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Correlation of Serum Malondialdehyde and Total Antioxidant Capacity Level among Stroke Patients: An Observational Study

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ABSTRACT

Background: Stroke is the leading cause of morbidity and mortality worldwide. Increase free radical formation accompanied by a low antioxidant level may play a crucial role in the pathogenesis of stroke.

Objective: This study was done to estimate the serum malondialdehyde (MDA) as a marker of lipid peroxidation and serum total antioxidant (TAC) level in stroke patients and also to find out any association of both with the severity of stroke.

Methods: Matched case-control study was conducted after approval from the institutional ethics committee. 80 case of stroke with matched controls (1:1) were selected by simple consecutive sampling technique as per predefined inclusion and exclusion criteria. Serum malondialdehyde, serum total antioxidant, and other biochemical parameters were estimated in all individuals. All the relevant data were collected and analyzed with help of SPSS v 25.0 (IBM, New York). For all statistical purposes $p < 0.05$ was considered as statistically significant.

Results: We found a highly significant increase in serum MDA and decrease in serum TAC levels of cases compared to controls ($p < 0.001$). There was a strong negative correlation between the serum malondialdehyde and total antioxidant level ($r = -0.702$, $p < 0.001$). Both serum MDA and TAC demonstrate a statistically significant difference between the three groups of stroke patients based upon severity ($p < 0.001$).

Conclusion: Existing oxidative stress is involved in the pathogenesis of stroke, and the oxidant-antioxidant disparity additionally contributes to its severity. Thus, intervention with antioxidants can play a valuable role in the outcome of stroke patients.

Key Words: Stroke, Serum malondialdehyde, Serum total antioxidant, Glasgow Coma Scale, Analysis of variance

INTRODUCTION

Stroke is defined as by the World Health Organization (WHO) as a "neurological debt of cerebrovascular cause that continues beyond 24 hours or is interrupted by death within 24 hours". It is the second most frequent cause of death worldwide followed by Ischemic Heart Disease (IHD); accounting for nearly 5.5 million deaths and about 116·4 million disability-adjusted life years (DALYs) in 2016.[1] The incidence rate of stroke in India is 119-145 per 100,000 populations and it has become one of the major cause of early death and disability in India.[2]

Strokes can be broadly classified into two major categories: ischemic and hemorrhagic. Ischemic stroke accounts for more than 80% of all stroke cases.[3] While the underlying causes of cellular injury following ischemia are found to be multi-factorial, there is considerable evidence to suggest that the reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, singlet oxygen and highly reactive hydroxyl radical may play a key role in its pathogenesis.[4]

Oxidative stress arises due to an imbalance between increased free radical formations with reduced antioxidant defence mechanisms that quenched the free radicals. This

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disparity leads to over-accumulation of reactive intermediates that contribute to the pathogenesis of stroke associated neuronal injury.[5,6] MDA is formed via peroxidation of polyunsaturated fatty acids (PUFAs) and can be measured as thiobarbituric acid-reactive substances (TBARS). It is the most frequently used marker of oxidative damage for various diseases.[7] Total antioxidant capacity (TAC) assay has been used to establish the overall antioxidant power.

Glasgow Coma Scale (GCS) is the most common scoring system used to outline the level of consciousness or to determine the severity of acute brain injury. It is established to guide the early management of patients with an acute brain injury. The GCS divides into three parameters: best eye response (E), best verbal response (V), and best motor response (M). The total GCS has a score between 3 and 15, 3 being the lowest or worst score and 15 being the highest one. The brain injury is classified as Severe: GCS ≤8, Moderate: GCS 9-12 and Mild: GCS 13-15.[8] There is extremely limited data regarding both oxidative and antioxidant status among stroke patients in India.

MATERIAL AND METHODS

This present study was a hospital-based observational analytical matched case-control study, being conducted in the department of Biochemistry in collaboration with the Department of Medicine and Neurology, SCB Medical College, Cuttack, Odisha, India from November 2016 to June 2018, after approval from Institutional Ethics Committee (IEC) with the letter-number IEC/IRB No: 150/7.9.15. As per previous study serum TAC level was significantly lower among stroke patients as compared to controls (0.77 +/- 0.38 vs. 0.95 +/- 0.30, p <0.015).[9] Based upon these facts, by applying hypothesis testing for two means (equal variance) method and assuming power (1-ß) to be 90%, Type I error (α) to be 2.5% for 1 sided test, minimum sample required for our study was 76 in each group (n master v2.0; BRTC, Vellore). 160 patients with a ratio of 80:80was enrolled by a simple consecutive sampling technique method after receiving written informed consent. The clinically diagnosed and radiologically confirmed cases of stroke (cerebrovascular accident; CVA) presenting within 24 hours of the onset of either gender between the age group of 30 to 80 years were included in the study. Patients with a history of chronic liver or renal disease, alcoholics, smokers, ischemic diseases, new-onset angina, acute myocardial ischemia, advanced heart failure, patients with a history of surgery for cardiovascular diseases and patients with major comorbid conditions were excluded from the study. Age and sex-matched healthy normotensive individuals without a history of transient ischemic attack or stroke were taken as control. The exclusion criteria of controls were the same as cases. A detailed history was collected from all individuals. The physiological parameters at the time of admissions such as age, height, weight, duration of disease, and systolic blood pressure (SBP) were recorded. A computerized tomography scan (CT scan) was performed in all patients on arrival. Only in those where CT scan was not conclusive, magnetic resonance imaging (MRI) of the brain with stroke protocol was used for confirmation. Glasgow Coma Score (GCS) of each patient was documented on a coma scale chart and the severity of stroke was gauged accordingly and classified into mild, moderate, and severe.

Blood sample (5 ml) was collected from both cases and controls from the median cubital vein without producing venous stasis with a 20-gauge needle in plastic disposable syringes. Blood samples were immediately sent to the SCB Biochemistry laboratory for evaluation. The biochemical parameters such as plasma FBS, serum lipid profile, urea, creatinine, and uric acid were estimated by Toshiba TBA-120FR Autoanalyzer, Japan using standard kits. Serum sodium, potassium, and ionized calcium were measured using the HDC-Lyte electrolyte analyzer, India. The serum MDA was evaluated by the method of K Satoh.[10] The serum TAC was evaluated by using Automated microplate ELISA reader Erba LisaScan EM, Japan-based on biotin double antibody sandwich technology.

The continuous variables were represented as a mean ± standard deviation (SD) and categorical data as percentages. The **unpaired t-test (**student's t-test) was used to compare the mean of two independent continuous variables. The relationship between two continuous variables is analyzed using Pearson's correlation coefficient. Analysis of variance (ANOVA) was used to compare the serum MDA and TAC among the different groups of stroke patients. Statistical analysis was done using SPSS v25.0 (IBM, New York). p-value <0.05 was considered to be statistically significant.

RESULTS

The present study includes 80 stroke cases and 80 age- and sex-matched healthy controls. Out of 80 patients, 55 are male (68%) and 25 are female (32%) whereas out of 80 controls 49 are male (61%) and 31 are female (39%). The age [mean (SD)] of patients and controls is 61.41 (7.02) and 59.08 (6.73) years respectively. 35 out of 80 cases were in the age group of 51-60 years and 32 were in between 61-70 years of age. Out of 80 controls, 32 were in the age group of 51- 60 years and 37 were in the age group of 61-70 years. Mean height (cm) between cases and control were not statistically significant [168.6 (7.2) vs 167.2 (6.8); p >0.05]. Mean body mass index (kg/m^2) between cases and control were not statistically significant [24.7 (6.3) vs 23.8 (7.6); p >0.05]. Mean weight (kg) between cases and control were not statistically significant [72.8 (10.3) vs 70.6 (10.8); p >0.05]. Mean SBP (mm Hg) between cases and control were statistically

significant [182.8 (32.4) vs 152.24 (28.5); p <0.05]. The mean duration of disease (days) among cases was13.3 (8.7). A comparison of biochemical parameters among cases and controls were summarized (Table 1).

There was a strong negative correlation between serum MDA (nmol/ml) and serum TAC (IU/ml) level as evidenced by the Pearson correlation coefficient = -0.799, p <0.001 (Figure1). There was a statistically significant difference in mean serum TAC (IU/ml) between three groups of stroke patients based upon severity as determined by one-way ANOVA [F (2,77) = 9.999, p<0.001]. A Tukey post hoc test revealed that the mean serum TAC (IU/ml) level among a severe group of stroke patients was significantly lower [29.01 (9.5)] as compared to moderate [37.41(10.5), p <0.01] & mild [44.15 (12.3), p <0.01] stroke groups. We found no statistically significant difference between mild & moderate categories (p >0.05). There was a statistically significant difference in mean serum MDA (nmol/ml) between three groups of stroke patients based upon severity as determined by one-way ANOVA: F (2,77) = 14.305, p <0.001. A Tukey post hoc test revealed that the mean serum MDA (nmol/ml) level among a severe group of stroke patients was significantly higher [2.78 (0.97)] as compared to moderate [1.67 (0.9), p <0.001] & mild [1.31 (0.8), p <0.001] stroke groups. There was no statistically significant difference between mild & moderate categories (p >0.05).

DISCUSSION

Male outnumbered the female in both cases and control and most of the cases belong to more than 60 years of age. There is no difference in weight, height, and BMI between the two groups of the population. As the serum level of MDA was increasing, serum TAC level was decreasing among the stroke patients and this negative correlation was also significant. Among three groups of stroke patients (based upon Glasgow coma scale) the level of serum MDA was highest among the severe group, whereas serum TAC level was low and this association was also significant.

India is now facing an extensive socioeconomic burden to meet the costs of rehabilitation of "stroke victims" because the population now survives through the peak years (age 55–65 years) of the occurrence of stroke.[11] In our study male predominance of stroke incidence was found (68% male versus 32% female). Similarly, Appelros 'et al.' found a worldwide male predominance of stroke.[12] The lower incidence of stroke in the female is probably due to genetic factors as well as the positive effects of estrogen on the cerebral circulation.[13] Another cause may be the higher blood pressure in men than women of similar ages.[14,15] Majority of patients (67 out of 80) belong to the older age group (51-60 years and 61-70 years). Similar observations were also found by Dalal 'et al.' in their study.[11] The mean age of stroke patients in our study was found to be 61 years. The mean age of stroke onset in India (i.e., 63 years) comes out with lower than the Western countries (68 years and 71 years in USA and Italy respectively).[16]

The routine biochemical investigations in this case-control study revealed a significant rise in FBS, urea, cholesterol, TG, and uric acid levels in cases as compared to control whereas serum HDL revealed significantly lower values in cases (Table-1). Elevated FBS is under the findings of Kaur 'et al.' and Stahl 'et al'.[17,18] Elevated blood glucose level has been implicated as a poor prognostic factor for stroke. Various studies have shown that elevation of serum cholesterol, triglyceride, and low HDL are associated with stroke.[19,20] The derangements in lipid profile may accelerate the development of atheroma in the carotid artery wall and thickening of intima-media leading to increased incidence of stroke. However, the role of hyperlipidemia in cerebrovascular diseases is still being evaluated.

Our study revealed serum urea level is significantly elevated in patients but serum creatinine is not significant when compared to controls. Woo 'et al.' have demonstrated that elevated plasma urea and creatinine level is associated with the severity of stroke. However, these parameters did not reveal any independent effect on mortality.[21] Following cerebral ischemia and reperfusion, the production of oxygen free radical and uric acid production stimulated via the xanthine oxidase pathway. Though uric acid is a powerful antioxidant, in high concentrations it can act as a prooxidant and exacerbate tissue damage. Storhaug 'et al.' have shown that increased serum uric acid level was associated with a 31% increased risk of stroke in men.[22]

We found a statistically highly significant elevation of serum MDA (nmol/ml) in patients than controls (2.31±1.11 Vs 1.63±0.31, p<0.001) and highly significant reduction of serum TAC (IU/ml) in patients as compared to controls (32.88 ±11.12Vs 57.99±10.91, p<0.001). There is a strong negative correlation of serum MDA with TAC (r = −0.702, p <0.001) in stroke patients. Similar results of serum MDA were obtained by Cojocaru 'et al.' and Abdullah 'et al.' in patients with acute stroke when compared to controls (p <0.05 and p <0.001 respectively). Serum TAC studies by Abdullah 'et al.' and Cojocaru 'et al.' show significantly low serum TAC level in stroke patients than controls (p <0.015 and p <0.05 respectively).[9,23] Srikrishna 'et al.' and Lorenteet 'et al.', found an association between higher serum TAC levels and severe ischemic stroke.[24,25] Oxidative stress is one of the mechanisms contributing to the neuronal damage potentially induced by ischemia and reperfusion. The antioxidant capacity may protect the brain against the deleterious effects of the free radicals produced during ischemia and reperfusion.[26]

There was a statistically significant difference in mean serum MDA as well as mean TAC between three groups of stroke patients based upon severity ($p<0.001$). The mean serum MDA level among a severe group of stroke patients was significantly higher as compared to moderate and mild stroke groups ($p<0.001$). There was no statistically significant difference between mild and moderate categories ($p >0.05$). We observed the mean serum TAC level among a severe group of stroke patients was significantly lower as compared to moderate and mild ($p <0.01$) stroke groups, but no statistically significant difference between mild and moderate categories ($p >0.05$). It is highlighting the fact that oxidants formed before and during ischemia might impair the neuronal functions and might play a major role in the pathophysiology of stroke. Zainab et al. revealed the levels of MDA correlate positively with the severity of stroke as measured by the NIHSS score. MDA on the day of admission correlates with the short-term prognosis (around one week) of the patients.[27]

We have conducted a matched case-control study, thereby trying to reduce the bias of study variables to some extent. The minimum sample size was estimated and with a short period, we have got satisfactory results which will stimulate us to conduct robust research shortly. Our study is also not devoid of limitations. We have got only an association, but a causal relationship could not be established. No, follow up could be done due to the time constraint of the project. We could not able to do a matched analysis, which might have otherwise given us some different outcomes. So, in the future, a longitudinal study with a larger sample size is awaited in this era of evidence-based medicine.

CONCLUSION

Stroke is a major cause of mortality and disability in an aging population involving excitotoxic mechanisms, inflammatory pathways, oxidative damage, ionic imbalance, and apoptosis. Our study revealed that oxidative stress is involved in the pathogenesis of stroke and is the major contributing factor for its severity. Estimation of serum malondialdehyde and total antioxidant capacity is simple and economic methods and may be employed as routine laboratory investigations to screen the high-risk persons so that early antioxidant intervention can be implemented. However, for a better level of evidence long term follow up studies are awaited in the future.

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