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International Journal of Clinical Biochemistry and Research

Journal homepage: www.innovativepublication.com

Original Research Article

Evaluation and comparison of antioxidant status in ischemic and haemorrhagic cases of stroke

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ARTICLE INFO

Article history:
Received 05-05-2020
Accepted 02-06-2020
Available online 30-06-2020

Keywords:
Oxidative stress
Antioxidant
Ischemic
Haemorrhagic

ABSTRACT

Introduction: The World health organization (WHO) has defined stroke as "rapidly developing clinical signs of focal (or global) cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than vascular region."
Material and Methods: This case-control study was conducted on 145 individuals (58 ischemic strokes and 29 haemorrhagic strokes as the case groups; 58 healthy individuals as the control group).
Diagnosis: The diagnosis of stroke was based on history, clinical examination and brain CT scan was used to confirm and classify as ischemic or haemorrhagic stroke cases.
Results: A total of 87 cases of stroke (58 ischemic stroke, 29 haemorrhagic strokes, and 58 healthy individuals as the control group) were identified during the study follow-up. Glutathione peroxides (GPX) levels are reduced significantly in Ischemic Stroke Patients (ISPs) and haemorrhagic stroke Patients (HSPs) equated with control subjects ($p<0.001$). Extreme decrease in GPX is seen in ISPs than HSPs($p<0.001$).
Conclusion: Finally, positive direct relationship was seen in MDA along with infarct size. So, it could consider as a bio marker for diagnosis of stroke. This could be valued for improving the dose frequency for improvement of patient health. From these studies, we can conclude that antioxidant defence is reduced in ischemic stroke patients as a significance of inclined oxidative stress.

1. Introduction

The World health organization (WHO) has defined stroke as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than of vascular origin."[1] It is one of the main reasons of adult disability and the second most common reason of death.[1] Stroke is a main reason of morbidity and mortality in an old age people. In the ageing, ischemic stroke accounts for >80% of all stroke cases.[2]

The causes of cellular injury subsequent ischemia are multifactorial, but there is rising indication suggesting the character of reactive oxygen species (ROS) in its pathogenesis. Oxidative stress resultant from generation of ROS is involvement in the neuronal damage produced by ischemia and reperfusion, one of the main goals in stroke treatment because the recanalization of an occluded artery and restoration of the blood flow can save brain tissue.[3] However, reperfusion might have some deleterious effects because oxidative stress can rapidly take place on reoxygenation.[4] ROS generated during ischemia/reperfusion can react with unsaturated lipids of bio membranes, thereby generating malondialdehyde (MDA), an end-product of lipid peroxidation. MDA could be a biomarker of tissue injury and reflect oxidative damage; indeed, several studies have shown increased MDA concentrations in acute stroke patients.[5]

Natural antioxidants include enzymes and non-enzyme antioxidants. Antioxidant enzymes include SOD, catalase (CAT), peroxidase, glutathione peroxidase (GSH-Px), and NADPH, and enhancing the activities of these can result in

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https://doi.org/10.18231/j.ijcbr.2020.038

antioxidant effects. Non-enzymatic antioxidants are mostly derived from natural plants or their extracts and include vitamin C, vitamin E, glutathione, melatonin, carotenoids, resveratrol, ursolic acid, and microminerals such as copper, zinc, and selenium. These are extremely important in minimizing oxidative stress.[6–9]

Taking into thought of the overhead evidences, this study was done to investigate the correlation of prognostic factors in stroke and haemorrhagic patients with serum malondialdehyde (MDA), Nitric oxide (NO), Glutathione peroxides, Uric acid, Superoxide dismutase (SOD), Catalase, Vitamin C (ascorbic acid) and Vitamin E (α- tocopherol) in patients with ischemic and haemorrhagic stroke cases. Until now there have been few studies that compared the differences between two types of strokes.

2. Objective of the Study

The current research work is planned to study oxidative stress and anti-oxidant status in stroke and compare it in cases of ischemic and haemorrhagic stroke.

3. Materials and Methods

3.1. Study design

Prospective, observational study.

3.2. Study type

Hospital based Case Control study.

3.3. Ethics, consent and permissions

1. After approval from Institutional Ethics Committee for Medical Research, study was initiated.
2. All the case and control were provided written, vernacular, informed consent to participate in the study.
3. Study was conducted as per ICH good Clinical Practice (GCP) guidelines.

This case-control study was conducted on an overall population of 143 individual's human participants (58 ischemic strokes and 29 haemorrhagic strokes as the case groups; 58 healthy individuals as control group).

The control group was selected from 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.

To study the antioxidant potential of ischemic stroke patients we have included Glutathione peroxides (GPX), Uric acid and SOD, Catalase in our study.

3.4. Sample size

145.

3.5. 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.

3.6. Subjects

Cases with acute ischemic stroke and cases with haemorrhagic stroke were recruited within the first 24 hours of onset of symptoms who were hospitalized at the emergency Ward of Index Medical College & P. G. Institute, Indore.

3.7. Inclusion criteria

Cases of both Ischemic and haemorrhagic stroke.

3.8. Exclusion criteria

1. Previous history of a cerebrovascular event.
2. History of a recent infectious or inflammatory disease.
3. Cancer.
4. Autoimmune disorder.
5. Haematological disorder.
6. Renal or hepatic disease.
7. Use of immune-suppressive or anti-inflammatory drugs in the previous two months.

3.9. Analyses assayed

3.10. Sample collected

Venous blood samples were obtained on admission.

4. Method

Blood samples were immediately centrifuged and analysed by semi-autoanalyzer following standard operating procedure.

Table 1:

S.No	Test	Method
1	Glutathione peroxidase (GPX)	Spectrophotometric assay Method
2	Uric Acid	Uricase Method
3	Superoxide dismutase (SOD)	Marklund and Marklund (1974) Method
4	Catalase	Aevi (1984) (Spectrophotometric assay) Method
5	Vitamin C (ascorbic acid)	Indophenol Method
6	Vitamin E (Alpha tocopherol)	Baker & Frank method

4.1. Statistical analysis

The collected data were compiled in MS Excel sheet for analysis. Analysed in Statistical Package for the Social Sciences (SPSS) version 25^{th} 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 in the form of pie diagram and bar diagram. p-value < 0.05 indicates statistical significant.

5. Results

A total of 87 cases of stroke (58 ischemic stroke, 29 haemorrhagic strokes) and 58 healthy individuals as the control group were identified during the study follow-up. Their age varied between 41 and 81 (52.41±9.49 and 54.85±9.57 in IS and HS, respectively) and in control group 33 male, 19 IS male and 31 HS male. In addition, female was 25 in control, 10 in IS, 27 in HS group. Whereas, BMI 23.4 ±3.3 in control group, 24.3 ±3.6 in IS group and 22.1±3.4 in HS group. Moreover, hypertensive 23 were in control group, 11 in IS and 19 in HS group. Systolic BP 134.6±12.5 in control group, 141.1±13.4 in IS and 139.5±14.3 in HS group. Diastolic BP 82.5±9.35 in control group, 81.2±9.21in IS group and 83.1±8.24 in HS group (Table 2).

The GPX levels are reduced significantly in ISPs and HSPs compared to control subjects($p<0.001$). Extreme decrease in GPX is seen in ISPs with HSPs($p<0.001$). The Uric acid levels are considerably increased in ISPs and HSPs when equated to control subjects($p<0.003$) and the more upsurge seen in ISPs with HSPs (Table 3). The SOD levels are reduced drastically ($p<0.05$) in ISPs and HSPs when equated to control subjects, whereas its levels are marginally reduced in ISPs with HSPs. Similarly, Catalase levels also decreased in both ISPs and HSPs.

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

6. Discussion

The current study depicts significant upsurge in lipid peroxides in Ischemic Stroke Patients (ISPs) and haemorrhagic stroke Patients (HSPs) as equated with control subjects. A study by Milanlioglu et al. determined that patients with acute ischemic stroke exposed increased oxidative stress reaction, and weakened antioxidant enzyme activity, signifying that imbalance of oxidant/antioxidant status could be a part of the pathogenesis of acute ischemic stroke.[10–17] To study the antioxidant potential of ischemic stroke patients we have included Glutathione peroxides (GPX), Uric acid and SOD, Catalase in our study.

Our results indicate that GPX levels are decreased significantly in ISPs and HSPs but extreme decrease is seen in ISPs. GPX reduction increases cerebral ischemic injury. Shivakumar et al. and Akila et al. have revealed that GPX levels have reduced in brain regions during reperfusion for 1 hours after moderate or severe ischemia for 0-5 hours.[18,19] The GPX was exhibited to decrease lethality, rise brain water levels and decline MDA levels in cerebral ischemic rats when given rapidly after ischemia signifying that its anti-ischemic results are due, in part to inhibition of lipid peroxidative reactions.[19–22] In our study, we have noticed reduced GPX levels in ISPs and HSPs which specifies that antioxidant capacity is declined in these patients. So, management with anti-oxidant could be helpful to decrease MDA in ischemic stroke patients.

We have also seen in our study that SOD levels are reduced in ISPs and HSPs when compared with control subjects, where as its levels are reduced in ISPs with HSPs. Similar to the current study, El Kossi et al. (2000) found significant difference between IS group and control group, concerning serum SOD activity.[23] Moreover, Cherubini et al. and Demikaya et al. found that SOD activity decreases significantly in IS patients.[24] SOD is an endogenous antioxidant that catalyses the dismutation of the superoxide anion radical. SOD plays an important role in the defense against fee radical damage in reperfusion injury a help in reducing the infarct size during ischemia and reperfusion.[24,25]

In our study that Catalase levels are reduced in ISPs and HSPs when equated with control subjects, where as its levels are declined in ISPs with HSPs. Similar to the current study Cherobini et al. (2000) reported that the levels of CAT, activity in plasma and red blood cells in patients at the onset of stroke were lower than the control group.[26]

7. Conclusion

The prospective cohort study on the evaluation of correlation of prognostic factors in stroke and haemorrhagic patients Glutathione peroxides, Uric acid, Superoxide dismutase (SOD), Catalase, Vitamin C (ascorbic acid) and Vitamin E (α- tocopherol) in patients with ischemic and haemorrhagic stroke cases. The antioxidative parameters like superoxide dismutase and Catalase was declined both ischemic and hemorrhagic stroke when equated with control. The sign for endothelial dysfunction nitric oxide level was declined significantly in ischemic stroke not in hemorrhagic stroke when compared to normal healthy volunteers. This may be beneficial for enhancing the dose regimen for improvement of patient health. Frequent exposure to CT scan could generate numerous problems to patient and some patients could not afford to take CT scan because of expensive. In this circumstance, our study consequences could be helpful for doctor and patient health. From these studies, we can conclude that antioxidant

Table 2: Characteristics of the whole group of patients

Characteristics	Control group	Ischemic stroke	Haemorrhagic stroke
n	58	29	58
Age, y, Mean±SD	53.64±9.43	52.41±9.49	54.85±9.57
Males	33 (56.8%)	19 (65.5%)	31 (53.4%)
Female	25 (43.1%)	10 (34.4%)	27 (46.5%)
BMI (kg/m^2)	23.4 ±3.3	24.3 ±3.6	22.1±3.4
Hypertension n (%)	23 (39.6)	11 (37.9)	19 (32.7)
Systolic BP (mmHg) Mean±SD	134.6±12.5	141.1±13.4	139.5±14.3
Diastolic BP (mmHg) Mean±SD	82.5±9.35	81.2±9.21	83.1±8.24

Table 3: Distribution of the anti-oxidants in patients

Characteristics	Control group Mean±SD	Ischemic stroke Mean±SD	Haemorrhagic stroke Mean±SD
Glutathione peroxides (GPX) (μmol/mg)	9.93 ± 2.31	4.02 ± 1.32 P < 0.001	4.22 ± 1.32 P < 0.001
Uric acid (mg/dl)	4.34 ±0.4	7.16±0.9 P < 0.001	6.23±0.7 P < 0.001
Superoxide dismutase (SOD) (U/mg)	14.3 ±0.3	9.3 ±0.6 P < 0.001	8.9±0.4 P < 0.001
Catalase (IU/mg)	13.3 ±0.6	8.3±0.7 P < 0.001	9.5 ±0.2 P < 0.001

Table 4: Distribution of the Vitamin C and E 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)	1.43 ± 0.24	0.56 ± 0.63	0.98 ± 0.71
Vitamin E (mg/L)	11.64±0.53	7.41±0.65	8.85±0.72

defence is impaired in ischemic stroke patients as a result of increased oxidative stress.

8. Source of Funding

None.

9. Conflicts of Interest

Nil.

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Cite this article: Shukla RK, Agrawal BK, Kumar A. Evaluation and comparison of antioxidant status in ischemic and haemorrhagic cases of stroke. *Int J Clin Biochem Res* 2020;7(2):176-180.