Original Research Article

Comparative study of oxidative stress and antioxidant status between ischemic and haemorrhagic stroke patients

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Abstract

Introduction: The World health organization (WHO) has defined stroke as "rapidly developed clinical signs of focal and at times global disturbances of cerebral function lasting more than 24 hours or leading to death, with no apparent cause other than vascular origin." Stroke is an interruption in the cerebral vasculature and is classified as being either ischemic stroke (IS) or haemorrhagic stroke (HS). **Material and Methods:** This is a Prospective, observational and Case Control study conducted in the Department of Biochemistry and Casualty Unit at Mahavir Institute of Medical Sciences over a period of 1 year on an overall population of 140 individuals (40 ischemic strokes and 30 haemorrhagic strokes as the case groups; 70 healthy individuals as the control group). The diagnosis of stroke was based on history and clinical examination and brain CT scan were used to confirm and classify ischemic and haemorrhagic stroke cases. **Results:** In our study, we observed that mean serum levels of Malondialdehyde (MDA) were increased in both ischemic 2.96 ± 0.51 nmol/mL and hemorrhagic stroke 2.41 ± 0.42 nmol/mL as compared to controls 1.38 ± 0.26 nmol/mL. We found reduced mean level of serum Superoxide dismutase (SOD) in cases of ischemic (9.41 ±2.52 U/mg) and hemorrhagic stroke (8.86±2.73 U/mg) as compared to controls (15.51 ±3.62 U/mg). The Catalase levels are decreased significantly in ISPs and HSPs compared to control subjects. Maximum decline in Catalase is found in ISPs with HSPs. **Conclusion:** Result showed a direct positive correlation with infarct size (Ischemic stroke) but less in hemorrhagic stroke when comparted with control group. The antioxidative parameters like Catalase and superoxide dismutase was decreased both ischemic and hemorrhagic stroke when compared with control.

Keywords: Ischemic stroke, Haemorrhagic stroke, Oxidative stress, antioxidant

Introduction

The World health organization (WHO) has defined stroke as "rapidly developed clinical signs of focal and at times global disturbances of cerebral function lasting more than 24 hours or leading to death, with no apparent cause other than vascular origin." It is one of the leading causes of adult disability and the second most common cause of death[1]. Stroke is a major cause of morbidity and mortality in an aging population. In the elderly, ischemic stroke accounts for more than 80% of all stroke cases[2].Stroke is an interruption in the cerebral vasculature and is classified as being either ischemic (lack of blood flow to an area of the brain) or haemorrhagic (leakage of blood in the brain)[3]. Ischemic stroke occurs because of a loss of blood supply to part of the brain, initiating the ischemic cascade. Brain tissue ceases to function if deprived of oxygen for more than 60 to 90 seconds and after approximately three hours were suffer irreversible injury possibly leading to the death of the tissue, i.e., infarction[4]. Haemorrhagic strokes are classified based on their underlying pathology. Some causes of haemorrhagic stroke are hypertensive haemorrhage, ruptured aneurysm, ruptured AV fistula, transformation of prior ischemic infarction, and drug induced bleeding[5].Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products[6]. ROS can play, and in fact they do it, several physiological roles (i.e., cell signalling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress)[7].

An *antioxidant* is the substrate that prevents the oxidation of molecules inside a cell. It is a well-known chemical process that allows the removal of electrons or hydrogen from a substance. Free radicals are produced during the biological oxidation reaction[8]. Because the radicals are reactive, they start the chain reaction simultaneously. This can lead to the damage or even the death of a cell. Hence, antioxidant agents are capable of terminating a chain reaction by eliminating free radical intermediates[9]. They perform the antioxidant behaviour by being oxidized, hence antioxidants can be considered reducing agents. Some examples are ascorbic acid, thiols, or polyphenols[10].

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Material and Methods

This is a Prospective, observational and Case Control study conducted in the Department of Biochemistry and Casualty Unit at Mahavir Institute of Medical Sciences over a period of 1 year on an overall population of 140 individuals (40 ischemic strokes and 30 haemorrhagic strokes as the case groups; 70 healthy individuals as the control group).

The control group was chosen from the healthy population, which matched for age and gender with the same exclusion criteria. Blood samples were obtained from the controls at the given time spans.

Diagnosis

The diagnosis of stroke was based on history and clinical examination and brain CT scan were used to confirm and classify ischemic and haemorrhagic stroke cases.

Inclusion criteria

Cases of both Ischemic and haemorrhagic stroke

Exclusion criteria

Previous history of a cerebrovascular event**,** History of a recent infectious or inflammatory disease**,** Autoimmune disorder**,** Haematological disorder**,** Use of immunosuppressive or anti-inflammatory drugs in the previous two months.

Sample collection

Venous blood samples were obtained on admission and were immediately centrifuged and analysed by semi-autoanalyzer following standard operating procedure.

Parameters	Method
Glutathione peroxidase (GPX)	Spectrophotometric assay Method
Malondialdehyde (MDA)	Jean CD et al Method
Nitric oxide	Sandwich enzyme immunoassay (ELISA) technique
Uric Acid	Uricase Method
Superoxide dismutase (SOD)	Marklund and Marklund (1974) Method
Catalase	Aevi (1984) (Spectrophotometric assay) Method
Vitamin C (ascorbic acid)	Indophenol Method
Vitamin E (Alpha tocopherol)	Baker & Frank method

Statistical Analysis

The collected data were compiled in MS Excel sheet for analysis. Analysed in Statistical Package for the Social Sciences (SPSS) version 25th were applied. Quantitative data were represented in the form of mean and standard deviation. To check significance difference between case and control group comparison unpaired 't' test was applied quantitative data was represented. p-value < 0.05 indicates statistical significant.

Results

A total of 140 individuals (40 ischemic strokes and 30 haemorrhagic strokes as the case groups; 70 individuals as the control group) were identified during the study follow-up.

Table 1: Characteristics of the Whole Group of Patients

Characteristics	Control group	Ischemic stroke	Haemorrhagic stroke
n	70	40	30
Age, y, Mean±SD	54.42±6.73	51.73±6.35	53.27±6.85
Males	43 (61.4)	26 (65)	19 (63.3)
Female	27 (38.6)	14 (35)	11 (36.6)

In table 1, their age varied between 41 and 80 (51.73±6.35 and 53.27±6.85 in IS and HS, respectively) and there was no significant difference of age among three groups.

Table 2: Distribution of Characteristics of the Patients

Characteristics	Control group	Ischemic stroke	Haemorrhagic stroke
Systolic BP (mmHg) Mean±SD	136.35±13.35	142.53±13.64	140.43±13.54
Diastolic BP (mmHg) Mean±SD	84.43±8.43	82.53±8.53	84.43±7.43

Systolic BP 136.35±13.35 in control group, 142.53±13.64 in IS and 140.43±13.54 in HS group. Diastolic BP 84.43±8.43 in control group, 82.53±8.53 in IS group and 84.43±7.43 in HS group (Table 2).

Table 3: Distribution of the oxidative biomarkers in control group, Ischemic stroke group and Haemorrhagic stroke

Characteristics	Control group Mean±SD	Ischemic stroke Mean±SD	Haemorrhagic stroke Mean±SD
Malondialdehyde (MDA) (nmol/mL)	1.38 ± 0.26	2.96 ± 0.51	2.41 ± 0.42
Nitric oxide (NO) µg /ml	3.79±0.52	4.53±0.38	4.94±0.49
Glutathione peroxidase (GPX) (µmol/ml)	10.23 ± 2.43	4.23 ± 1.03	4.43 ± 1.14
Uric acid (mg/dl)	4.74 ±0.43	7.24±1.53	6.53±1.29
Superoxide dismutase (SOD) (U/mg)	15.51 ±3.62	9.41 ±2.52	8.86±2.73
Catalase (IU/mg)	14.52 ±3.6	8.54±1.75	9.48 ±1.65

In table 3, In our study, we observed that mean serum levels of MDA were increased in both ischemic 2.96 ± 0.51 nmol/mL and haemorrhagic stroke 2.41 ± 0.42 nmol/mL as compared to controls 1.38 ± 0.26 nmol/mL. There is an increase in Nitric oxide in ischemic stroke (ISPs) and Haemorrhagic stroke patients when compared to control subjects. This indicates that lipid peroxidation is significantly increased in HS than IS patients.

The GPX levels are decreased significantly in ISPs and HSPs compared to control subjects. Maximum decline in GPX is found in ISPs with HSPs. The Uric acid levels are significantly increased in ISPs and HSPs when compared to control subjects and the increase is more in ISPs with HSPs. We found reduced mean level of serum SOD in cases of ischemic (9.41 ±2.52 U/mg) and haemorrhagic stroke (8.86±2.73 U/mg) as compared to controls (15.51 ±3.62 U/mg). The Catalase levels are decreased significantly in ISPs and HSPs compared to control subjects. Maximum decline in Catalase is found in ISPs with HSPs in table 3.

Table 4: Distribution of the Antioxidant marker in control group, Ischemic stroke group and Haemorrhagic stroke

Characteristics	Control group Mean±SD	Ischemic stroke Mean±SD	Haemorrhagic stroke Mean±SD
Vitamin C (mg/L)	1.52 ± 0.37	0.63 ± 0.03	1.01 ± 0.09
Vitamin E (mg/L)	12.54± 2.37	7.54± 0.97	9.64± 1.37

In table 4, it was observed that the serum levels of Vitamin C were significantly lowers in both ISPs and HSPs than those of control. It was observed that the serum levels of Vitamin E were significantly lowers in both ISPs and HSPs than those of control.

Discussion

A stroke occurs when a blood vessel in the brain ruptures and bleeds, or when there's a blockage in the blood supply to the brain. The rupture or blockage prevents blood and oxygen from reaching the brain's tissues. Ischemic stroke involves a blockage caused by either a clot or plaque in the artery. The symptoms and complications of ischemic stroke can last longer than those of a TIA, or may become permanent. Haemorrhagic stroke is caused by either a burst or leaking blood vessel that seeps into the brain. There is increasing evidence that stroke incidence rates in developing countries have increased by more than 100% during the last four decades[11].

In our study, we observed that mean serum levels of MDA were increased in both ischemic 2.96 ± 0.51 nmol/mL and haemorrhagic stroke 2.41 ± 0.42 nmol/mL as compared to controls 1.38 ± 0.26 nmol/mL. Sarkar *et al.* observed significantly higher concentration of MDA in stroke patients compared with controls, similar to our study and suggested that increased level of lipid peroxides may be due to oxidation of blood or neural lipids by ischemia. They suggested that increased level of lipid peroxide may be due to oxidation of blood or neural lipids by ischemia and rise in lipid peroxide in haemorrhagic stroke was due the compressive effects producing ischemia. These studies match with findings of our study, that serum MDA levels are increased after stroke suggesting involvement of lipid peroxidation in the pathophysiology of ischemic as well as haemorrhagic stroke[12].

Our study also indicates that uric acid levels are significantly increased in ISPs and HSPs and the increase is more in ISPs compared with control. Milanlioglu et al. found similar results to that of the present study; significant differences in serum Uric acid was found between stroke patients and healthy controls[13]. Uric acid plays an important role in acute ischemic stroke, as a consequence of its antioxidant properties. It is particularly effective in quenching hydroxyl superoxide and peroxynitrite radicals and may serve a protective physiological role by preventing lipid peroxidation[14].

We found reduced mean level of serum SOD in cases of ischemic (9.41 ±2.52 U/mg) and hemorrhagic stroke (8.86±2.73 U/mg) as compared to controls (15.51 ±3.62 U/mg). Srikrishna R *et al*. observed reduced SOD in cases 4.04±0.03U/ml as compared to 9.01±1.04 U/ml in controls. Spranger M *et al.* found that mean serum levels of SOD in cases of both ischemic and haemorrhagic stroke were significantly lower as compared to controls suggesting that antioxidants are depleted as a consequence of an excessive production of oxygen free radicals very early after the onset of stroke[15].

Cherubini *et al.* found that antioxidants including SOD are reduced immediately after an acute stroke, possibly as a consequence of increased oxidative stress and A specific antioxidant profile is associated with a poor early outcome[16]. Thus, our findings of serum SOD levels matched with previous studies. Hence increasing the anti-oxidative capacity in serum within the first day after the onset of symptoms might be a therapeutic option to minimize the oxidative injury caused by oxygen free radicals until the endogenous free radical scavenging systems recovers.

In our present study, the serum Vitamin C levels were decrease significantly in ischemic stroke and haemorrhagic stroke patients (decreases significantly in large vessels infarcts than in small vessel infarcts) compared to controls. It may be due to the exhaustion of this antioxidant in the neutralization of free radicals which are formed in excess during ischemia and reperfusion**.** Many studies show that reduced Vitamin C levels are associated with increased risk of both ischemic and Haemorrhagic strokes. In this regard, Sheikh et al. reported similar findings[17].

In the present study serum vitamin E levels were significantly decreased in ischemic stroke cases when compared to controls. Vitamin E, a potent chain breaking lipid soluble antioxidant, reacts with lipid peroxyl radicals eventually terminating the peroxidation chain reaction and thereby reducing oxidative damage. Some studies have shown reduced serum vitamin E levels in stroke patients and this may be due to high lesion volume resulting in production of more number of free radicals from a large ischemic injury. It is also shown that reduced vitamin E levels resulted in poor clinical outcome in stroke patients[18].

Conclusion

Oxidative stress may play a role in the pathogenesis of both ischemic stroke and haemorrhagic stroke in terms of oxidants. The study results showed significant increase in oxidative stress levels with infarct size. Result showed a direct positive correlation with infarct size (Ischemic stroke) but less in haemorrhagic stroke when comparted with control group. The antioxidant parameters like Catalase and superoxide dismutase was decreased both ischemic and haemorrhagic stroke when compared with control. The marker for the endothelial dysfunction nitric oxide level was decreased drastically in ischemic stroke not in haemorrhagic stroke when compare to normal healthy volunteers.

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