Original Article

A Comprehensive Assessment of Oxidative Stress from Hematological Parameters in Alzheimer's Patients

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Abstract

Objective: To evaluate the relationship between oxidative stress and hematological parameters in patients with Alzheimer's disease.

Material and Methods: This was a cross-sectional study conducted at Liaquat University Hospital from May to July 2022 aimed at assessing Alzheimer's disease (MCI-AD) that causes mild cognitive impairment and also using NIA-AA diagnostic criteria in this study. The study included 300 participants, divided into case and control groups based on cognitive function and neuroimaging. Exclusions were made for individuals with any other additional brain pathology or mental illness that falsifies the study results. Peripheral blood samples were taken under standardized conditions for flow cytometry analysis and also for measuring reactive oxygen species (ROS) and various oxidative stress markers. Flow cytometry data was examined using FCS Express software.

Results: The correlation between oxidative stress and Alzheimer's disease is significant (P = 0.001), with odds ratios ranging from 1.8 to 2.2, sensitivity and specificity values of 0.50 and 0.6, and a robust likelihood ratio of 1.4.

Conclusion: This study underscores the potential relationship between Alzheimer's disease and oxidative stress.

Keywords: Alzheimer's disease, Antioxidants, Free radicals, Neurodegenerative disease, Oxidative stress

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Introduction

Alzheimer's disease is a neurodegenerative ailment that is widely widespread. It is defined by a loss of cognitive function caused by the deposits of beta-amyloid peptide and neurofibrillary tangles in memory and cognitive centers such as the cerebellar cortex and the hippocampal regions.[1] There are several mechanisms underlying the development of Alzheimer's disease, but the most potent one is oxidative stress, which modifies the structures of biomolecules such as proteins, lipids, and nucleic acids in the brain. These then build up within brain cells and cause the degeneration of neural cells[2]. Multiple research investigations provide substantial proof that the brain becomes particularly susceptible to oxidative stress because it uses oxidative processes at a fast pace and has inadequate antioxidant levels.[3-4]

For several oxidative activities, including respiration, the brain has substantial quantities of mitochondria. Utilizing mitochondrial respiration results in the production of superoxide's, which are free radicals that kill brain cells and are also linked to the pathogeneses of Alzheimer's disorders.[5] Many investigations have shown that mitochondrial fragmentation is another feature in patients with Alzheimer's disease. This occurs due to reduced expression of fission-related genes like Drp1 and Fis1, as well as elevated expression of fusion-

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related genes like Mfn1, Mfn2, and Opa1, which cause neuronal injury that causes mitochondrial fragmentation.[6-7]

Alzheimer diases is a highly prevalent disease that is widely spreading around the world, with an increasing rate of 117% in the last 26 years and keeps increasing havoc. It is still most common in Turkey and Brazil.[8] According to recent research, it is estimated that by the end of 2050, 152 million people around the world will be affected by Alzheimer's disease because of an increase in risk factors and the genetic composition of the population.[9]

Many therapies are developed to treat Alzheimer's disease either fully or partially as medical research advances. At the moment, cholinesterase inhibitors and rhivastigmine are used to treat AD symptoms, however not totally.[10-11] Although antioxidants are theoretically the greatest option for treating AD, their inability to pass the blood-brain barrier limits its applicability. However, because to advancements in nanotechnology, it is now feasible to treat AD by administering significant amounts of antioxidants effectively to the brain[12-13]. Mesenchymal stem cell therapy is additional excellent technique for treating AD owing to its anti-inflammatory properties, which lower the rate of neuronal cell damage.[14-15]

The aim of this research is to show relation between oxidative stress and development of Alzehmeirs disease.

Material and Methods

This was a cross sectional study which was conducted in Out Patient Department of Neurology in association with Department of radiology of Liaquat University Hospital. This study was conducted from May 2022 to July 2022 after approval from ethical review committee. The National Institute on Aging-Alzheimer's Association (NIA-AA) has recently revised its MCI-AD diagnostic criteria, which were used in this study. According to its criteria, the diagnosis of MCI-AD (Mild Cognitive Impairment due to Alzheimer's disease) is based on cognitive assessment, CSF biomarkers, and structural neuroimaging, which include computed axial tomography (CAT) and nuclear magnetic resonance (NMR). This study consists of 300 subjects who were divided into two groups: a control group and a case group. In the control group, 200 subjects were included, including all people without any cognitive impairment (normal function and cognition as confirmed by comprehensive neuropsychological testing) and also showing negative neuroimaging (CAT (Computed Axial Tomography) and NMR (Nuclear Magnetic Resonance)). On the other side, the selection criteria for the case group included 100 people with any cognitive impairment revealed in neuropsychological tests (CDR and altered RBANS-DM) but no impairment in daily routine activities, and they were also positive for neuroimaging). Individuals who failed to meet all the requirements specified for each group or who had any additional brain pathology including high grade vascular Sub- corticoid brain, hydrocephalous or any other additional brain deformity or abnormality that was determined by Neuroimaging were not allowed to participate in this study, and patients with significant mental illness, moderate to severe dementia , significant sensory system impairment or had history of any brain disorder that falsified this investigation were also excluded. To mitigate potential confounding variables, peripheral blood samples were taken under standardized circumstances following an overnight fast. Flow cytometry was used to quantify the amount of ROS, with a FACSCanto II (Becton Dickinson, BD, CA, USA) device being used to evaluate the results. The levels of Nitric oxide, Superoxide anion, hydrogen peroxide and peroxunitrite/ hydroxyl radical were measured by monitoring variations in the median fluorescence intensity (MFI) generated by diaminofluorescein (DAF), hydroxyphenyl fluorescein (HPF), dichlorofluorescein (DCF), and dihydroethidine (DHE), respectively. 106 cells were incubated for 180 minutes (for DAF) or 30 minutes (in case of DCF, HPF and DHE) at 37°C in the dark with 10 μmol/L of HPF, 160 mmol/L of DHE, 2 μmol/L of DAF and 20 mmol/L of DCF. The samples were then washed, reconstituted in PBS, and maintained cold until flow cytometry was able to collect 10,000 events. After that, the data was examined using FCS Express software.

The normal values of oxides in CSF and Blood plasma are:

1. NO (Nitric Oxide) (CSF 0.2-0.4μM, plasma 0.1-0.5 μM)
2. O2 – (Superoxide Anion) (CSF0.02-0.02 μM, Plasma 0.01-0.1 μM)
3. ONOO (Peroxunitrite) (CSF 0.02-0.1 μM, Plasma 0.01-0.05)
4. OH (Hydroxyl Radical) (CSF0.002-0.02 μM, Plasma 0.001-0.01 μM)

Results

Tabel-1 compares oxide biomarker levels between a 100-person Control group and a 200-person Case group. Notably, the Case group, particularly individuals with elevated levels, showed significantly higher levels of Nitric Oxide (NO), Peroxynitrite (ONOO), Superoxide (O2), and Hydroxide (OH) compared to the Control group. Statistical significance (P values ranging from 0.001 to 0.04) emphasizes these differences. Elevated NO levels in the Case group demonstrated a strong association (Odds Ratio: 2.2, 95% CI: 1.1 to 1.5), with similar associations found for ONOO, O2, and OH. Sensitivity/Specificity ratios of 0.5/0.6 and Likelihood Ratios ranging from 1.313 to 1.5 .

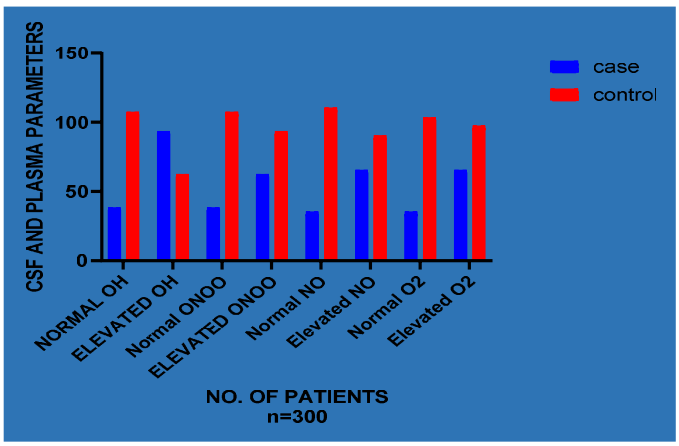

Figure 1: *Compares the different Levels of Oxides in Plasma and CSF between the Control Group and the Case Group*

Discussion

Among people over the age of 65, Alzheimer's disease is one of the most prevalent diseases associated with dementia. It causes neurodegeneration of neuronal cells in the brain by amassing beta-amyloid aggregates and tau protein tangles.16 The main contributory factor invol-ved in many brain neurodegenerative disorders is oxi-dative stress. It happens when a variety of superoxide or oxide species, such as OH, ONOO, O2, and NO, start to build up inside the neuronal cells of the brain. Many studies have shown that mitochondrial malfunction, inflammation, or elevated metal levels that interfere with electron transport chain activity cause their levels to become abnormally high, causing neurodegenera-tion.17 Our study results concur with the findings of Andra et al. that mitochondrial damage leads to the accumulation of amyloid bodies in neurons by elevating the levels of oxides, which increases the risk of develo-ping alzehmeirs disease in the elderly population.18 The study results of Elena Temango et al. also show that people with elevated levels of free radicals in their neu-ronal cells have a higher risk of developing alzehmeirs disease than a normal population.19 Carmen Peña-Bautista et al.'s study supports our findings, which indicate that Alzheimer patients' plasma and CSF had higher levels of oxides—such as OH and ONOO—than those of the normal control group. This indicates a strong correlation between oxidative stress and Alzheimer's disease, with an Odd ratio of more than 1 to 2.2.20

Alzheimer's disease is currently treated with many approved medications, such as memantine, rhivastig-mine, galantamine, and donepezil, that inhibit an enzyme called acetylcholinesterase, which increases the levels of acetylcholine in the brain and improves cognitive functions. Another therapy called monoclonal antibodies includes Aducanumab, which directly removes excess amyloid beta from the brain and lessens the buildup of neuritic plaques in the brain. Recent advancements in the field of nanotechnology also help to treat Alzheimer's disease to a great extent by directly delivering the anti-oxidants to the brain and lessening the development and complications of Alzheimer's disease.21-22-23

The present discourse posits that oxidative stress is a critical factor in the pathogenesis of Alzheimer's disease, and that the mitigation of this illness is contingent upon the amounts of oxides present in neural cells.

Table 1: *Association of serum / CSF levels of Oxides in normal versus Alzehmeirs patients.*

Biomar kers	Control with normal levels of oxides	Control with elevated levels of oxides	Case with normal levels of oxides	Case with elevated levels of oxides	Total	P value	ODD Ratio	95%CI	Sensitivity /specifity	Like hood ratio
NO	110	90	35	65	300	0.001	2.2	1.1 to 1.5	0.5/0.6	1.5
ONOO	107	93	38	62	300	0.01	1.877	1.04 to 1.45	0.5/0.6	1.408
O2	103	97	37	62	300	0.007	1.247	1.06/1.46	0.5/0.6	1.471
OH	105	95	40	60	300	0.04	1.65	1.00 to 1.39	0.5/0.6	1.313

Conclusion

This study underscores the significant implications of oxidative stress in the progression of Alzheimer's disease.

Conflict of Interest: *None*

Funding Source: *None*

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Authors Contribution

SFU: Conceptualization of Project
SS: Data Collection
HRC: Literature Search
KW: Statistical Analysis
AHS: Drafting, Revision
SB: Writing of Manuscript