OXIDATIVE STRESS IN ALZHEIMER'S DEMENTIA

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Abstract

The aim of this study was to evaluate oxidative stress on a group of patients with Alzheimer's dementia (AD) and compare its parameters with a control group composed of mentally healthy subjects.

This research involves patients diagnosed with AD from the Clinical Hospital for Neurology and Psychiatry of Oradea and from nursing homes for the elderly, whose levels of malondialdehyde (MDA), carbonylated proteins (CP) and ceruloplasmin (CER) have been determined.

The results have been compared with those of a control group consisting of 40 persons of comparable age and educational levels and who had visited their general practitioner (GP) for regular checkups.

Patients exhibited high levels of oxidative stress reflected by their elevated levels of malondialdehyde (MDA), carbonylated proteins (CP) and ceruloplasmin (CER).

Rezumat

Scopul acestui studiu a fost de a evalua stresul oxidativ pe un grup de pacienți cu demență Alzheimer (AD) și de a compara parametrii testati cu un grup martor format din subiecți sănătoși mintal.

Această cercetare implică pacienți diagnosticați cu boala Alzheimer de la Spitalul Clinic de Neurologie și Psihiatrie din Oradea și de la azile de bătrâni, ale căror niveluri de malondialdehidă (MDA), proteine carbonilate (CP) și ceruloplasmina (CER) au fost determinate.

Rezultatele au fost comparate cu cele ale unui grup de control format din 40 de persoane cu vârstă și educație comparabile și care au vizitat medicul de familie pentru controale regulate.

Pacienții au prezentat un nivel crescut de stres oxidativ reflectat de concentrațiile ridicate ale malondialdehidei și a proteinelor carbonilate.

Keywords: Alzheimer's dementia, oxidative stress, carbonylated proteins.

Introduction

Alzheimer's disease is the most common form of mental decline in the elderly. Prince and collaborators in a recent study estimated that a total

of 36.6 million people aged over 60 are suffering of this disease and in the future this number is expected to almost double every 20 years [19].

In Alzheimer's disease there is a progressive deterioration of memory. Intra- and extracellular histological alteration were described. These include the presence of extracellular deposits of amyloid-β peptides forming senile plaques and the intracellular neurofibrillary tangles of hyperphosphorylated tau in the brain and there are commonly considered pathognomonic for the disease [8].

Many studies have shown that oxidative stress is an early event in Alzheimer's disease and appears before cytopathological changes and can have an essential role in disease pathogenesis [26].

Oxidative stress causes an imbalance between the production of reactive oxygen and the ability of a biological system to rapidly scavenge the reactive intermediates [13].

Reactive oxygen species are highly reactive. They are able to attack proteins, carbonylating them, lipids - by oxidation of which results in a stable compound, malondialdehyde, carbohydrates and nucleic acids [7].

The objective of our study was to investigate the oxidative stress markers on a group of patients with Alzheimer's dementia and compare its parameters with a control group composed of mentally healthy subjects in our county.

Materials and Methods

The research was carried out between 2003-2008 on a group of 171 patients diagnosed with Alzheimer's dementia (AD), of whom 148 were in-patients of the Clinical Hospital for Neurology and Psychiatry of Oradea and 23 resided in nursing homes for the elderly within Bihor County and the Nucet Hospital, 5 were patients of the Medical and Social Centre of Ciutelec and 6 were patients of the Philadelphia Foundation Sălard. Out of those 171 patients, we have selected 40 Alzheimer's dementia patients for whom oxidative stress was evaluated. The values were compared to those obtained from a number of 40 healthy individuals of similarly age and education, visiting their general practitioner (GP) for their regular checkups.

The study was approved by the institutional ethical committee and all patients/tutors gave written informed consent.

Inclusion criteria for patients with AD

Age: 50 – 58.

Diagnosis: Alzheimer's dementia (AD), according to criteria International Statistical Classification of Diseases, 10th Revision (ICD-10;

1992) and Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; 1994) [1, 28].

A minimal score on the Mini Mental State Exam (MMSE) of 25 points.

Exclusion criteria for the control group

- Concurrent neurological issues
- Severe anemia (hemoglobin < 9 g/dL)
- Severe and unchecked arterial hypertension
- Severe malnutrition
- Concurrent psychiatric issues or a history of psychological illness
- Mental deficiency
- System diseases (cancer, HIV-AIDS)
- Stroke (cerebrovascular accident CVA) in the last 6 months
- Alcoholism or others

The oxidative stress level was assessed by measuring in ser malondialdehyde (MDA) using a method with thiobarbituric acid (TBA), carbonylated proteins (CP) with guanidine hydrochloride method, and the concentration of ceruloplasmin (CER), the most powerful plasma antioxidant with the Ravin method [7].

The statistical analysis was carried out using SPSS software (version 12).

Results and Discussion

The data for measured biomarkers (MDA, CER and CP) are presented in Table I. All results are presented as means ± standard deviation.

Table I

Mean and standard deviation of oxidative stress biomarkers

Parameter	Study Group	Control Group
CER	32.7±3.7 mg%	31.6±1.5 mg%
MDA	4.63±0.52 mmol/mL	2.12±0.27 mmol/mL
CP	4.02±0.45 mmol/mg	1.53±0.12 mmol/mg

There was no significant difference of the serum ceruloplasmin concentration between patients with Alzheimer's dementia (32.7±3.7 mg%) and the reference group (31.6±1.5 mg%) ($p>0.05$) (Figure 1).

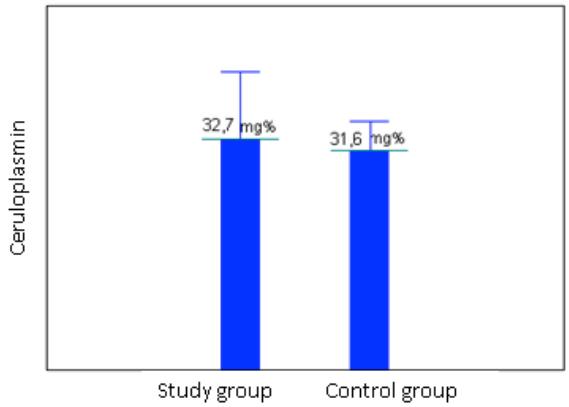

Figure 1
Comparative mean values of CER for the study and control groups

The serum levels of MDA in studied patients (4.63±0.52 mmol/mL) were significantly increased than in the control group (2.12±0.27 mmol/mL) ($p<0.001$) (Figure 2).

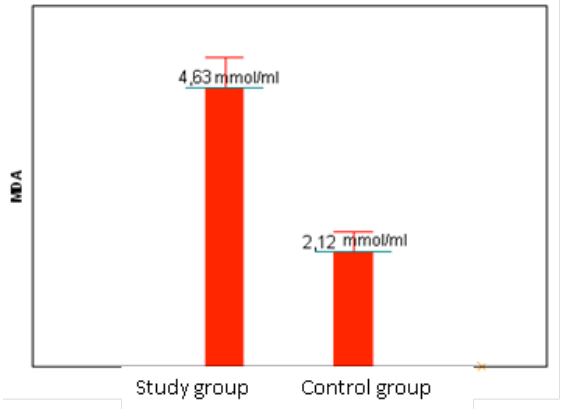

Figure 2
Comparative mean values of MDA for the study and control groups

In comparison with the control group, carbonylated proteins serum concentration in Alzheimer's dementia patients were considerable higher (1.53±0.12 mmol/mg, *versus* 4.02±0.45 mmol/mg) ($p < 0.001$) (Figure 3).

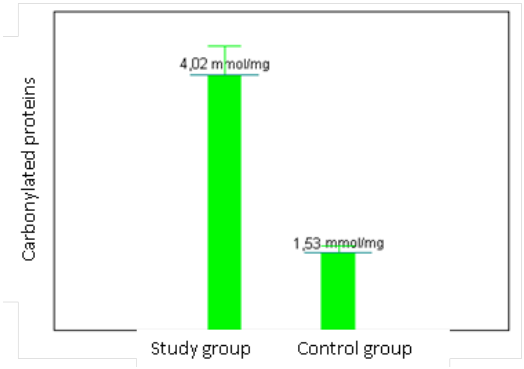

Figure 3
Comparative mean values of CP for the study and control groups

Performing an integrated analysis of the obtained result for the three biomarkers assessed for the studied patients and healthy volunteers, we observed that all patients registered increased values for MDA and carbonylated proteins, and regarding ceruloplasmin concentration, the recorded values were low in 47.5% of the studied patients, normal in 10.0%, and increased in 42.5% of cases (Table II, Figure 4).

Table II
Case distribution based on values of the CER, MDA and CP

	Low values		Normal values		Increased values	
	No.	%	No.	%	No.	%
CER	19	47.5	4	10	17	42.5
MDA					40	100.0
CP					40	100.0

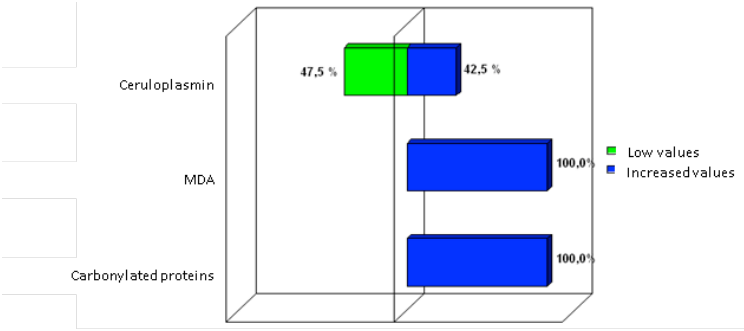

Figure 4
Case distribution based on values of the assessed biomarkers of oxidative stress

Recent studies have indicated that metals such as copper, zinc, and iron constitute factors of the pathogenesis of Alzheimer's disease. Increased concentrations of copper, zinc and iron were found in the senile plaques and

neurofilaments of the brains of Alzheimer patients [21, 22]. The metals are involved in free radicals generation, causing functional and structural alterations on macromolecular levels [22].

Ceruloplasmin is a α_2 - glycoprotein carrying copper atoms with a ferroxidase activity and plays a role in iron metabolism. Our study showed mean concentration of ceruloplasmin in AD patients almost similar to the control group ($p>0.05$). Distribution of values was dispersed, in 10% of cases the concentrations of ceruloplasmin was in normal ranges, in 47.5 % of cases the level was low and in 42.5 % was increased.

Brewer et al 2010 measured both concentration and ceruloplasmin activity and also free copper in the plasma of patients with mild AD. The results reveal a low activity of ceruloplasmin in AD patients, but the ceruloplasmin concentration was the same in both patients and controls. At the same time, the free copper in plasma was increased. The authors explain this by the reduced capacity of fixing copper in ceruloplasmin molecule [6].

Amyloid precursor protein APP is an important regulator of copper homeostasis [2] and of abnormal homeostasis between metals and it contributes to the formation of amyloid deposits.

The debate regarding the toxic or protective role played by copper in Alzheimer's dementia is still ongoing. In contrast to some studies in support of copper toxicity [27] and of the growth of copper levels in Alzheimer patients [24, 25], other studies support the theory of copper playing a protective role [3].

Although previous studies have failed to show the difference between copper levels in Alzheimer patients and in control groups [14], more recent studies have shown higher values as well as decreases in copper levels [11, 16], probably owing to the fact that the absolute level of copper must also take into consideration the copper fraction not bound to ceruloplasmin.

According to Pulido *et al.*, the neural degeneration involved in Alzheimer's disease is the result of oxidative stress and of lesions occurred at the level of the vulnerable cerebral tissue [20].

The brain has an increased consumption of oxygen and glucose, making it more vulnerable to oxidative damage [5, 9, 16]. The free radicals can attack the poliunsaturated fatty acids of the phospholipidic membrane of cells, yielding peroxidation products: one of such is MDA [7].

In our study, CP and MDA had higher in the study group compared to the controls; the same data were obtained in others research [10, 15, 18].

These results reflect that in patients with AD there is an oxidative aggression. Oxidative stress is a phenomenon associated with the aging

process, but it is more increased in people with AD, probably due to the different response of the nervous cell to free radicals action.

The imbalance caused by increased production of reactive oxygen species and decreased antioxidant mechanisms creates dysfunction in cellular and molecular level.

Although antioxidant therapy in AD research results were not encouraging, further studies are required to elucidate the molecular mechanisms of disease and to find new therapeutic formulas prophylactic and curative.

Conclusions

The mean values of CER in the study group were insignificantly higher than in the control group.

The mean values of MDA in the study group were significantly higher compared to the control group.

Statistically significantly increased values were also recorded for CP.

Our study reveals an oxidative stress in patients with Alzheimer's disease.

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