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Assessment of the levels of oxidative and anti-oxidative stress markers in subjects with cerebrovascular accident

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Abstract: *Background:* Stroke, a leading cause of global mortality and long-term disability, is characterized by a disturbance in cerebral blood supply, often leading to significant oxidative stress and inflammatory responses. *Objectives:* This study investigated the levels of oxidative and antioxidative stress markers in cerebrovascular accident (CVA) patients to elucidate their roles in disease progression and potential for intervention. *Methods:* Sixty CVA patients (30 males, 30 females, aged 40-80 years) and forty age-matched healthy controls were enrolled. Serum concentrations of malondialdehyde (MDA), Vitamin C (Ascorbic acid), and Vitamin E (Alpha-tocopherol) were determined using spectrophotometric methods. Statistical analysis was performed using SPSS version 18, with significance set at $p<0.05$. *Results:* The findings showed significantly higher serum MDA concentrations in CVA patients (7.14±0.03) compared to controls (6.83±0.03), indicating increased lipid peroxidation and oxidative stress. Conversely, CVA patients exhibited significantly lower serum Vitamin C (Ascorbic acid) levels (1.18±0.04) than controls (1.81±0.04), suggesting a compromised antioxidant status. While Vitamin E (Alpha-tocopherol) showed a slight, non-significant decrease in CVA patients, β-carotene levels were not statistically different between groups. Furthermore, female CVA patients had significantly higher serum MDA levels than male CVA patients, while male CVA patients presented with significantly higher serum Vitamin C concentrations. *Conclusion:* These findings provide strong evidence for the presence of significant oxidative stress and a diminished antioxidant defense in CVA patients, underscoring the critical role of free radical-mediated injury in stroke pathogenesis. The observed sex-based differences warrant further investigation. This study highlights the potential for therapeutic strategies targeting oxidative stress to improve outcomes in stroke management.

Keywords: Anti-Oxidant, Malondialdehyde, Cerebrovascular Accident, Oxidative Stress, Sex, Stroke.

Abbreviation: CVA - Cerebrovascular Accident; MDA – Malondialdehyde; DNA - Deoxyribonucleic Acid; NO - Nitric Oxide; RNS - Reactive Nitrogen Specie; ROS - Reactive Oxygen Specie; ONOO - Perooxynitrite Radical; NADPH - Reduced Nicotinamideadenine dinucleotide; NOX - Nitrogen Oxide; XO - Xanthine Oxidase; SOD - Superoxide dismutase; TBA - Thiobarbituric Acid

Introduction

Stroke, also known as a cerebrovascular accident (CVA) or brain attack, represents a critical medical emergency characterized by the acute loss of brain function due to a disturbance in the cerebral blood supply [1]. This disruption can stem from either ischaemia, a reduction in blood flow caused by thrombosis, arterial embolism, or systemic hypoperfusion [2], or haemorrhage, which involves bleeding from cerebral blood vessels [3]. The resulting

neurological deficits can be profound, including impaired motor function, speech difficulties, and visual field disturbances [4]. Stroke is a leading cause of long-term disability and mortality globally, with risk factors encompassing advanced age, hypertension, diabetes mellitus, dyslipidemia, and tobacco smoking [5-6].

Ischaemic stroke, accounting for approximately 87% of all stroke cases, is particularly associated with significant oxidative stress and inflammatory responses in the acute post-ischaemic period [7-8]. Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, leading to potential cellular damage [7, 9]. The brain, despite constituting less than 2% of total body weight, consumes about 20% of the body's oxygen, rendering neuronal cells highly vulnerable to oxidative injury [7]. Free radicals, such as superoxide anion (O^{2-}), hydroxyl radical (HO·), and reactive nitrogen species (RNS) like nitric oxide (NO), are major contributors to this oxidative burden [10]. These species can directly oxidize cellular components, including proteins, lipids, and DNA, leading to cellular impairment and disease progression [11].

Experimental stroke studies consistently demonstrate the pivotal role of oxidative stress in cerebral damage following ischaemia. The overproduction of ROS beyond the capacity of biological systems to counteract their effects results in cellular injury [9]. Furthermore, the interaction of ROS with nitric oxide can produce highly toxic reactive nitrogen species like peroxynitrite (ONOO−), which causes further oxidative and nitrative damage to proteins [12]. The brain's inherent low levels of antioxidants make neurons particularly susceptible to this oxidative assault [13]. Increased activity of nitric oxide synthase (NOS) and the generation of superoxide from various sources, including NADPH oxidases (NOX), xanthine oxidase (XO), and the mitochondrial electron transport chain, contribute significantly to the heightened oxidative state during ischaemia and reperfusion [12]. This cascade of events ultimately leads to energy failure and neuronal death [14].

Conversely, antioxidants play a crucial role in mitigating the damaging effects of free radicals. These substances counteract oxidation by neutralizing harmful free radicals, thereby preventing oxidative damage to cells [15-16]. The body's antioxidant defense systems are multifaceted, comprising antioxidant enzymes such as superoxide dismutase (SOD), catalase, and peroxidases, as well as small molecule antioxidants like lipid-soluble Alpha-tocopherol (Vitamin E) and water-soluble Ascorbic acid (Vitamin C) compounds [17]. These antioxidants work in synergy to scavenge free radicals and protect against oxidative injury (Good and David, 2004). Malondialdehyde (MDA), a product of lipid peroxidation, is a commonly used biomarker for assessing oxidative injury in vivo, reflecting the extent of free radical attack on polyunsaturated fatty acids in cell membranes [18].

Given the critical involvement of oxidative stress in stroke pathophysiology, understanding the balance between pro-oxidant and antioxidant markers is essential for developing effective therapeutic strategies. This study aims to investigate the levels of oxidative and antioxidative stress markers in cerebrovascular accident patients, specifically focusing on malondialdehyde (MDA), Vitamin C (Ascorbic acid), and Vitamin E (Alpha tocopherol), to further elucidate their roles in the disease progression and potential for intervention.

Material and Methods

Subjects of Experiment: The study involved sixty individuals, both males (n=30) and females (n=30), diagnosed with cerebrovascular accident (CVA) attending the Clinic at the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State. Nigeria. Subjects ranged in age from forty to eighty (40-80) years. For comparative analysis, forty age-matched, apparently healthy individuals without CVA or other known ailments were recruited as control subjects.

Collection and Processing of Samples: Blood samples (5mL) were collected from each participant using syringes after disinfecting the mid-cubital area and applying a tourniquet. A drop of blood was immediately used for rapid blood sugar testing. The remaining blood was gently discharged into

clean, plain test tubes and allowed to clot. Subsequently, the samples were centrifuged at 5,000 rpm for five minutes to separate the sera. Sera were either analyzed immediately or stored at 4^{o}C and analyzed the following day for the determination of malondialdehyde (MDA), Vitamin C, and Vitamin E concentrations.

Determination of Biochemical Parameters: Determination of Malondialdehyde (MDA) Concentration Lipid peroxidation was assessed by measuring the level of malondialdehyde (MDA) spectrophotometrically, as described by [19]. This method is based on the reaction of MDA with thiobarbituric acid (TBA) to form a red or pink colored complex that absorbs maximally at 532 nm in an acidic solution.

Determination of Vitamin C Concentration: Vitamin C concentration was estimated using the method described by [20]. This method relies on the oxidation of ascorbic acid to diketoglutamic acid, which then reacts with 2, 4-dinitrophenylhydrazine to form a diphenylhydrazine derivative. This derivative, when dissolved in strong sulphuric acid solution, produces a red color measurable Spectrophotometrically at 500 nm.

Determination of Vitamin E (Alpha tocopherol) Concentration: Vitamin E (Alpha tocopherol) concentration was estimated by the method of [20]. This method involves the reduction of ferric ions to ferrous ions by α-tocopherol, followed by the formation of a red-colored complex with αα-dipyridyl. The absorbance of this chromophore was measured at 520 nm using a spectrophotometer.

Determination of β-Carotene Concentration: Beta-carotene was determined spectrophotometrically at 450 nm using the method of [20].

Statistical Analysis: he data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 18. Results were expressed as mean ± standard deviation of the mean. Significant differences between results were established by one-way analysis of variance (ANOVA), with the acceptance level of significance set at $p<0.05$.

Results

Comparative Study between Control and Cerebrovascular Accident Subjects: The analysis of pro-oxidant and antioxidant indices revealed significant differences between control subjects and cerebrovascular accident (CVA) patients. As shown in Table 1, serum malondialdehyde (MDA), a marker of pro-oxidant status, was significantly higher ($p<0.05$) in CVA patients (Group 2) compared to control subjects (Group 1).

Table-1: Malondialdehyde concentration of normal and cerebrovascular accident subjects

Groups	Malondialdehyde
Group 1 (Control)	6.83±0.03^{a}
Group 2 (Test)	7.14±0.03^{b}

Results are expressed as Mean ± SD; n = 60.

Mean values having different letters as superscripts across the row are considered significant ($p<0.05$)

Regarding antioxidant indices (Table 2), serum Vitamin C was significantly lower ($p<0.05$) in CVA patients (Group 2) (1.18±0.04) compared to control subjects (Group 1) (1.81±0.04). Serum Vitamin E showed a significant increase in Group 1 (0.99±0.01a) when compared with Group 2 (0.95±0.00b). No statistical difference was observed in β-carotene concentration between Group 1 and Group 2 subjects (0.01±0.00a for both groups).

Table-2: Anti-oxidant concentration of normal and cerebrovascular accident subjects

Parameters	Treatment Groups	
	Group 1 (Control)	Group 2 (Test)
Vitamin C	1.81±0.04^{a}	1.18±0.04^{b}
Vitamin E	0.99±0.01^{a}	0.95±0.00^{b}
β-carotene	0.01±0.00^{a}	0.01±0.00^{a}

Results are expressed as Mean ± SD; n = 60

Mean values having different letters as superscripts across the row are considered significant ($p<0.05$).

Comparison between Male and Female Cerebrovascular Accident Test Subjects: Further analysis compared the oxidative and

antioxidative stress markers between male and female CVA patients. As presented in Table 3, serum MDA concentration was significantly higher ($p<0.05$) in female CVA patients (7.10±0.305b) compared to male CVA patients (7.07±0.254a).

Table-3: Malondialdehyde concentration of male and female cerebrovascular accident test subjects

Groups	Malondialdehyde
Male (Test)	7.07±0.254[a]
Female (Test)	7.10±0.305[b]

Results are expressed as Mean ± SD; n =60

Mean values having different letters as superscripts across the row are considered significant ($p<0.05$).

For antioxidant indices in male and female CVA patients (Table 4), there was no statistical difference in serum Vitamin E concentration (1.00±0.000 for both male and female groups) or β-carotene concentration (0.00±0.000 for both male and female groups). However, mean serum Vitamin C was significantly higher ($p<0.05$) in male CVA patients (1.17±0.379a) than in female CVA patients (1.07±0.254).

Table-4: Anti-oxidant concentration of male and female cerebrovascular accident test subjects

Parameters	Treatment Groups	
	Male (Test)	Female (Test)
Vitamin C	1.17±0.379[a]	1.07±0.254[b]
Vitamin E	1.00±0.000[a]	1.00±0.000[a]
β-carotene	0.00±0.000[a]	0.00±0.000[a]

Results are expressed as Mean ± SD; n =60

Mean values having different letters as superscripts across the row are considered significant ($p<0.05$).

Discussion

This study aimed to investigate the levels of oxidative and antioxidative stress markers in cerebrovascular accident (CVA) patients compared to healthy controls, and to assess potential sex-based differences. Our findings demonstrate a clear association between CVA and altered oxidative and antioxidative profiles, consistent with the established role of oxidative stress in stroke pathophysiology.

The analysis revealed a significantly higher serum malondialdehyde (MDA) concentration in CVA patients compared to control subjects. MDA is a widely recognized marker of lipid peroxidation, which occurs when free radicals attack polyunsaturated fatty acids in cell membranes [18]. This elevated MDA level in CVA patients is attributed to increased oxidative stress resulting from ischaemic stroke, supporting the notion that free radical production is a crucial mechanism of brain injury following ischaemia and reperfusion [21]. The brain's susceptibility to oxidative damage is amplified by its high oxygen consumption, rich polyunsaturated fatty acid content in cellular membranes, and relatively low levels of endogenous antioxidant enzymes [7, 22]. Our results align with previous studies that have reported enhanced levels of oxidative stress markers in ischaemic stroke patients [23-24].

Conversely, serum concentrations of key antioxidants, namely Vitamin C and Vitamin E, were observed to be lower in CVA patients compared to control subjects. While Vitamin E showed a slight, non-significant decrease, Vitamin C was significantly reduced in CVA patients. Antioxidants, including vitamins C and E, play vital roles in scavenging free radicals and protecting against oxidative damage [15-16]. The diminished antioxidant levels in CVA patients suggest an increased utilization of endogenous antioxidants to combat the heightened free radical load and oxidative stress during ischaemic stroke [25]. This reduction in serum antioxidants can exacerbate redox-sensitive vascular changes associated with conditions like hypertension, a known risk factor for stroke [23].

Furthermore, our investigation into sex-based differences within the CVA patients' group revealed that the female CVA patients exhibited significantly higher serum MDA concentrations than their male counterparts. This finding suggests a potentially greater degree of lipid peroxidation and oxidative stress in females experiencing CVA, which could imply a differential impact of oxidative injury based on sex. In terms of antioxidants, male CVA patients had significantly higher serum Vitamin C concentrations than female

CVA patients, while no significant difference was observed for Vitamin E or β-carotene between sexes. The higher Vitamin C levels in males might suggest variations in antioxidant defense mechanisms or dietary intake between male and female CVA patients.

Conclusion

The findings of this study provide strong evidence for the presence of significant oxidative stress and a compromised antioxidant status in patients suffering from cerebrovascular accidents. The elevated MDA levels and reduced antioxidant concentrations underscore the critical role of free radical-mediated injury in stroke pathogenesis. These results highlight the potential for therapeutic interventions targeting oxidative stress to enhance patient outcomes in stroke management.

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