ In India about 3.7 million people are affected by dementia at present according to ARDSI report and it will double by 2030.⁴

The etiopathology is still unclear of Alzheimer's disease.⁵ The cellular events of oxidative stress may be a basic mechanism of neurodegenerative disorders in recent findings. The acute oxidative stress accelerate cell

dysfunction and leads to cell death. The oxidative stress is an imbalance between pro-oxidant and free radicals on one hand and antioxidant system on the other hand.⁶ Oxidative stress is due to generation of oxygen free radicals, H₂O₂, hydroxyl radical, hydroperoxide and nitric oxide collectively named as reactive oxygen species (ROS). ROS is main etiologic factor and assume acute and specific neuronal degeneration which is observed in Alzheimer's disease.⁷ The ROS formation are highly reactive towards proteins, lipids, DNA molecules causing damage to these molecule and leads to dysfunction, death of cell.⁷

There are many free radical scavengers in the system which involve enzymes and non-enzymatic reactions. One of such enzymatic antioxidant defence system is superoxide dismutase which converts superoxide radicals to hydrogen peroxide (H₂O₂). These H₂O₂ converts in to water molecule by another enzymatic antioxidants GSH-Px and Catalase. Therefore SOD, GSH-Px and Catalase are called as primary defence system.^{7,8}

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The non-enzymatic antioxidant defence system is Ascorbic acid (Vit C), Alpha-Tocopherol (Vit E), and Pyridoxal Phosphate (Vit B₆). There is balance between both the activities and intracellular levels of these antioxidants which are required for survival of organism and their health.⁹ The brain has high content of polyunsaturated fatty acids (PUFA) and low levels of antioxidants. The brain PUFA are very much vulnerable attack by free radical and makes it prone to increased lipid peroxidation.¹⁰

The Amyloid- β (A β) is main culprit of Alzheimer's disease. The aggregation of A β and their deposition in brain leads to plaque formation. A β induces tau phosphorylation and ROS formation in brain. The peptidyl radicals' damages mitochondrial DNA, RNA, lipids and proteins leads to synapse damage and death of neuronal cell. This is taking place at memory centre, the hippocampus of the brain. The β -amyloid peptide produces free radicals outside the neurons and these free radicals become neurotoxic to synaptosomal membrane and the hippocampal cells.¹¹ This study was evaluated the oxidative stress markers in Alzheimer dementia patients.

2. Materials and Methods

1. To evaluate Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), Catalase, Ascorbic acid (Vit C), Alpha-Tocopherol (Vit E), Pyridoxal Phosphate (Vit B₆) and Malondialdehyde (indicator of lipid peroxidation) in Senile dementia.
2. To find out relationship between enzymatic antioxidants and non-enzymatic antioxidants in Alzheimer's dementia patients and control cases.

2.1. Study design

This study was designed as randomised Case controlled study.

2.2. Subjects and method

The present study was carried out in the department of Biochemistry Grant Medical College and JJ hospital Mumbai and BRIMS Bidar. The blood samples was collected and analysed in clinical Biochemistry laboratory. The total duration of study was three months. The patients selected for the present study were attending indoor/outdoor patient department from J J Hospital Mumbai and BRIMS Teaching Hospital Bidar.

2.3. Inclusion criteria

1. Newly diagnosed cases, not on treatments.
2. Male subjects, above 70 to 75 years.
3. MMSE Score of less than 12.

2.4. Exclusion criteria

1. Patients addicted to alcohol or drug abuse.
2. Patients suffering from major psychiatric disorder, chronic illness.
3. Any other concurrent drug intake.

3. The Healthy Control Subjects

50 healthy control subjects in the age group of 70 to 75 years were included in the study. The physical examination of subjects carried out in General Medicine and Psychiatric department such as Systolic blood pressure, Diastolic blood pressure, body mass index, and Mini Mental State score examination. The healthy control subjects shown all tests within normal limit, their MMSE Score was normal (26) and were completely free from psychiatric disorder, addicted to alcohol or intake of any other drug

4. The Dementia Subjects

50 Alzheimer's type of dementia in the age group of 70 to 75 years were included in the study. The physical examination of Patients carried out in General Medicine and Psychiatric department such as Systolic blood pressure, Diastolic blood pressure, body mass index, and Mini Mental State score examination. The Physical examination included Systolic blood pressure, Diastolic blood pressure, body mass index were within normal limits except Mini Mental State score examination Score in Alzheimer's dementia subjects. The Alzheimer's dementia was diagnosed by DSM-IV criteria and Mini Mental State examination score. The Mini Mental State score examination less than 12 of patients were selected in our study. This Mini Mental State score examination test administered by senior resident of psychiatric department, which includes questionnaires' related to place, time, attention and calculation, recall, language etc. A score of less than 23 points on the Mini Mental State score examination indicated cognitive impairment.¹² The informed written consent was taken from the dementia patients with the help of patients family who are very close to are generally most knowledgeable about what the patient want.¹³ The study was approved by institute ethical committee.

The fasting blood samples was collected from patients and healthy controls with all aseptic precautions in plain polythene tubes for the estimation of lipoproteins. Serum was separated by centrifuging the samples at 3000rpm for 10 minutes and preserved in freezer till the laboratory estimation proceeds.

5. Estimation of Enzymatic Antioxidants

The 12 hours fasting blood samples were collected using EDTA as anticoagulant. The hemolysates were used to determine Superoxide dismutase, Glutathione peroxidase

and Catalase. Erythrocyte Superoxide dismutase, Glutathione peroxidase and Catalase activities were measured using commercially available kits (Randox Laboratories Limited Ireland cat No. SD125 and RS 505 respectively). Erythrocyte Catalase activity was measured by the method of Aebi et al.¹⁴

6. Estimation of Non-Enzymatic Antioxidants

The Plasma was separated by centrifuging the samples at 3000rpm for 10 minutes. These plasma samples were preserved in freezer till the laboratory estimation starts. Malondialdehyde (MDA) was determined by using Thiobarbituric acid method.¹⁵

The estimation of Ascorbic acid was done by the colorimetric method of Ayekyaw (1978).¹⁶ The estimation of Alpha-Tocopherol was done by colorimetric method of Baker and Frank (1949).¹⁷ The estimation of Pyridoxal phosphate was done by enzymatic Bhulmann method.¹⁸ The statistical analysis was carried by Microsoft office 2019 and SPSS software version 18.1- 2017. The Pearson correlations were used as measures of association for the variables. The probability values $P < 0.0001$ was considered as significant and also data were expressed in mean $\pm$ SD form.

7. Result

Standard Characteristics of the two groups enrolled in the study are reported in Table 1. No difference appeared in age, systolic, diastolic blood pressure, BMI between Alzheimer's dementia subject and control group. The subjects in Alzheimer's dementia groups had significantly lower MMSE score compare to control group. ($P < 0.0001$). As presented in Table 2 . Negative correlation was observed but not significant correlation between GSH-Px, Vitamin C, and MDA and MMSE in Alzheimer's dementia subjects. The Catalase and Vitamin E observed small correlation with MMSE in Alzheimer's dementia. The Vitamin B₆ observed moderate correlation with MMSE in Alzheimer's dementia. The enzymatic Antioxidants Superoxide dismutase, Glutathione peroxidase and Catalase levels were lower in Alzheimer's dementia (1133.6 ± 63.32 , 50.09 ± 2.83 , 105.7 ± 7.96 , p value < 0.001) compared to healthy Controls (1406.2 ± 46.46 , 57.13 ± 3.12 , 129.48 ± 3.66). Similarly Ascorbic acid, Alpha-Tocopherol and Pyridoxal phosphate levels were lower in Alzheimer's dementia (0.79 ± 0.02 , 6.03 ± 0.47 , 18.36 ± 1.92 , p value < 0.001) compared to healthy Controls (1.7 ± 0.20 , 8.16 ± 0.47 , 27.95 ± 0.21). The levels of MDA were higher in Alzheimer's dementia patients (4.14 with SD ± 0.41 nmol/ml, ($p < 0.001$) as compared to healthy controls (2.51 with SD ± 0.21 nmol/ml). All the subjects in Alzheimer's dementia were male with mean age of cases being 73.42 ± 3.72 and that of controls 74.56 ± 4.30 .

Fig. 1: The Serum levels of Enzymatic Antioxidants in U/gm in healthy controls and Alzheimer's Dementia subjects. (* $p < 0.001$ as compared to healthy controls)

Fig. 2: The serum levels of non-enzymatic antioxidants in mg/dl in healthy controls and Alzheimer's Dementia subjects. (* $p < 0.001$ as compared to healthy controls)

8. Discussion

The present study we evaluated oxidative stress markers in Alzheimer's dementia patients in comparison with age and sex matched controls. The results showed that a decrease in Serum levels of enzymatic and non-enzymatic antioxidants such as Superoxide dismutase, Glutathione peroxidase, Catalase, Ascorbic acid (Vit C), Alpha-Tocopherol (Vit E) and Pyridoxal phosphate (Vit B₆) in patients with Alzheimer's dementia, with higher levels of MDA. Most of the previous studies on evaluation of these oxidative stress in brain region. This is in contrast of our study where the levels of these markers were assayed in blood and not in brain region. The oxidative stress plays an important role in several neurological disorders in recent research. The pathophysiology of neurodegenerative disorders like Alzheimer's disease involves oxidative stress.¹⁹ In the brain, production of free radicals in Alzheimer's disease patients is unique, which comes from sources of Alzheimer's disease-affected brain. It has been shown that the free radical production by β -amyloid peptide and

Table 1: Standard characteristics of Alzheimer's Dementia (AD) patients and Control groups

S. No	Parameters	AD group MMSE	Control group MMSE	P value
1	Age (Year)	73.42±3.72	74.±3.72	Not Significant
2	SBP (mm in Hg)	132.24±2.72	128.24±1.18	Not Significant
3	DBP (mm in Hg)	84.14±1.39	83.33±2.08	Not Significant
4	BMI (Kg/m ²)	24.68±1.12	26.04±0.32	Not Significant
5	MMSE Score	6.24±1.12	27.45±1.72	0.0001*

Data are expressed as Mean ±SD, SBP: systolic blood pressure, DBP: diastolic blood pressure, BMI: Body mass index. MMSE: Mini Mental State Examination, AD: Alzheimer's dementia group.

Table 2: Pearson correlation analysis between Antioxidants Vitamins and MMSE score in patients of Alzheimer's Dementia (AD) and Control group

S. No	Parameters	AD groups MMSE	Control group MMSE
1	Superoxide dismutase (SOD)	r = 0.08	r = 0.03
2	Glutathione Peroxidase (GSH-Px)	r = -0.05	r = 0.13
3	Catalase	r = 0.10	r = 0.02
4	Ascorbic Acid (Vit C) mg/dl	r = -0.08	r = -0.18
5	Alpha-Tocopherol (Vit E) mg/dl	r = 0.15	r = 0.19
6	Pyridoxal phosphate (Vit B ₆) nmol/L	r = 0.34	r = 0.003
7	MDA (Malondialdehyde) nmol/L	r = -0.02	r = -0.001

MDA (Indicator of lipid peroxidation).

Table 3: The Serum Levels of Antioxidant Vitamins in Alzheimer's dementia (AD) and Control group

S. No.	Parameter	Groups	
		Healthy Control Mean Age (73.42 ± 3.72)	Alzheimer's Dementia Mean Age (74. ± 3.72)
1	Superoxide Dismutase (SOD) U/gm Hb	1406.2±46.46	1133.6 63.32
2	Glutathione Peroxidase (GSH-Px) U/gm Hb	57.13±3.12	50.09±2.83
3	Catalase U/gm Hb	129.48±3.66	105.7±7.96
4	Ascorbic Acid (Vit C) mg/dl	1.77±0.20	0.79±0.02
5	Alpha-Tocopherol (Vit E) mg/dl	8.16±0.47	6.03±0.47
6	Pyridoxal phosphate (Vit B ₆) nmol/L	27.95±0.21	18.36±1.92
7	MDA (Malondialdehyde) nmol/L	2.51±0.21	4.14±0.41

All values are expressed as mean ± SD. (*p < 0.001). MDA (Indicator of lipid peroxidation).

advanced glycation end products.^{20,21}

The components of cells and mitochondrial DNA, RNA, Proteins and lipids damages by increased reactive oxygen species leads to synapse damage and finally neuronal cell death. This whole process taking place in to the memory centre, the hippocampus, and related key brain areas of Alzheimer's dementia patients. The critical step in the pathogenesis of several diseases is lipid peroxidation. The underlying mechanism of oxidative damage due to free radicals for many pathological conditions. The cellular component is damaged by free radicals. Lipid peroxidation is often the first parameters to which researchers turn when they wish to prove the involvements of free radicals in cell damage. Free radical involvement in neurodegenerative disorder is through distinct stages; initiation, elongation & chain propagation.²²

Table 3 & Figures 1 and 2 clearly shows significantly elevated levels of lipid peroxide and decreased levels of Superoxide dismutase, Glutathione peroxidase, Catalase, Ascorbic acid (Vit C), Alpha-Tocopherol (Vit E) and

Pyridoxal phosphate (Vit B₆) in Alzheimer's dementia patient as compared to control groups.

The G sell et al. evaluated enzymatic antioxidants like catalase and SOD in patients with dementia and found reduced activity of catalase in basal ganglia, parieto-temporal, and cortex.²³ Marcus et al. found decreased levels of SOD in frontal and temporal region.²⁴ In recent study, Padurarul et al. found decreased levels of SOD with increase in MDA in serum of Alzheimer's disease patients.²⁵ Similarly results found by Casado et al. that decreased levels of SOD and catalase and changes in MDA levels in blood samples of Alzheimer's disease.²⁶

Perrin et al. showed that superoxide dismutase, catalase and Glutathione peroxidase were the main enzymes involved in protection against the free radical induced damage.²⁷ Serum MDA are taken as substitute indicator of lipid peroxidation. The decrease in superoxide dismutase, glutathione peroxidase, catalase, Ascorbic acid (Vit C), Alpha-Tocopherol (Vit E), Pyridoxal Phosphate (Vit B₆) with increase in MDA in our study suggest there is shift in

the oxidant-antioxidant balance.

Baldeiras et al. Greilberger et al 2008 studies found that higher levels of lipid peroxidation in the central nervous system and peripheral tissues both in patients and Alzheimer's dementia and mild cognitive impairment.²⁸ Hence one can postulate the accumulation of free radicals which leads in to stimulate antioxidant defences, leads to depletion of antioxidant reserves. Hence our study showed there was increased lipid peroxidation (MDA). Thus we conclude the free radical produced by β -amyloid is neurotoxic and vascular endothelial cells produce abundance superoxide radicals by interacting with β -amyloid causing lipid peroxidation. The surrounding of senile plaque contains deactive microglia cells which become active macrophage can generate ROM and increases susceptibility of lipid peroxidation membrane causing loss of cholinergic neurodegeneration and dopaminergic neurons in the Alzheimer's brain.²⁸

Riviere S et al, Montilla Lopez P et al. studies shows plasma levels of vitamin C were significantly lowered in patients of Alzheimer's disease even after adequate amount of this vitamin in the diet. Further invitro and invivo research shows vitamin C can decrease oxidative stress and inhibits structural progression of Alzheimer's disease by arresting the oligomerization of A β peptides.²⁹ Our results also shows similar of this study. Therefor we suggest increased oxidative stress increases consumption of Ascorbic acid (Vit C) for countering the free radicals leads to decreases the antioxidants Ascorbic acid (Vit C) Alpha-tocopherol (Vit E) in Alzheimer's dementia patients.

Mangialasche F, Xu W, Kivipelto M et al. studies reported that decreased levels of plasma Vitamin E with increased risk of associated neurodegenerative disorders like Alzheimer's disease and mild cognitive impairment.³⁰ Further Aoki K, et al. postulate that vitamin E deficiency leads in to destruction of neurons causes cerebral atrophy.³¹ Alpha-Tocopherol (Vit E) act as chain breaking antioxidant which reduces the progression of Alzheimer's disease. Increased oxidative stress by A β plaques is well known risk factor for neuronal damage. Alpha-Tocopherol act as scavenger for these free radical and provides neuroprotection.³¹ Therefore Alpha-Tocopherol (Vit E) plays a role in protective plasma lipids from oxidative stress. In our study due to increased oxidative stress, consumption of Alpha-Tocopherol (Vit E) is increased. It breaks the chain reaction by trapping free radicals that damages cells. This leads decreased antioxidant Alpha-Tocopherol (Vit E) in Alzheimer dementia may cause oxyradical mediated injury. Thus, the important role of Alpha-Tocopherol (Vit E) is in inhibition of lipid peroxidation in Alzheimer dementia.^{30,31}

PLP deficiency causes loss of body functions such as poor sleep, behaviour, cardiovascular function, loss of hypothalamus pituitary control of hormone secretion. It has been recently shown that Vitamin B₆ is highly antioxidant

effects. It was manifested that Vitamin B₆ act as a quencher of hydroxyl radical (*OH) and further up to scavenging of eight (*OH) molecules.³² Pyridoxine deficiency leads to fatty acids biosynthesis, and impaired defence mechanism and increases lipid peroxidation.³³

M. Keles, B. et al. and B. K. Ohta et al. showed that lower levels of Vitamin B₆ in patients suffering from mild cognitive impairment (MCI) or Alzheimer disease (AD) leads into progression of disease, they further found improvement in cognitive function by supplementation of pyridoxal phosphate (Vit B₆) in their patients groups.^{34,35} Therefor due to antioxidant property of pyridoxal phosphate (Vit B₆) we suggest the hypothesis oxidative stress may reduce vitamin B₆ due to high rate of consumption, which results in to deficiency of pyridoxal phosphate (Vit B₆). The decreased levels of Pyridoxal phosphate leads to increased homocystein in circulation which increases toxicity of neurons by generation of ROS and increases the oxidative stress.

In conclusion our results suggest that there is change in production and metabolism of reactive oxygen species. In normal biological process generation of oxidants are powerful molecules is balanced by antioxidants. In neurodegenerative disorders like Alzheimer dementia there is imbalance of oxidant-antioxidant, results in elevated oxidative stress. Elevated oxidative stress may be responsible for loss of cholinergic, noradrenergic, dopaminergic neurons in Alzheimer dementia.

Initial increase in lipid peroxidation is due to β -amyloid protein is neurotoxic and vascular endothelial cells produce abundance superoxide radicals, with reduction in enzymatic and non-enzymatic antioxidants by exposing the cells to the effect of superoxide radical, hydroxyl radical and hydrogen peroxide. The oxidant mediated neurotoxicity and loss of neurons occur in neurodegenerative disorders, thus the initiation of progression of Alzheimer dementia is due to free radical injury is important, then the therapy of agument endogenous antioxidant to reduce oxidative injury which might prevent delay the disease process.

Thus, we suggest antioxidant agents may be useful in treatment of neurodegenerative disorders like Alzheimer dementia. In Alzheimer dementia β -amyloid is toxic to neurons, which can be diminish by antioxidants Vitamin C and vitamin E and vitamin B₆. Ascorbic acid (Vitamin C) with multidrug therapy may be useful for neurodegenerative disorders like Alzheimer dementia. Thus, we suggest antioxidant therapy is most promising field in oxygen free radical research.

9. Source of Funding

None.

10. Conflict of Interest

None.

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Cite this article: Jadhav BH, Kalekar MG, Jaweed SA, Shelke P. Comparative evaluation of enzymatic and non-enzymatic antioxidants in alzheimer dementia. *Int J Clin Biochem Res* 2020;7(2):232-237.