Original Article

Cystatin C serum level in acute ischemic stroke

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ABSTRACT

Background: Cerebrovascular ischemic stroke is one of the key neurological diseases. Its myriad of risk factors and complexity of pathogenesis keep the door open for new biomarkers to be studied. Cystatin C is a sensitive biomarker of preclinical renal dysfunction. There is a correlation between the vascular disease of the kidney and brain, so cystatin C is considered as an important biomarker for acute ischemic stroke. Aim: We assessed the correlation of cystatin C serum levels with vascular risk factors, clinical severity, and short term outcome of first-ever acute ischemic stroke. Patients and methods: In this prospective cohort study, we included 174 adult patients with first-ever acute cerebrovascular ischemic stroke of not more than 48 hours duration with normal kidney functions (78 males and 96 females with age ranged from 33 to 90 years). Serum cystatin C was determined by enzyme-linked immunosorbent assay. All patients were followed up for one week to detect a short-term outcome. Results: Cystatin C serum level had a statistically significant positive relationship with some vascular risk factors such as diabetes mellitus, dyslipidemia, and atrial fibrillation. Cystatin C serum level tended to be positively associated with the national institutes of health stroke scale (NIHSS) at admission. Higher scores of NIHSS indicating more severe neurological impairment were correlated with higher mean levels of serum cystatin C with a $p < 0.01$. There was highly statistically significant correlation between cystatin C serum level and outcome of the investigated patients. It was highest in the patient group with a poor outcome ($p < 0.001$). Conclusion: Serum cystatin C is an indicator of severe neurological impairment and prognostic biomarker for poor outcome.

Keywords: Cystatin C, Ischemic stroke, Severity, Outcome

Introduction

Based on the report by World Health Organization (WHO), for many years, the main cause of morbidity and mortality in the world (accounting for 55% of the total population) has been cardiovascular diseases which have been ischemic heart disease, stroke, and peripheral artery disease.[1, 2] It is a rapidly developing clinical manifestation of focal or generalized brain dysfunction, with no obvious source other than that of vascular source.[3] Stroke is caused by two types of vascular disorders in the brain including ischemia or bleeding, and ischemic stroke is more prevalent.[4] Stroke survivors may experience numerous neurological impairments.[5] The brain is a rich source of different biomarkers and any injury like stroke to the brain could cause an increase in levels of these biomarkers in cerebrospinal fluid and serum. Evaluation of those biomarker levels is an accessible technique for the assessment of severity, course, outcome, and to some extent the differential diagnosis of different types of cerebrovascular disorders.[6]

Cystatin C is one of the cysteine proteinase inhibitors. It is filtered by the glomerulus and is largely reabsorbed and metabolized in the renal proximal tubules.[7] Cystatin C is a key biomarker of preclinical renal functional state.[8] There is a link between vascular diseases of the kidney and brain because of similarities in the vascular supply to both of them; so cystatin C is a key biomarker for acute ischemic stroke.[9]

This investigation was undertaken to assess the correlation of serum cystatin C levels with first-ever acute ischemic stroke and

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to relate it with vascular risk factors, severity, and short-term outcome.

Patients and Methods

This investigation was conducted in the intensive care and stroke units, Neurology Department, Zagazig University Hospitals, during August 2017 and March 2019. In this prospective cohort study, we included 174 adult patients with first-ever acute cerebrovascular ischemic stroke who were diagnosed based on the World Health Organization (WHO) criteria,[10] confirmed by brain computed tomography (CT) and/or magnetic resonance image (MRI), of not more than 48 hours duration, with normal kidney functions.

Exclusion criteria:

Those who suffered from hemorrhagic stroke (intracerebral or subarachnoid hemorrhage), any central nervous system complication other than acute cerebral arterial infarction, patients with a history of head injury, patients under thrombolytic therapy, patients with chronic kidney illness, and patients with liver diseases were excluded from the investigation.

Patients were subjected to the complete history taking, as well as full general and neurological assessment with an evaluation of stroke severity utilizing national institutes of health stroke scale (NIHSS) on admission. The stroke severity of the investigated patients was categorized into:[11]

Mild stroke severity if NIHSS less than 6.

Moderate stroke severity if NIHSS 6-15.

Moderate to severe stroke if NIHSS 16-20.

Severe stroke if NIHSS 21-42.

Evaluation of baseline vascular risk factors:

The determination of major vascular risk factors was based on history and laboratory findings. Hypertension was supposed to be present if subjects had been previously diagnosed (according to guidelines set by the World Health Organization and International Society of Hypertension) whether they were receiving antihypertensive treatment or a three measurements of systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg.[12]

Diabetes mellitus was recorded in patients under anti-diabetic therapy or two fasting venous plasma glucose measurements ≥ 126 mg/dl.[12]

Dyslipidemia was considered as those having the previous diagnosis of dyslipidemia, the utilization of lipid-lowering drugs or abnormal fasting lipid profile as follows: Total cholesterol level > 200 mg/dl, Low density lipoprotein (LDL) ≥ 100 mg/dl, High density lipoprotein (HDL) <40 mg/dl or Triglycerides level > 150 mg/dl.[13]

History of smoking was noted if the patient smoked during the 3 months before the stroke onset.[12]

The patients were supposed obese if the patients' body mass index ≥ 30 kg/m2. It was determined by dividing weight (Kg) by height (m^2).[14]

History of atrial fibrillation (AF) was considered as the history of AF or 24 hour Holter electrocardiogram showed AF.[12]

Laboratory investigations:

Both routine and special laboratory investigations were performed at the Clinical Pathology Department, Zagazig University Hospitals.

- Routine laboratory tests: Complete blood count, random blood sugar (RBS), liver and kidney function tests, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), lipid profile, and glomerular filtration rate (GFR).
- Special laboratory tests: Determination of cystatin C serum level by three ml of venous blood which were drawn from all ischemic stroke patients using standard venipuncture methods, within 48 hours of stroke onset. Blood samples were kept at room temperature for 4 hours to clot, then centrifuged to obtain the serum which was deposited frozen. The levels of cystatin C were assessed utilizing the double-antibody sandwich enzyme-linked immune-sorbent assay technology (ELISA).
- Radiological investigations: All patients were subjected to a brain CT scan and/or magnetic resonance image (MRI) to verify the diagnosis of acute stroke.

Ethical consideration: A written informed consent was obtained from each patient or his/her relative to be included in the investigation. This research was verified by the institutional research board (IRB) of the Faculty of Medicine, Zagazig University.

Follow up after one week:

The outcome was assessed after one week according to NIHSS as follows:[15]

a) Improvement in NIHSS.
b) No change.
c) Deterioration: decrease in NIHSS by 2 points or more.
d) Death.

Statistical analysis:

The data were analyzed utilizing the statistical package SPSS. The data presented as mean and SD or median and IQ range for quantitative data, and number and percentage for qualitative data. Student's t-test was used to assess statistical differences between the two groups of quantitative data. Nonparametric Mann–Whitney (MW) and Kruskal–Wallis (KW) tests were used for quantitative variables, which were not normally distributed. Pearson's correlation was used to study the relationship between quantitative data. P-values less than or equal to 0.05 were supposed statistically significant.

Results

In this prospective cohort study, we included 174 adult patients with first-ever acute cerebrovascular ischemic stroke of not more than 48 hours duration with normal kidney functions (78 males and 96 females with age ranged from 33 to 90 years). The mean age was 61.2 ± 14.5 years. There was a statistically

significant positive correlation between cystatin C and some vascular risk factors including diabetes mellitus, dyslipidemia and atrial fibrillation (Table 1).

Table 1: The relationship between cystatin C serum level and vascular risk factors of the studied patients:

Variables	Cystatin C (x103 ng/ml)		MW	P
	Median	IQ-Range		
Sex:				
Males (n=78)	4.0	2.5 – 17.4	275.0	0.2
Females (n=96)	4.4	3.6 – 8.9		
Obesity:				
Yes (n=60)	4.3	3.5 – 8.9	337.0	0.6
No (n=114)	4.6	3.7 – 29.3		
Hypertension:				
Yes (n=120)	4.2	2.5 – 9.7	337.0	0.6
No (n=54)	4.5	3.6 – 18.5		
Diabetes mellitus:				
Yes (n=99)	4.6	3.6 – 10.9	300.0	0.04
No (n=75)	4.2	2.5 – 6.0		
Dyslipidemia:				
Yes (n=60)	4.6	3.6 – 10.9	300.0	0.04
No (n=114)	4.2	2.5 – 6.0		
Atrial fibrillation:				
Yes (n=30)	7.0	3.5 – 28.9	229.0	0.03
No (n=144)	4.3	3.4 – 6.9		
Smoking:				
Yes (n=30)	3.8	2.9 – 29.3	197.0	0.3
No (n=144)	4.4	3.5 – 8.9		

There is a statistically significant positive relationship between cystatin C serum level and total leukocytic count (TLC), random blood sugar (RBS), C reactive protein (CRP), total cholesterol and triglycerides (Table 2).

The levels of serum cystatin C were positively associated with NIHSS scores. The higher levels of serum cystatin C were correlated with higher scores of NIHSS revealing more severe neurological impairment with a $p < 0.01$. The median serum cystatin C level (ng/ml) was 13.6 with an IQ range of 11.6-22.2 in the severe neurological impairment group (NIHSS: 21-42), versus the 9.3 and IQ range 6.1-12.4 in moderately severe neurological impairment group (NIHSS: 16-20) (Table 3).

There was a highly statistically significant correlation between cystatin C serum levels and the outcome of the investigated patients. It was the highest in the patient group who died (Table 4).

Table 2: The relationship between cystatin C serum level and continuous variables of the studied patients:

Spearman's correlation	r	P
Age	0.04	0.8
SBP	0.1	0.2
DBP	0.2	0.2
TLC	0.3	0.03
RBS	0.4	0.01
CRP	0.3	0.02
ESR	0.06	0.7
Total cholesterol	0.4	0.01
Triglycerides	0.5	0.01
LDL	0.1	0.2
HDL	0.2	0.2
NIHSS (at admission)	0.6	≤0.001
NIHSS (after 7 days)	0.3	0.04
GFR	-0.4	<0.01

CRP: C reactive protein, DBP: diastolic blood pressure, ESR: erythrocyte sedimentation rate, GFR: glomerular filtration rate, HDL: high-density lipoprotein, LDL: low-density lipoprotein, RBS: Random blood Sugar, SBP: systolic blood pressure, TLC: total leukocyte count.

Table 3: The relationship between cystatin C serum level and NIHSS at admission:

NIHSS	Cystatin C (x103 ng/ml)		KW	P
	Median	IQ-Range		
Moderate(6-15) (n=102)	6.4	5.2-7.5	182	<0.01
Moderate to severe (16-20) (n=45)	9.3	6.1-12.4		
Severe(21-42) (n=27)	13.6	11.6-22.2		

n: number of patients.

Table 4: Association between cystatin C serum level and outcome of the studied patients:

Outcome	Cystatin C (x103 ng/ml)		KW	P
	Median	IQ-Range		
Improved (n=84)	4.2	2.5 – 6.0	157.3	<0.001
No change (n=51)	4.6	3.6 – 10.9		
Deterioration(n=12)	6.9	5.9 – 29.5		
Death (n=27)	8.8	7.9 – 31.4		

Discussion

There is an idea that one organ may give us an idea about the other. Many previous investigations have been noted the correlation between impaired renal function and stroke and this is according to the evidence that there is a correlation between vascular diseases of both of them.[15] High levels of cystatin C may directly influence the remodeling of the vascular wall by adjusting the equilibrium between proteolytic and antiproteolytic activities.[15]

There is a relationship between cystatin C and some vascular risk factors leading to an ischemic stroke. Because cystatin C levels may reflect the duration and severity of identified risk factors and have a notable role in atherosclerosis pathogenesis and progression. Serum level of cystatin C may indicate atherosclerosis status, but could not expect asymptomatic atherosclerosis.[16]

In our investigation, there was no statistically significant correlation between cystatin C and age or sex. This is in line with Dharnidharka et al.,[17] Mathews and Levy [18] and Zhu et al. [19], so cystatin C is a better indicator of renal functional state than creatinine as serum creatinine is affected by age, sex, and muscle bulk.[18]

In our study, there was no association between serum level of cystatin C and a history of hypertension. This is in line with Xiao et al.,[20] Huang et al.,[16] and Umemura et al.[21] On the contrary, Hoke et al. [22] and Kim et al. [15] reported that the patients from the group with the highest levels of cystatin C were significantly more hypertensive. This difference may be because of the different cohort characteristics from Kim et al.,[15] as they included only elderly patients, or that serum level of cystatin C may fluctuate in different stages of stroke.

Diabetes mellitus was significantly related to ischemic stroke and resulted in poor short-term outcomes.[16] A possible explanation is that the post-ischemic inflammatory responses have been revealed to be significantly higher in diabetic patients than in non-diabetic patients.[23] Acute hyperglycemia results in enhanced brain lactate production (intracellular acidosis), the development of brain edema, breakdown of the blood in brain barrier (endothelial damage), enhanced risk of hemorrhagic transformation, and enlarged infarct size, which may contribute to the risk of poor outcome.[24]

In our investigation, we noticed a statistically significant relationship between cystatin C serum level and diabetes mellitus. Besides, a statistically significant positive relationship was noticed between cystatin C serum level and random blood sugar (RBS). In line with these observations, Danthala and Lakshmaiah [25] and Kim et al. [15] showed that higher quartiles of cystatin C are associated with the increase in RBS. This results mismatch with Huang et al. [16] and Umemura et al. [21] who noted that there is no statistically significant relationship between cystatin C serum level and random blood sugar (RBS). This difference may be because of different research designs as Umemura et al. [21] that included only non-cardioembolic ischemic stroke.

Abnormal lipid profile has been previously determined in acute ischemic stroke. LDL plays a critical role in emerging of inflammation and formation of plaques in the blood vessel wall which reduces blood flow in arteries.[16]

Regarding dyslipidemia, we noticed a statistically significant correlation between cystatin C serum level and history of dyslipidemia. Besides, a statistically significant positive relationship was noticed between cystatin C serum level and total cholesterol (TC) and triglycerides (TG). In accordance with these observations, Danthala and Lakshmaiah [25] and Kim et al. [15] showed that higher quartiles of cystatin C ware associated with an increase in triglycerides serum level.

Moreover, Zhu et al. [19] found a linear association between GFR according to cystatin C (eGFRCysC) and neurological deterioration in patients with LDL >4.14 mmol/l, namely, the risk of poor functional outcome at 1 year increased with a reduction of eGFRCysC at baseline in ischemic stroke patients with LDL >4.14 mmol/l. In contrast, Xiao et al.,[20] Huang et al.,[16] Umemura et al. [21] and Ercan et al. [26] reported no association between cystatin C and TG, TC, HDL, and LDL serum levels. Kim et al. [15] reported that HDL levels were significantly lower in patients with higher levels of cystatin C compared with the lowest levels of the cystatin C group.

Our findings revealed that there was a statistically significant positive relationship between cystatin C serum level and total leukocytic count (TLC). In accordance with these findings, Danthala and Lakshmaiah [25] and Kim et al. [25] noted the same results.

Elevated TLC in the acute stroke was a significant indicator of initial stroke severity, more disability and neurological deterioration [27] but it did not have any prognostic value on mortality in ischemic stroke patients.[28]

There was a statistically significant positive relationship between cystatin C serum level and CRP. This is in line with Shlipak et al.,[29] Ichihara et al [30] and Umemura et al. [21] as C reactive protein is an inflammatory biomarker known to be related to the atherosclerosis pathogenesis and ischemic stroke development.[12] On the contrary, Ercan et al. [26] reported no association between cystatin C serum level and CRP.

There was a statistically significant negative correlation between cystatin C serum level and glomerular filtration rate (GFR). This finding is in line with those of Kim et al. [15] and Ercan et al.[26]

In our investigation, there was no association between cystatin C serum level and smoking. This is in accordance with Dong and Neo.[12] This is opposite to Kim et al. [15] and Umemura et al. [21] who noted that the patients from the group with the highest levels of cystatin C were significantly more prone to have a history of smoking. This difference may be because of the female predominance of our patients who are fewer smokers than males.

There was a highly statistically significant positive correlation between cystatin C and stroke severity as assessed by NIHSS in the studied patients at admission. This is in accordance with Siegler et al. [31] as well as Danthala and Lakshmaiah [25] who reported that NIHSS scores were correlated with the levels of serum cystatin C. The higher scores revealing more severe neurological impairment was correlated with higher mean levels of serum cystatin C. Under ischemic stimuli, high levels of cystatin C could be released from neuronal cells, including neurons and astrocytes.[32]

Considering the relationship between cystatin C serum level and short-term outcome of the investigated patients, we noticed a highly statistically significant correlation between them. It was the highest in the patient group who died. In line with this observation, previous investigations [29, 33, 34] stated that

follow-up of the patients revealed that high cystatin C levels were correlated with a high prevalence of cardiovascular complications or death from all causes. Prospective cohort research [35] reported that higher serum cystatin C concentrations were correlated with higher vascular and nonvascular mortality rates throughout the range assessed, and these relationships were independent of age, prior disease, and known vascular risk factors. Yang et al. [36] noted that elevated serum cystatin C level is a self-sufficient risk predictor of acute ischemic stroke. In view of our findings, we can conclude that there is a significant positive correlation between serum cystatin C level and vascular risk factors, severity and poor short-term outcome of acute ischemic stroke.

We suggest that specialists should consider adding the application of cystatin C serum level to their routine admission testing in acute ischemic stroke patients since it is significantly related to the vascular risk factors, severity, and short-term outcome of acute ischemic stroke. We also suggest further investigations with larger sample size and longer duration of follow up, with determination of cystatin C during follow up period to evaluate the fluctuation and variability in its value with time in stroke patients.

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Conflicts of interest:

There are no conflicts of interest.

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