ASSESSMENT OF ANTIOXIDANT STATUS IN CEREBRAL STROKE: A PROSPECTIVE STUDY IN BHILAI, CHHATTISGARH

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Abstract

Background: Stroke is a clinical condition with multifactorial origin where brain is depressed of oxygen and glucose supply lasting around 24 hours and may lead to permanent damage or death. The World Health Organization (WHO) referred it as a disturbance of cerebral vasculature apparently leading to death. **Materials and Methods:** 50 individuals (20 subjects of hemorrhagic stroke and 30 subjects of ischemic stroke were selected along with 50 healthy controls without any cardiac complications).Selection of patients: All patients either attending OPD or mostly admitted in IPD with clear history of stroke (hemorrhagic or ischemic stroke) were approached for enrollment for the study. Their clinical history, CT scan reports and legal consent was taken as the basis of selection and categorization for the study into hemorrhagic stroke or ischemic stroke participants. **Result:** In comparison to 50 stroke subjects (20 hemorrhagic stroke and 30 ischemic stroke) with 50 healthy controls, Glutathione peroxidase (GPx), Superoxide Dismutase (SOD), Catalase (CAT), Vitamin C and Vitamin E was perceived to be markedly lower in Stroke subjects when weighed against the healthy control group. ($p<0.001$). Again, a well-built linkage was studied among lipids peroxidation and mass of infarct in Stroke cases. The lipoxidation marker Malonaldehyde (MDA) was understood to have a good optimistic term with infarct size. **Conclusion:** The rocketing levels of MDA in literatures and the striking detoriation of antioxidant status seen could be a predictive signal for stroke. Henceforth a watchful check of antioxidant status could prove to be better evidence in planning medication regimen and keeping lipoxidation under control, for sustaining a much-improved quality of life for the stroke patients.

INTRODUCTION

Stroke is a clinical condition with multifactorial origin where brain is deprived of oxygen and glucose for nearly a day and may escort towards permanent damage or death. The World Health Organization (WHO) clearly reffers the same as a "disturbance in cerebral vasculature apparently leading to fatality".[1] Stroke has surfaced as a primal factor for loss of quality of life among adults and also a major cause of mortality among senior citizens1. Among the various categories of stroke, the ischemic variant of stroke is glimpsed to be more denting over hemorrhagic form of stroke in ripened citizens.[2]

Although stroke is of multifactorial origin, the strapping role of oxidative stress on the disease pathogenesis cannot be ignored. The free radical generated oxidative stress is understood to be the main rogue for the observed ischemia in cases of stroke. The re-oxygenation attempts in treatment often have reverse effects, since oxidative stress can re-establish itself very fast and leads to irreversible cerebral nerve damage. Therefore preventing the scenario of oxidative stress had become the primal goal for avoiding occlusion/ischemia in the treatment of stroke.[3] Since free radicals have the orthodox nature to re-estblish oxidative stress in no time, it theatres as a "death sentence to neurons".[4] ROS (Reactive oxygen species) like free radicals also has the tough ability to execute lipoxidation of the plasma membrane generating massive amounts

189

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of Malonaldehyde (MDA), a well-established marker for lipid peroxidation. MDA has already had a repute of being a meticulous indicator of tissue lipid peroxidation and damage. Various older literature and recent researchers have well indicated the augmented levels of MDA in subjects suffering from various tissue lipoxidation injuries like thyroidism and cerebral stroke.[5]

Antioxidants, on the other hand, have been effectively exploited by clinicians all over the world to reinstate the peril of oxidative stress in numerous diseases including induced stroke. Antioxidants has been established to have immense potential with mammoth defensive capabilities to cease free radicals induced injuries and has thus earned titanic respect as an oxidative stress buster.[6,7,8,9] There are a number of enzymatic as well as non-enzymatic antioxidants that has dished us up with their capacities to gobble up free radical injuries. Natural antioxidants such as super-oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione-S-Transferase (GST) Glutathione Reductase (GR), Glucose-6-P-Dehydrogenase ect had been tirelessly used as defence against Reactive oxygen species (ROS). While, non-enzymatic antioxidants include Vit C, Vit E, Ceruloplasmin Carotenoids, Albumin, Bilirubin and few more, have proved their importance in hand to hand.

All the above had led us to consider the issue of scrutinizing the interrelationship between the different forms of stroke (ischemic and hemorrhagic) along with other parameters like lipid peroxidation marker (Malonaldehyde-MDA) with the status of various antioxidant status standing against them.

Objective of the study
This present research study was taken up to comprehend the inter relationship between Antioxidants with various forms of stroke (ischemic and hemorrhagic) and the effectiveness of antioxidants as a tool to safeguard brain.

MATERIALS AND METHODS

This Hospital based Case Controlled study type was planned with a prospective and observational design format involving patients who were pre-diagnosed cases of stroke, both ischemic and hemorrhagic, under the supervision and treatment of doctors in the hospital attending either OPD or IPD departments. A total number of 50 participants were enlisted for the research after getting written consent. The age group cutoff was taken between 45-70 years. Age and sex matched control group of 50 volunteers was also enrolled for reference. All the participants were enrolled within 24 hours of inception of warning sign at the emergency ward of Sankaracharya Institute of Medical Sciences, Bhilai, and Chhattisgarh. They were ensured of the categorization into hemorrhagic or ischemic stroke

by their clinical history and brain CT scan to validate the selection criteria. The duration of the enrolment was maintained for a period of 1 year. All Certified patients of ischemic or hemorrhagic stroke were approached for participation and consent for the study. Approval for the study was simultaneously proceeded from the Institutional Ethical Committee (IEC) for the same. All selected participants who were ready to participate were informed from time to time, about the study developments. The entire research was conducted as per Good Clinical Practice (GCP) Guidelines.

Sample Size
The present case-controlled study was taken up on overall 100 participants. They were categorized in 3 groups:

Group I: 30 subjects. These participants were cases of Ischemic stroke.
Group II: 20 subjects. These participants were suffering from Hemorrhagic stroke.
Group III: 50 Control participants with no history of stroke or any major neural complications.
Venous blood samples were collected from all participants and analyzed immediately after processing following the regular protocol. Semi-autoanalysers and Fully-automated analysers were utilized for analysis.

Serial number	Test	Method
1	Glutathione peroxidase (GPX)	Spectrophotometric assay.
2	Uric Acid	Uricase Method
3	Superoxide dismutase (SOD)	Marklund and Marklund (1974) Method
4	Catalase	Spectrophotometric assay.
5	Vitamin C (ascorbic acid)	Indophenol Method
6	Vitamin E (Alpha tocopherol)	Baker & Frank method

Statistical Analysis
All statistical data were collected and entered into Excel sheet. Calculation and variations in parameters was done by using SPSS version 22. Quantitative data was shown as mean $\pm$ SD and suitable tables. Appropriate t-test and Person correlation was applied to show the association between stroke and other variables in study. P-value less than 0.05 were considered to be statistically significant.

Inclusion Criteria
Confirmed cases of Stroke as hemorrhagic as well as ischemic stroke.
Patients ready to participate in the study and willing to give written consent.

Exclusion Criteria
Patients unable to participate or give written consent.

Patients expecting financial benefit from the study.
Any other neural events or complications.
Cancer, Immuno-compromised or under immunosuppressive drug.
Chronic liver, kidney or pancreatic diseases.

RESULTS

A total of 50 cases of stroke was analyzed and assessed. Out of this 30 were ischemic category stroke and 20 were of hemorrhagic type of stroke. Along with 50 healthy controls were also analyzed. Age group cutoff was taken between 45-70 years (52.41±9.49 and 54.85±9.57 in Ischemic stroke and Hemorrhagic stroke respectively).

Among the gender categorization, 33 were healthy males while 19 had IS and 12 were suffering from HS. Among females 17 were healthy while 11 females had IS and 8 females had HS.

BMI was seen to be marginally affected in all the groups (24.3 ±3.6in IS group 22.1±3.4 in HS group and 23.4 ±3.3 in reference group). Apart from BMI, slight higher levels of Systolic BP was seen in stroke cases compared to normal controls although the increased changes was more predominant over the IS subjects when compared to HS cases(134.6±12.5 in control group, 141.1±13.4 in IS group and 139.5±14.3 in HS group). On the other hand, the changes seen in the Diastolic BP was a trivial variation (82.5 ± 9.35 in control group,

81.2±9.21in IS group and 83.1±8.24 in HS group [Table 1].

The GPx levels in our study was seen to be radically reduced in stroke cases compared to the control group (p<0.001). The predominance was seen more over the IS subjects when compared to the HS subjects (p<0.001). Again the situation of Uric acid was also very identically opposite to GPx levels. Uric acid levels were observed to be noticeably augmented among the cases when evaluated against controls (p<0.001). However it was also pragmatic that the augmentation of uric acid was more in the IS patient group than the HS patient group. SOD levels, on the other hand had minor variations among the patient groups ie. IS and HS categories, but when compared to controls, a drastically reduced levels was witnessed (p<0.001). Likewise, levels of Catalase was also witnessed to be reasonably poorer in patients group when compared to controls even though not much disparity was seen in-between the IS and HS groups. [Table 2].

Separately, it was also evidenced that the blood levels of other antioxidants like Vitamin-C was lower in IS and HS subjects comparatively to the control group with irrelevant variations among each other. Moreover the levels of Vitamin-E also had slightly more intense scenario of much lower levels among patient group in contrast to control group but among themselves they had negligible differences. [Table 3].

Table1: Characteristics of the whole group.

Parameters	Control group	Ischemic stroke	Hemorrhagic 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/m2)	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 2: Distribution of the anti-oxidants in whole group.

Parameters	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
Superoxidedismutase (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 3: Vitamin C and E distribution HS, IS and control group

Parameters	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

DISCUSSION

This recent learning illustrates a noteworthy surge in peroxidation of lipid in cases of Stroke as compared to healthy volunteers. Milanlioglu et al had earlier demonstrated elevated free radical stress, and undermined antioxidant activity in stroke subjects, which was strongly suggestive of discrepancy in oxidant/antioxidant status, which could be the

answer for the observed pathogenesis seen in stroke.[10,11,12,13,14,15,16,17]

To understand the defensive potency of antioxidant in stroke, Glutathione peroxides (GPx), Uric acid, SOD and Catalase was taken under our research for evaluation.

The outcome of the project pointed out significantly diminished Peroxidase levels in stroke cases, more extremely in Ischemic stroke category than

Hemorrhagic group. This fall in GPx levels unquestionably enhances ischemic injury to the brain. National researcher groups like Shiva kumar et al. and international scientists like Akila et al. have clearly exposed the episode of reduced GPx levels in the brain after fair or brutal ischemia.[18,19] This terminal collapse in GPx levels along with water invasion into the cranium by the side of decline in MDA levels, signify anti-ischemic results approximating silencing of lipid peroxidation reactions in rats.[19,20,21,22]

In this present project, we observed strongly bargained GPx levels in ISPs and HSPs, stipulative of bowed down antioxidant defensive capacity among patients category. Hence it is predictive that, supervision by anti-oxidant supplementation could materialize to reduce MDA levels in ischemic stroke patients.

We also landed up to the understanding that the levels of SOD levels are also severely trimmed down in stroke subjects predominantly in Ischemic stroke subjects than in Hemorrhagic stroke subjects when weighed against healthy control group.

Analogous to our recent findings, in the year 2000 El Kossi et al. had noticed important variation in serum activity of SOD among Ischemic Stroke subjects as well as the control subjects.[23] What is more is that, Demikaya et al and also Cherubini et al. found distinctly decreased SOD activity in Ischemic stroke patients.[24] SOD is well understood to be an strong player as an endogenous antioxidant grounding dismutation of superoxide anion radicals. SOD demonstrates immense business in shielding fee radical damage in cases of reperfusion injury and also play trivial role in aiding infarct size reduction during crisis.[24,25]

In this study, it was evidenced that in cases of either Ischemic stroke or Hemorrhagic stroke, the levels of the enzyme Catalase were strikingly dejected when equated with control subjects, where the dejection is more dominant over the Ischemic group than in Hemorrhagic stroke cases. To add to the resemblance with our findings, literature had clear evidences from Cherobini et al, who in the year 2000 described poor CAT activity levels in plasma and RBC at the inception of stroke were evaluated against control group.[26]

CONCLUSION

The futuristic approach for finding the prognostic parameter in stroke patients throws light for better understanding of the disease. The antioxidants were evidenced to be lower in stroke patients than the volunteer control group. As per other previous studies, Nitric oxide level was also majorly understood to be depressed in ischemic stroke cases than hemorrhagic stroke when evaluated against normal volunteers. All these above may help in organizing smooth management and dose regimen progression for better patient health. Frequent CT scan exposure again is both a psychological and financially saddle to patient and their family. Hence, our findings could be comforting both for doctor and patient recovery. Therefore, a dedicated watch, over the detoriating antioxidant defense due to increased oxidative stress could emerge as a noble way in managing stroke cases.

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192

International Journal of Academic Medicine and Pharmacy (www.academicmed.org)
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193

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