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Original Research Article

PREDICTIVE ROLE OF CELL FREE DNA AND HS-CRP AS A BLOOD BIOMARKER IN ACUTE ISCHEMIC STROKE PATIENTS

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ABSTRACT

Background: Stroke being an important health issue, several blood biomarkers of stroke are being evaluated. High sensitivity C-reactive protein (hsCRP), an indicator of inflammation and cell free- DNA (cf-DNA) released from damaged neurons in stroke patients can be helpful to assess stroke prognosis. We planned to assess the role of. Cell free DNA (cf-DNA) and High sensitivity CRP. Previous studies showed that C-reactive protein (CRP), an inflammatory marker, was associated with stroke severity and long-term outcome. **Material & Methods:** This study comprised of 154 acute ischemic stroke patients. The plasma (cf-DNA level) was estimated with real-time PCR assay for the β-globin gene (Qiagen- Roter-Gene Q MDX, Germany), while hsCRP was measured by immunoturbidimetric method (Roche Cobas C311, Fully automatic). The clinical assessment was done with National Institutes of Health Stroke Scale (NIHSS) at the time of admission. After a period of three months from the onset of stroke, the modified Rankin scale (MRS) scores were estimated. **Results:** Elevated levels of cf-DNA and hsCRP were found in patients with higher NIHSS admission score and MRS 3-month scores ($p<0.05$). Favorable stroke outcome was consistent with cf-DNA level <10000 kilogenome-equivalents/L or hsCRP <6mg/L ($p<0.05$). **Conclusion:** The estimation of Cf-DNA and hsCRP can contribute to the clinical evaluation and optimal management of ischemic stroke patients.

Keywords: Cell-Free DNA, hsCRP, Modified Rankin Scale, Acute Ischemic Stroke, Prognosis.

INTRODUCTION

Inflammation plays a critical role in the pathogenesis and prognosis of ischemic stroke. Acute-phase protein C- reactive protein (CRP), or high-sensitivity c-reactive protein (hsCRP, CRP measured with a high-sensitivity assay), is a nonspecific biomarker of inflammation, which is reported to be positively associated with higher risks of stroke recurrence and functional damage for stroke survivors as well Evidence also showed that stroke recurrence was highly associated with functional disability. Biologically, on the one hand, post-stroke inflammation biomarker hsCRP would cause cell death, brain injury, and blood-brain barrier disruption, which result in functional damage directly **(1).** Due to the modern-day lifestyle changes linked with stroke risk factors, India is witnessing distressing rise in incidence of stroke patients **(2).**

In recent years, an increased level of CRP remarkably associated with the functional prognosis of AIS was observed in multiple studies. Nevertheless, most of the previous studies investigating the prognosis of patients with acute ischemic stroke were mainly focused on new stroke attack and mortality. In addition, Halvor et al. **(3)**

found that CRP and homocysteine were associated with long-term mortality in young ischemic stroke patients. Huang et al. **(4)** revealed that hs-CRP was related to a worse prognosis risk of all-cause death within three months after AIS in Chinese patients. The objective of this study was to evaluate the significance of blood biomarkers like cell free DNA as well as high sensitivity C-reactive protein in assessing severity and long-term prognosis of acute ischemic stroke, for these reasons, there is need for evaluating blood markers of acute cerebral ischemia that are sensitive, specific, feasible and affordable. Thus, the new biomarkers of stroke prognosis are anticipated to aid clinician in assessing severity, prognosis and overall management of acute stroke.

MATERIALS AND METHODS

This study comprised of 154 acute ischemic stroke patients. Inclusion criteria: were as follows, first episode of acute ischemic stroke, age limits 18-90 years, and no history associated incapacitating medical condition. Exclusion criteria: were cases of trauma to the central nervous system, meningitis, encephalitis, systemic infections, hypertensive encephalopathy, tumors, migraine, post-cardiac arrest, drug overdose, organ failure, psychiatric syndromes, and shock. For the participation in this study, a written, informed consent was received from the patient's relative or attendant. It was conducted subsequent to the university ethics committee sanction. The neurologic consequence was determined with modified Rankin scale (MRS), a function assessment scale for the estimation of neurologic deficit **(5)**.

In the hospital emergency room, five mL of venous blood sample was collected in EDTA vial from each patient. The samples were centrifuged for 20 min at 14000g to separate the plasma. The extraction of cell free DNA was done from 1ml plasma sample by circulating nucleic acid isolation protocol (QIAamp, Qiagen). The estimation of this cf-DNA was done with real-time PCR assay for the β-globin gene (Qiagen- Roter- Gene Q MDX, Germany). It is based on amplification of beta-globin gene, The approximate turnaround time for quantifying the cf-DNA was 3-4 hours and the units were kilogenome equivalents/L. Further, hsCRP was measured on C-311 analyzer by immunoturbidimetric method (Roche Cobas C311, Fully automatic) and the results were expressed as mg/L.

Statistical analysis

The measured biochemical parameters of cell free DNA (cf-DNA) and high sensitivity C-reactive protein (hsCRP) was presented as mean ± SD. To compare two data sets, t-test was employed. Mann-Whitney U-test was used for data not-normally distributed. Correlation was assessed by Spearman Rank test and expressed as r-value. Multiple logistic regression was applied to model relationship between blood markers and outcome variable (MRS 3-month score) while adjusting for possible confounders. The SPSS (Version 25.0; IBM,) 2016 was used for statistical analyses of the data at 5% significance level.

RESULTS

The median patient age was 61 years and the mean duration between onset of stroke symptoms and blood collection was within 24 hours. There was no significant association between these blood marker levels and stroke risk factors. (Table 1)

Table 1: Patients Characteristics

Risk factors	Subjects (n= 154) %
Gender- Male	94 (61.03%)
Diabetes mellitus	30 (19.48%)
Hypertension	80 (51.94%)
Ischemic heart disease	15 (9.74%)
Hyperlipidemia	63 (40.90%)
Smokers	40 (25.97%)
Alcoholics	36 (23.37%)

The multiple logistic regression analysis revealed these markers to be independently associated with MRS outcome when compared with other stroke risk factors (R^2=0.224, Adjusted R^2=0.065). A weak positive correlation was found between the biomarker levels with NIHSS admission as well as MRS 3-month score. (Table 2) The receiver operating characteristic (ROC) curve set cf-DNA threshold of 10000 kilogenome equivalents/L at 76% sensitivity and 86% specificity when modelled for MRS scores , while for hsCRP the threshold was 6mg/l at 60% sensitivity and 76% specificity. The patients with cf-DNA level more than 10000 or hsCRP level more than 6 had severe presentation consistent with high NIHSS score or poor clinical outcome with high MRS score at three months

($p<0.05$). (Table 3) Further analysis of twenty-six patients who received treatment like thrombolysis or thrombectomy showed good clinical outcome when the biomarker level was on lower side, cf-DNA level less than 10000 and hsCRP level less than 6 ($p<0.05$).

Table 2: Association between blood biomarker and Clinical outcomes

Blood marker level	**Mean± SD**	**Range**
Cell free DNA [#]	8790±1855.73	729-41170
hsCRP (mg/L)	4.82 ± 3.78	1- 12
Clinical evaluation	Mean± SD	Range
NIHSS score (at admission)	12.9 ± 8.6	0- 30
MRS score (at 3 months)	2.56 ± 1.6	0- 5
Correlation (r)	NIHSS	MRS
Cell free DNA	0.222	0.396*
hsCRP (mg/L)	0.350*	0.328*

kilogenome equivalents/L, * $p < 0.05$

cf-DNA *cell-free DNA, hsCRP *high-sensitivity C-reactive protein

Table 3: Comparison of severity and poor outcome with blood parameters.

Blood Parameter	**NIHSS >15 (n=22)**	**p-value**	**MRS ≥3 (n=28)**	**p-value**
cf-DNA	16078.00 ± 8483.60	0.02	15637.29 ± 8990.64	<0.02
hsCRP	6.75 ± 4.35	0.03	6.30 ± 4.17	0.02

DISCUSSION

To our knowledge, few of the previous studies investigated the relationship between Cf- DNA, hs-CRP levels and the stroke outcome. However, the assessment of prognosis of stroke is often tricky, depending on severity of the event and associated comorbidities. Hence, there is an enormous interest to search markers of stroke severity as well as prognosis. Biomarker of stroke is defined as a physiological or pathological substance measured in blood sample that marks the occurrence of stroke **(6).** These biomarkers are studied to characterize stroke and its outcome. Many of them have shown promising results in assessing stroke pathophysiology.

However, the result interpretation is confounded by multiple issues like diverse etiopathogenesis of stroke slow release, latent rise, effect of blood brain barrier on transport of molecules, clearance by various mechanism, this study is an effort to elucidate the role of cf-DNA as well as hsCRP in early course of acute ischemic stroke, within 24 hours from the onset of the event.

On the other hand, hsCRP is considered as acute phase reactant synthesized by the hepatic tissue in response to ongoing inflammatory process. This protein promotes thrombotic events through activation of monocytes that lead to expression of procoagulant tissue factor. HsCRP directly stimulate vascular endothelial cells to generate adhesion molecules leading to the intrusion of inflammatory cells into the vessel wall **(7, 8).**

On similar lines, we too observed higher cell-free DNA and hsCRP in patients with severe presentation of stroke or poor outcome. Chronic inflammatory processes associated with traditional risk factors of atherosclerosis like aging, hypertension, diabetes, dyslipidemia, smoking play key role in the development of atherosclerotic plaque leading to ischemic stroke. Hence, study of inflammatory markers like hsCRP can help in better understanding of pathogenesis of ischemic stroke. It will be interesting to do serial measurements of hsCRP in stroke patients for better analysis of its impact on disease severity and long-term prognosis. In the literature, we could find few studies on cf-DNA or plasma DNA level in stroke patients. Demonstrated plasma DNA concentration correlating with stroke severity and suggested it to be used for predicting outcome in the emergency room. Formerly, the authors have shown that cf-DNA correlated well with the stroke severity and it can also predict neurological outcomes subsequent to therapeutic intervention in acute ischemic stroke patients **(9, 10).**

In this study we evaluated diagnostic capability of cf-DNA and hsCRP as stroke biomarkers for getting corroborative and prognostic information at the time of admission. This will not only guide clinicians in optimizing management, but also help patient's attendants to take informed decision about further

intervention. It would be ideal to measure these two markers from the initial blood sample collected in the emergency department.

CONCLUSION

This study suggests that cf-DNA and hsCRP can adjunct clinical assessment in acute ischemic stroke. The estimation of these biomarkers may help objectively to predict prognosis and optimal management of stroke patients.

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