



## Clinical Investigation

# Correlative study of serum cystatin C levels with severity of acute ischemic stroke.

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Received: 12<sup>th</sup> october-2013 Accepted: 18<sup>th</sup> November-2013 Published: 30<sup>th</sup>-December 2013

## Abstract

**Background:** The attractiveness of cystatin C as a biomarker for acute ischemic stroke is obvious, in the background of accumulating evidence indicating a link between vascular disease of the kidney and brain. We investigated the association of serum cystatin C levels with first onset acute ischemic stroke and the correlation of serum cystatin C levels with severity of acute ischemic stroke as assessed by NIH stroke scale. **Methods:** A total of 50 patients with first onset of acute ischemic stroke, aged more than 45 years, presenting within 72 hours after onset, by interview, history, clinical examination, neuroimaging and other investigations. Serum Cystatin C was measured by means of a particle-enhanced immunonephelometric assay (N Latex Cystatin C) with a nephelometer (Siemens BN Prospec). **Results:** Serum cystatin C levels were significantly raised in 62% (n=31) patients of acute ischemic stroke compared with controls 14% (n=7), with a  $p < 0.001$ . Compared with controls, the mean serum cystatin C level was significantly higher in stroke patients ( $1.33 \pm 0.71$  versus  $0.83 \pm 0.24$  mg / L,  $p < 0.001$ ). The diagnostic accuracy of serum cystatin C as a marker of acute ischemic stroke evaluated using Receiver Operating Characteristic (ROC) curve analysis, showed a sensitivity of 66.00 and specificity of 84.00 with an area under curve of 0.80 (and 95 % CI [0.71 - 0.87]). **Conclusion:** Serum cystatin C was a better marker for acute ischemic stroke and indicator of severe neurological impairment.

**Key words:** Acute ischemic stroke, Cystatin C, NIH Stroke scale.

## Introduction

Cystatin C is a 122-amino acid, 13-kDa protein that is a member of the family of cysteine proteinase inhibitors<sup>(1)</sup>. It is encoded by the 'housekeeping type' CST3 gene (belonging to the type 2 cystatin gene family), and produced by all nucleated cells at a constant rate<sup>(2)</sup>. It is freely filtered by the glomerulus and is largely reabsorbed and catabolised in the proximal tubules<sup>(3)</sup>. Cystatin C is a better estimate of renal function, particularly within the "normal" range of kidney function<sup>(4)</sup>. The attractiveness of cystatin C as a biomarker for acute ischemic stroke is obvious, in the background of accumulating evidence indicating a link between vascular disease of the kidney and brain. There are a lot of similarities in the vascular

supply to kidney and brain. Both are low resistance end-organs and are exposed to high-volume blood flow throughout the cardiac cycle, explaining their pulsating nature. It therefore seems logical that microvascular disease in the kidney and brain might travel together<sup>(5)</sup>.

This study was undertaken to evaluate the association of serum cystatin C levels with first onset acute ischemic stroke, and to correlate the severity of acute ischemic stroke as assessed by the National Institute of Health Stroke Scale with levels of serum cystatin C. It also aimed at evaluating serum cystatin C as a potential marker for acute ischemic stroke.

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## Materials and Methods

A total number of 50 patients with first onset acute ischemic stroke, aged more than 45 years, presenting within 72 hours after onset. 50 age and sex matched controls were selected from inpatients with minor illnesses from other departments following the same exclusion criteria after obtaining institution ethical clearance. Other types of stroke as well as patients with chronic kidney disease, liver disease, malignancy and hypothyroidism are excluded from the present study. The study was conducted from January 2011 to March 2012. Serum Cystatin C was measured by means of a particle-enhanced immunonephelometric assay (N Latex Cystatin C) with a nephelometer (Siemens BN Prospec). The severity of acute ischemic stroke was assessed by the National Institute of Health (NIH) stroke scale. Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean $\pm$ SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5% level of significance. Student "t" test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Chi-square/Fisher exact test has been used to find the homogeneity of samples on categorical scale.

## Results

The maximum number of patients in this study were in the age group between 51-60 years (42%) followed by 61-70 years (28%). The mean age was  $62.88 \pm 9.67$ . There was a male preponderance in patients with stroke in the present study with a male to female ratio of 2.33: 1. With regard to risk factors, 10% were diabetic, 18% were hypersensitive, 30% had both diabetes and hypertension, 38% were current smokers, 30% consumed alcohol and 12% had ischemic heart disease. There were three cases of cardio-embolic stroke in the present study (6%), who had rheumatic heart disease with mitral stenosis among two had atrial fibrillation. The majority of cases i.e.60% (n=30) had severe neurological impairment with NIHSS scores of 15-24, followed by 38% (n=19) of cases who had moderately severe neurological impairment with NIHSS scores of 5-14. There were no cases in the very severe neurological impairment group with NIHSS scores of >25. The mean duration of hospital stay of the cases that were admitted was  $6.24 \pm 4.02$ , among which 60% (n=30) were discharged in 3-7 days and 22% (n=11) in 7-14 days. The total number of deaths in the series was 6, with a case fatality rate of 12%. Among the survivors 78% (n=39) were discharged home and the

remaining 10% (n=5) went against medical advice. There was a slight preponderance of left sided stroke, which was present in 50% (n=25) of cases, in comparison with right sided stroke in 38% (n=19).The remaining 12% of cases were posterior circulation stroke (n=4) and multi infarct state (n=2).

Serum cystatin C levels were significantly raised in 62% (n=31) patients of acute ischemic stroke compared with controls 14% (n=7), in the present study with a  $p < 0.001$ . Compared with controls, the mean serum cystatin C level was significantly higher in stroke patients ( $1.33 \pm 0.71$  versus  $0.83 \pm 0.24$  mg/L,  $p < 0.001$ ). NIHSS scores were correlated with the levels of serum cystatin C, higher scores indicating more severe neurological impairment were associated with higher mean levels of serum cystatin C with a  $p < 0.001$ . The mean serum cystatin C levels (mg/L) were 1.68 in the severe neurological impairment group (NIHSS - 15 - 24), versus 0.83 in the moderately severe neurological impairment group (NIHSS - 5 - 14). Serum cystatin C levels were raised in only 9.7% (n=3) of cases with moderately severe neurological impairment (NIHSS scores: 5 - 14), whereas in cases with severe neurological impairment (NIHSS scores: 15 - 24) it was raised in 90.3% (n = 28). This indicates that serum cystatin C is a better indicator of more severe neurological impairment.

A comparison of NIHSS score and serum cystatin C levels according to outcomes showed a positive correlation with a  $p < 0.001$ . The mean serum cystatin C levels and the NIHSS score were  $1.75 \pm 0.81$  and  $21.00 \pm 2.53$  respectively, in cases which expired, versus the mean serum cystatin C of total cases  $1.33 \pm 0.71$ . There were no individual clinical variables in the present study, which had a significant correlation with serum cystatin C levels. The only laboratory parameters which had a significant correlation with serum cystatin C levels were serum creatinine ( $p < 0.001$ ), serum triglycerides  $p = 0.093+$ , and urine albumin ( $p = 0.095+$ ). Serum creatinine values were elevated ( $> 1.40$  mg/dl) in 38% (n=19) cases and were significantly associated with higher mean serum cystatin C levels of  $1.79 \pm 0.9$  ( $p < 0.001$ ). In comparison, cases with normal serum creatinine values had mean serum cystatin C levels of  $1.04 \pm 0.34$ . With regard to lipid subfractions, higher serum triglyceride levels ( $> 150$  mg/dl) correlated with serum cystatin C with a  $p = 0.093+$ . The levels of serum cystatin C were raised in 61.3% (n = 19) in cases with elevated triglycerides ( $> 150$  mg/dl) which were present in 54% (n = 27). Total cholesterol was elevated ( $> 200$  mg/dl) in 32% (n=16), LDL was elevated ( $> 130$  mg/dl) in 36% (n=18) and low HDL ( $< 40$  mg/dl) in 48% (n=24) of cases.



The diagnostic accuracy of serum cystatin C as a marker of acute ischemic stroke evaluated using Receiver Operating Characteristic (ROC) curve analysis, showed a sensitivity of 66.00 and specificity of 84.00 with an area under curve of 0.80 (and 95% CI [0.71 - 0.87]). This indicates that it is a good marker of acute ischemic stroke.

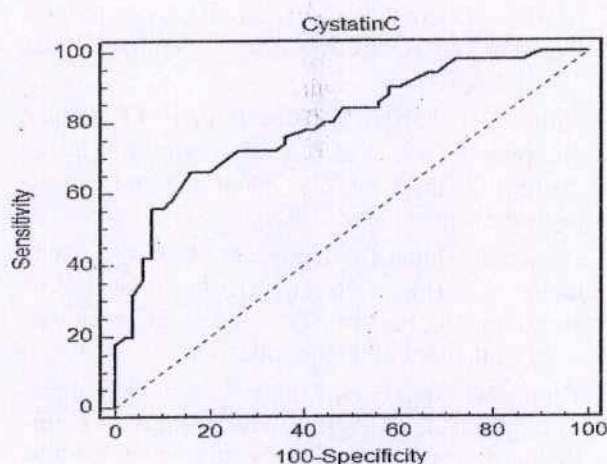


Fig: 1- ROC curve analysis to define Cystatin C as marker for Stroke

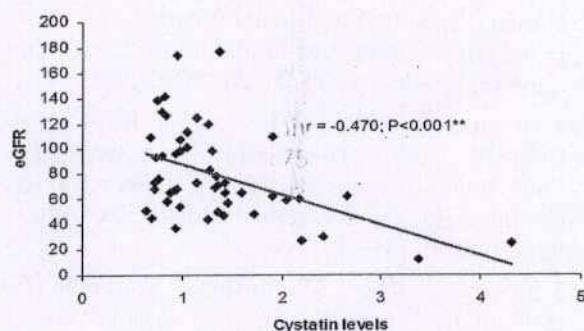


Fig: 2- Mean Cystatin C levels according to marker for Stroke

The presence of albuminuria was significantly associated with raised serum cystatin C levels with a  $p=0.095+$ . The eGFR was calculated for all cases using the Modified Disease in Renal Diet equation with four variables: serum creatinine, age, race and gender and correlated with NIHSS scores. The mean eGFR was 87.38 ml/min in cases with moderately severe neurological impairment (NIHSS scores: 5 - 14) versus an eGFR of 75.12ml/min in cases with severe neurological impairment (NIHSS scores: 15 - 24). The correlation between serum cystatin C levels and eGFR was statistically significant with  $p=0.001^{**}$  ( $r=-0.470$ ).

## Discussion

The association between impaired renal function and stroke has previously been noted in several studies and are based on evidence indicating a link between vascular disease of the kidney and brain. In a study by Freidman et al, involving 492 stroke survivors in New Zealand followed up for a mean of 18 months serum creatinine concentration independently predicted mortality even after adjustment for confounders<sup>(6)</sup>. A 7 year follow up study by Mac Walter et al in a large cohort of acute stroke patients assessed renal function as a long-term predictor of mortality, using several markers of renal function obtained on admission to hospital. The results of the study demonstrated that admission renal function as assessed by calculated creatinine clearance, serum concentration of creatinine, urea, and ratio of urea to creatinine all predicted mortality even when values were within conventional normal reference intervals<sup>(7)</sup>.

In support of the idea that one organ may tell us about the other, Ikram and colleagues described cross-sectional associations of renal function and MRI-defined findings of brain vascular disease in a population-based cohort of 484 older adults with a mean age of 73 years from the Rotterdam Scan Study.<sup>79</sup> Lower renal function was correlated with a smaller volume of normal white matter, especially deep rather than lobar, and a larger volume of white matter lesions<sup>(8)</sup>. In individuals with mild to moderate chronic kidney disease not requiring dialysis, 30% to 60% excess risk of clinically overt stroke has been reported, even after adjustment for common stroke risk factors<sup>(9-11)</sup>. Patients with end-stage renal disease, defined by an eGFR <15 mL/min per 1.73 m<sup>2</sup> or kidney replacement therapy, are at 3 to 9 times greater risk for stroke compared with the general population<sup>(12)</sup>. Endothelial dysfunction of the conduit arteries, in part as a result of this reduced nitric oxide availability is also common in kidney disease<sup>(13)</sup> and might plausibly contribute to small-vessel disease of the brain<sup>(14-16)</sup>.

The limitations of these studies were the inaccuracy of serum creatinine as a marker of renal impairment. Serum cystatin C is a more precise test of kidney function, a finding in several cross sectional studies<sup>(17,18)</sup>. It has been suggested that cystatin C might predict the risk of developing chronic kidney disease, thereby signaling a state of 'preclinical' kidney dysfunction<sup>19</sup>. In the Cardiovascular Health Study of Community dwelling adults age 65 years and older, prevalence of MRI-defined infarcts was higher in those with impaired kidney function compared with those without, as measured by cystatin C but not as measured by creatinine<sup>20</sup>. In the study by Li Ni et al in



plasma cystatin C level was associated with an increased risk for clinical stroke, other cardiovascular events and death from all causes<sup>21</sup>.

Cystatin C levels may reflect the duration and severity of other established risk factors like hypertension<sup>22</sup> and plays an important role in the pathogenesis of atherosclerosis. High levels of cystatin C may directly affect the process of vascular wall remodeling by regulating the balance of proteolytic and antiproteolytic activities<sup>23,24</sup>. However the limitation of the present study of less sample size and it was a unicentric study. In summary our findings indicated that serum cystatin C was a good marker of acute ischemic stroke and a better indicator of more severe neurological impairment as assessed by the NIH stroke scale. Further studies may provide greater understanding about the different expressions of vascular disease of the kidney and brain which act as biomarker.

We would like to acknowledge Dr K P Suresh, Scientist (Biostatistics), National Institute of Animal Nutrition & Physiology, Bangalore for statistical analysis and Anand labs for analysis.

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