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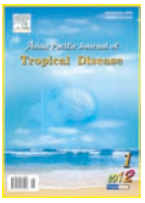

Document heading

Correlation of Serum Endothelial Dysfunction Markers with CT Angiographic Findings in Ischemic Stroke

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ABSTRACT

Objective: Endothelial dysfunction is considered as root cause of vascular diseases like stroke, myocardial infarction (MI) and venous thromboembolism. Soluble endothelial dysfunction markers are emerging as surrogate markers of disease risk. We aim to correlate the findings of Computed Tomography Angiography (CTA) with biochemical markers of endothelial dysfunction in patients of stroke. **Material and method:** 40 patients diagnosed to have ischemic stroke or Transient Ischemic Attack (TIA) based on clinical history, examinations and imaging were included. We assessed high sensitive C – reactive protein (hsCRP), total nitric oxide (NO) and superoxide dismutase (SOD) levels in all patients within 24 hours and CT-Angiography of bilateral neck vessels within 48 hours of hospital admission. **Results:** Increase in hsCRP, NO and decrease in SOD was significant in cases as compared to controls. These biochemical markers correlated significantly with CT Angiographic findings. **Conclusions:** This study demonstrates that hsCRP, NO and SOD is good surrogate biochemical markers for assessing disease risk and burden in ischemic stroke. These surrogate markers showed a linear correlation and statistical significance with CT angiography score. Specific intervention targeted to reduce the oxidative stress, as indicated by these markers, and imaging findings should be a part of stroke management protocol.

1. Introduction

Stroke incidences and mortality are increasing along with modernization and advancing longevity of life. Worldwide, 15 million people suffer a stroke each year. Five million of those die and 5 million are left permanently disabled[1]. It is estimated that by the year 2020, stroke mortality will be doubled as a result of aging population and future impact of current smoking patterns[2]. Globally, about 55 million people have had a stroke at some time in the past, either with or without residual disability; two-thirds of these individuals live in low- income and middle-income countries. Two-thirds of all stroke deaths occur in low and middle income countries[3-4]. As infectious diseases and malnutrition decline in developing countries, stroke incidence rises due to decreased physical activity, increased tobacco use, and dietary changes. By the year 2040, there will be a billion adults aged 65 years or older at risk for stroke, in low and middle income countries. In addition to the aging population, the major stroke risk factors worldwide are hypertension and tobacco use[5]. There are many cases of ischemic stroke which are unaccounted for by these known risk factors and risk stratification by these risk factors is largely incomplete and hence there is a need for other markers which can predict the disease risk better. Endothelial cell plays a key role in vascular homeostasis. Endothelial dysfunction has a central role in the pathogenesis of many vascular diseases related to atherosclerosis[6-7]. It is associated with a number of conventional risk factors including hypercholesterolemia, smoking, hypertension, diabetes mellitus, insulin resistance and obesity[8]. Apart from the risk factors involved, atherosclerosis is an inflammatory disease in which abnormal functioning of endothelial cells shows a crucial role at the all stages of atherosclerosis[9]. It is therefore useful to measure biological markers of vascular endothelial function in vivo because such markers might provide insight into the evolution and prognosis of vascular diseases.

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2.1 Materials and methods

This research work was a hospital based cross sectional study conducted from May 2010 to June 2011 in Department of Medicine and Department of Radiodiagnosis, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, in accordance with the declaration of Helsinki guidelines on good clinical practice. The Institutional ethical committee approved the study and written informed consent was obtained from each patient.

Patients diagnosed to have ischemic stroke or Transient Ischemic Attack (TIA) based on clinical history, examinations and imaging were taken up. A Non Contrast Computerized Tomography (NCCT) of head was performed in every patient. Patients with cerebrovascular hemorrhage and cardio-embolic ischemia were excluded from the study. Computed Tomography Angiography (CTA), using 64-slice CT scanner (GE Light speed) was performed in every patient within 48 hours. We divided our patients into those with known risk factors of stroke and patients without these risk factors. The risk factors we studied were hypertension, diabetes mellitus, smoking, coronary artery disease, alcoholism and dyslipidemia. Patients with age <40yrs, renal failure, hemorrhagic stroke, infection, patients on anticoagulants, antiplatelet agents, Angiotensin Converting Enzyme (ACE) inhibitors and statins were excluded from the study. A total of 40 patients and 40 controls were studied with and without risk factors.

A detailed clinical history and physical examination were conducted which included the age, sex, evaluation for the presence or absence of the known risk factors. Physical examinations included anthropometric measurements such as height, weight, body mass index and waist circumference. Venous blood samples were drawn from the cases within 24 hours and serum was separated from the blood and preserved in -80 degree Celsius temparature till the estimation. The serum hsCRP level was measured by sandwich enzyme-linked immune-sorbent assay (ELISA) kit (Diagnostic Biochem Canada) as per manufacturer's protocol. The total nitric oxide (NOx) estimation was done with help of Ghasemi et al. method[10]. We determined extracellular SOD in serum with a common and convenient indirect method which utilizes nitro blue tetrazolium (NBT) conversion to NBT diformazan (formazan dye) via superoxide radical. The rate of the reduction with O_2- is linearly related to the xanthine oxidase (XO) activity and is inhibited by SOD. The IC 50 (50% inhibition activity of SOD or SOD- like materials) was determined by the colorimetric method at 440nm.

We graded CTA findings by a scoring system:

0 -No changes

1 -Eccentric or focal changes without calcification without narrowing

2 -Eccentric changes or focal changes with calcification without significant stenosis

3 -Circumferential changes without calcification without stenosis

4 -Circumferential changes with calcification without significant stenosis

5 -Significant stenosis.

In case of multiple lesions, scores were summated. Each side was scored in this fashion and finally the scores of both sides were added

2.2 Statistical analysis

Sigma stat version 3.5 was used to calculate all statistical data. If the data were normally distributed than the measure of central tendency and dispersion used was mean±standard deviation. If the data were not normally distributed than the measure of central tendency and dispersion used was median±Inter Quartile range. Means of two groups were compared by using student t-test. Medians of two groups were compared by using Mann Whitney rank sum test. Correlations between two quantitative variables were achieved through Pearson's correlation. Comparison between two proportions was done by Chi-square test, but if 20% variables were below 5; Fisher exact tests was used.

3. Results

In our study, patients with recent stroke or TIA (cases) were compared with controls. Both the groups were comparable in baseline characteristics like age, sex ratio and BMI. Both cases and controls had a roughly equal population of subjects with one or more risk factors for stroke like hypertension, diabetes, smoking, CAD. (Table 1)

Table 1

Characteristics of the study subjects.

Characteristic	Cases	Control
Age	60.55±13.456	60.625±13.378
Sex(M/F)	18/22	22/18
BMI	29.576±3.273	28.691±2.602
WRF/WOTRF*	18/22	20/20
Diabetics/Non diabetics	6/34	10/30
Smokers/non smokers	4/36	2/38
Hypertensives/ non hypertensives	8/32	10/30
CAD/Non CAD	1/39	0/40
Alcohol/Non alcoholic	0/40	0/40

[The level of significance for all above mentioned characteristics are not significant among cases and control groups (P>0.05)]

* WRF- patients with one or more risk factors for stroke like hypertension, diabetes, smoking, alcoholism and CAD/CVD; WOTRF- without any risk factor.

Markers of oxidative stress like hsCRP and NO were significantly higher in cases (8.75±3.45 and 26.1±5.8 respectively) as compared to controls (1.15±1.0 and 23.5 ±2.75 respectively). Antioxidant markers like SOD was significantly low in cases than in controls (1.3±0.18 and 1.755 ±0.35 respectively with a p value of <0.001). (Table 2)

Table 2

Level of high sensitive C-reactive protein, nitric oxide and superoxide dismutase.

Parameters	CASE	CONTROL
hsCRP (mg/L)	8.75±3.45	1.15±1.0
NO (μM)*	26.1±5.8	23.5±2.75
SOD (U/mg)*	1.3±0.18	1.755±0.355

*values in median±IQR; The concentration of hsCRP & nitric oxide are significantly higher (P<0.005) and SOD is significantly lower (P<0.001) in cases as compared to control subjects.

Markers of oxidative stress like hsCRP and NO were not significant between cases with risk factors (8.633±2.507 and

25.8±5.0 respectively) and cases without risk factors (7.964± 2.260 and 26.1±6.0 respectively). However, antioxidant marker SOD was significantly lower in cases with risk factors (1.252 ±0.152) than in cases without risk factors (1.346±0.0926) with a p value 0.02. Multi-detector CT Angiographic (MDCTA) findings were more prevalent in cases with risk factors (Fig 1). The CTA score was higher in cases with risk factors (4 ±4) as compared to those without risk factors, but was not statistically significant (p= 0.186). (Table 3)

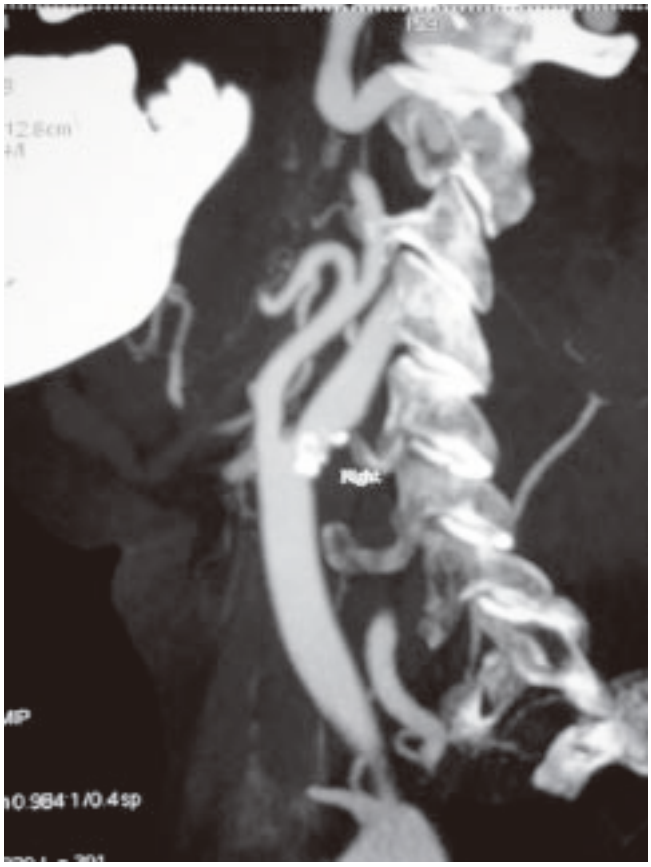

Figure 1. CT angiography saggital plane showing calcified plaque at right carotid bifurcation causing stenosis

Table 3

Levels of high sensitive C-reactive protein, nitric oxide, superoxide dismutase and Multi-detector CT Angiographic score in cases with and without risk factors

Parameters	CASE WRF	CASE WOTRF
hsCRP (mg/L)	8.633±2.507	7.964±2.260
NO (μM)*	25.8±5.0	26.1±6.0
SOD (U/mg)	1.252±0.152	1.346±0.0926
MDCTA	4±4	1±1

The hsCRP level, nitric oxide level & MDCTA score are not significant (P>0.05) while SOD level is significantly lower (P=0.02) in cases with risk factors as compared to cases without risk factors.

Biochemical markers of oxidative stress like hsCRP, NO and SOD significantly correlated with the CTA scores and the disease burden (Table 4), (Fig 2). Moreover, CTA score of patients with infarct on NCCT was significantly higher than in patients without NCCT findings. (1±3 Vs 0±2) (P= 0.049).

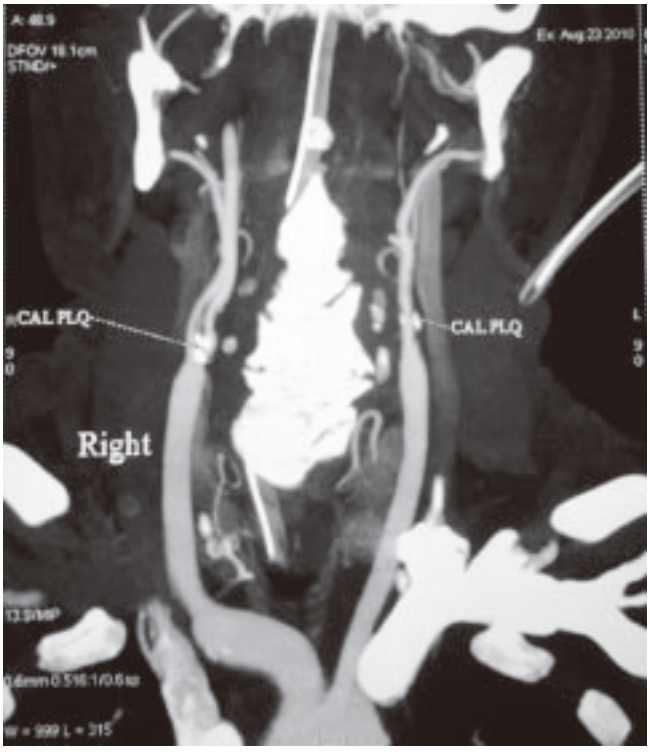

Figure 2. CT angiography coronal plane showing calcified plaque bilateral at right carotid bulb causing stenosis

Table 4

Correlation of biochemical markers with MDCTA findings in cases.

INDEX	PARAMETER	Correlation coefficient
MDCTA Score	hsCRP	0.589
MDCTA Score	NO	0.472
MDCTA Score	SOD	−0.792
MDCTA Score	NCCT	0.275

While correlating the parameters with MDCTA score hsCRP, nitric oxide & SOD levels showed the significance (P<0.005) and NCCT does not showed significance (P>0.05).

4. Discussion

The participants of this study have basic physical characteristics viz. age, height, weight, BMI and sex-ratio similar to other studies on biochemical markers of ischemic stroke and TIA performed in the past [7, 11-16]. In our study, we found that the markers of endothelial dysfunction like hsCRP and NO were significantly higher and level of antioxidant marker like SOD was significantly lower in cases with or without risk factors than in controls. This signifies that patients with ischemic stroke had a higher disease burden, more widespread atherosclerosis and more oxidative stress than in healthy controls and in controls with risk factor.

hsCRP and NO levels were not significantly different between cases with risk factor and cases without risk factors. This signifies that cases without any identifiable risk factor were at an increased risk of ischemic stroke due to oxidative stress. Hence, raised hsCRP and NO account

for this increased risk and are probably independent risk factors for ischemic stroke. Extensive work by Cao et al in 2003 and recently published work of Iso et al, 2012 also provided similar evidence that elevated hsCRP is predictive of stroke independent of carotid intima-media thickening (IMT) and development of risk factors like diabetes and hypertension[17,18]. The activation of neuronal nitric oxide synthase and augmentation of NO levels between 12-24 hours after ischemic stroke was observed by other researchers[19-21]. Our study also shows higher serum levels of NO detected in 40 acute stroke patients as compared to age and risk matched controls was consistent with the literature. However, in our study, cases with risk factors had a significantly lower SOD and a significantly higher disease burden in the form of a higher CTA score. Even after extensive search of literature, we could not find a study on analysis of correlation between biochemical markers of endothelial dysfunction and CTA findings.

CTA scores, which signify anatomical extent and severity of atherosclerosis, correlated significantly and linearly with biochemical markers of oxidative stress in patients of ischemic stroke. Rothwell PM et al, 2000 and Takaya N et al showed that some parameters of carotid artery atherosclerosis such as carotid artery stenosis, type of plaque, and presence of complications are identified factors for the future stroke risk[22-23].

Cases with stroke and risk factors had a higher oxidative stress in the form a significantly higher hsCRP and a lower SOD as compared to controls with risk factors. This suggests that these markers not only have a prognostic value in patients with stroke but may also play an etiological role in atherosclerosis and stroke. This finding corroborates with the fact that CRP is not only a marker of atherosclerosis but is actually involved in pathogenesis of early atherosclerosis as shown in the work by Torzewski M et al[24].

The most studied antioxidant enzyme in stroke is SOD. There are three major endogenous isoforms of superoxide dismutase; cytosolic, mitochondrial and extracellular SOD (EC-SOD). EC-SOD is also expressed in brain. EC-SOD activity was found to be lower in acute stroke patients but increased to the level of controls within 5 days from the cerebrovascular accident, possibly as a consequence of an increased release of free radicals[25]. SOD activity in serum was inversely correlated with the infarct size and the severity of neurological damage, while a reduction of SOD activities in red blood cells was correlated with infarct size, stroke severity, and poor short-term prognosis [25-26].

The lack of data regarding antioxidant levels before stroke onset hinders the possibility of ascertaining whether low antioxidants are a cause or a consequence of stroke. Several epidemiological studies have shown that low levels of ascorbic acid, a-tocopherol, and carotenoids in the diet or in blood are associated with an increased risk of stroke [27]. However, since almost all antioxidant levels increased in the days following the stroke, it seems reasonable to assume that at least in part they declined after the cerebral infarct.

Interestingly, cases without any risk factors had a higher hsCRP and significantly lower SOD than in control population with risk factors, i.e., raised hsCRP and low SOD confer an almost equal if not higher risk of stroke than even diabetes, hypertension and smoking on this population. Meta-analysis of seven prospective studies by Danesh J et al, 1998 also described similar risk predictability of hsCRP for ischemic stroke[28]. However, there are evidences against this risk predictability of hsCRP[29-30].

The importance of our study lies in the fact that specific therapeutic interventions can now be guided towards lowering hsCRP. Angiotensin converting enzyme inhibitor therapy has been associated with lowering of hsCRP in patients with a stroke[31]. Recently published results of HELENA study group showed that as the fitness increased the hsCRP levels reduced, means that the fitness is inversely proportional to the CRP level and risk of stroke[32]. However, whether this is a specific effect due to lowering hsCRP, or whether the two are parallel phenomena remain to be proven. The JUPITER trial on patients with baseline characteristics similar to as in our study has shown that lowering the levels of hsCRP in healthy men and women with no evidence of cardiovascular disease, dyslipidemia and hypertension but hsCRP $\geqslant$2.0 mg/L by rosuvastatin showed a significant reduction in the incidence of major cardiovascular events, including stroke[14]. However, there were several limitations to this study. First, the benefit was modest in absolute terms. Second, it was not tied to baseline levels of hsCRP, although there was some evidence that the greatest benefit was seen in those whose hsCRP was reduced to <2.0 mg/L. Third, it remains uncertain whether the mechanism through which statins appear to work is related to inflammation or to some other effect, with the effect on hsCRP a bystander or epiphenomenon. JUPITER provides only indirect evidence, therefore, that statins are of benefit among those with elevated hsCRP values, as the results may simply reflect a general benefit of statin therapy among all patients, with the greatest magnitude seen among those at higher risk.

Many epidemiological studies have shown that younger people suffer from stroke that have no known risk factors in form of smoking, dyslipidemia, diabetes mellitus, and hypertension. However, above markers can predict the onset of vascular catastrophe, especially in evolving stroke and non-hemorrhagic stroke in which initial NCCT Head findings may not be abnormal. In such cases, using various drugs which improves endothelial dysfunction may retard the evolution of stroke.

5. Conclusion

This study demonstrates that hsCRP, NO and SOD is good surrogate biochemical markers for assessing disease risk and burden in ischemic stroke. These surrogate markers showed a linear correlation and statistical significance with CT angiography score. Specific intervention targeted to reduce the oxidative stress, as indicated by these markers, and imaging findings should be a part of stroke management protocol.

Conflict of interest statement

We declare that we have no conflict of interest.

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