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ORIGINAL ARTICLE

Correlations of serum cystatin C level and gene polymorphism with vascular cognitive impairment after acute cerebral infarction

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Abstract

Background The aim of this study was to explore the possible correlations of serum cystatin C level and cystatin C gene (CST3) polymorphism with vascular cognitive impairment in patients who had acute cerebral infarction.

Methods A total of 152 patients with acute cerebral infarction were recruited in this case-control study. Patients were divided into vascular cognitive impairment (VCI) group ($n = 71$) and cognitive impairment no dementia (CIND) group ($n = 81$). The serum concentrations of cystatin C were measured with immunoturbidimetric assay while the gene polymorphisms of CST3 were determined by technique polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results In the VCI group, serum cystatin C level was significantly higher than that in the control group. The frequency of the B allele was found to be higher in the VCI group as compared with that of the CIND group (18.5% vs 7.7%, $p = 0.006$). In logistic regression analysis, significant associations of VCI with high serum cystatin C level (OR 3.837 (1.176–12.520), $p = 0.026$) and CST3 B allele (OR 2.038 (1.048–3.963), $p = 0.036$) were also found.

Conclusions A high cystatin C level and CST3 B allele confer risks for VCI after acute cerebral infarction. It is probable that measurement of the serum cystatin C level and detection of CST3 gene polymorphism would aid in the early diagnosis of VCI, but further studies are warranted.

Keywords Vascular cognitive impairment · Cystatin C · CST3 gene

Introduction

Vascular cognitive impairment (VCI) is a crucial and common complication of acute cerebral infarction. Lack of effective predictors may lead to diagnosis delay and poor prognosis of VCI. Cystatin C, an inhibitor of endogenous cysteine cathepsins, has received considerable attention in recent years. In our previous studies, cystatin C was statistically found to be an independent risk factor for acute cerebral infarction [1, 2]. Cystatin C has been thought to play roles in neuronal injuries and dysfunctions and possibly relate to the clinical manifestations of cognitive impairment [3]. There has been ample basic research [4, 5], clinical studies [6, 7], and genetic studies [8–10] demonstrating that cystatin C inhibits the aggregation of cerebral amyloid protein (Aβ) and potentially prevents cognitive impairment. In light of some fundamental and clinical studies [11–14], a high cystatin C level has been shown to be linked to cognitive impairment. So far, an increasing amount of clinical data has only focused on the relationship between cystatin C and Alzheimer's disease (AD). However, there was a paucity of evidence on the correlation between cystatin C and cognitive impairment after cerebral infarction, and whether cystatin C could be a possible predictive biomarker for VCI remained unclear.

Cystatin C is a protein coded by CST3 gene. It is generally accepted that CST3 belongs to "housekeeping genes" that are constantly transcribed and expressed in most tissues. Nevertheless, emerging research has noted that the expression

Highlights
- There were significant associations of VCI with high serum cystatin C level and CST3 B allele.
- High cystatin C level and CST3 B allele may confer risks for VCI after acute cerebral infarction.
- It is a probability that the measurement of serum cystatin C level and detection of CST3 gene polymorphism would aid in the early diagnosis of VCI, but further studies are warranted.

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of cystatin C is not stereotypical but can be influenced by the CST3 polymorphisms. A study [15] reported that the secretory amount of cystatin C from dermal fibroblasts was significantly small among individuals with CST3 BB homozygote, and this was believed to contribute to the occurrence of AD. Despite that, the linkage between serum cystatin C level and CST3 gene polymorphism is still poorly understood. VCI is a severe acute complication commonly seen in patients with acute cerebral infarction, yet not all cases of cerebral infarction lead to VCI. Differences in VCI severity, onset, and course, whether VCI correlates with genetic predisposition and whether CST3 gene polymorphism modulates the development of VCI or not, were issues that have been rarely addressed in previously published literature. Our study, thereby based upon molecular epidemiology, sought to investigate the correlations between the serum cystatin C level, CST3 polymorphism, and VCI in patients suffering from acute cerebral infarction.

Material and methods

Studied population

This was a case-control study. From January 2017 to April 2018, totally 152 patients who were hospitalized to the Neurology Department of The First Affiliated Hospital of Shantou University Medical College and diagnosed with acute cerebral infarction within 3 days after onset, were consecutively enrolled in our study. Acute cerebral infarct lesions were revealed by head computed tomography (CT) or magnetic resonance imaging (MRI). Patients were divided into the VCI group (case group) and cognitive impairment no dementia (CIND) group (control group). (1) In the VCI group ($n = 71$), which consisted of 45 males and 26 females, individuals must meet the DSM-V diagnostic criteria for dementia and satisfy the following criteria: the Montreal Cognitive Assessment (MoCA) score < 26 points (add 1 point if less than 12 years of education), the Hachinski Ischemic Score (HIS) ≥ 7 points, and the Hamilton Depression (HAMD) Scale < 7 points. Scores derived from these scales were determined by at least two neurologists [16–19]. (2) In the CIND group ($n = 81$), there were 61 males and 20 females. Inclusion criteria for this group included no cognitive impairment and MoCA score ≥ 26 points (one more point for those receiving education for less than 12 years). There were no significant differences in baseline characteristics of these two groups such as severity of cerebral infarction, therapeutic time, gender, and age ($p > 0.05$), rendering patients in these two groups comparable. Patients were excluded if they fulfilled any of the following criteria: (1) patients who were not cooperative in clinical examinations because of hearing, visual, or speaking difficulties, (2) patients who had cognitive impairment caused by other cerebral or systemic diseases instead of acute cerebral infarction, and (3) patients who had severe and complicated cardiovascular, hepatic, and/or renal diseases. The survey was fully approved by the ethics committee of The First Affiliated Hospital of Shantou University Medical College. All participants had signed the informed consent. All procedures conformed to the tenets the Declaration of Helsinki.

Laboratory investigations

Serum cystatin C concentration was determined by a high-sensitivity latex-enhanced immunoturbidimetric method with an automatic biochemical analyzer (COULTER LX20; BECKMAN, USA). The reference ranges of serum cystatin C levels were 0.63 mg/l to 1.10 mg/l. Blood urea nitrogen (BUN), serum creatinine (Cr), uric acid (UA), homocysteine (HCY), glycosylated hemoglobin (HbA1c), and serum lipids including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assessed by an automatic biochemical analyzer (COULTER LX20; BECKMAN, USA).

Genotyping

Anticoagulated peripheral venous blood (2 mL) was collected from each patient under fasting condition. Genomic DNA was then extracted from blood sample as template DNA by using a DNA Extraction Kit (Shanghai Sangon Biotechnology Co Ltd., Shanghai, China). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was carried out to determine the CST3 genotype. The CST3 gene was amplified by using PCR primer sequences, CST3 sense 5′-TGGG AGGGACGAGGCGTTCC-3′ and CST3 antisense 5’-TGCTGGCTTTGTTGTACTCG-3′. PCR-amplified fragments were 389 bp in length, which subsequently underwent digestion by restriction enzyme Sac II. The agarose gel electrophoresis was then utilized to separate these restricted DNA products into multiple DNA bands that were visualized by an ultraviolet transilluminator (GelDoc2000), and ultimately, genotypes could be determined. Three fragments with 41, 226, and 51 bp in size were observed in haplotype A, while two segments with 127 and 191 bp in haplotype B. And lastly, bidirectional DNA sequencing with an automated analyzer (Beijing Meiyimei Biological Engineering Co Ltd., Beijing, China) was applied to validate the accuracy of the genotype.

Statistical analysis

Statistical analyses were computed using SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA). Measurement data were presented as $\chi \pm s$. A two-independent-sample t test or analysis of variance (ANOVA) was adopted to analyze the measurement date among the groups. Enumeration data between groups

were analyzed by a χ^2 test. Pearson correlation analysis was applied to evaluate the association between the serum cystatin C and the MoCA score. To assess the correlation of the serum cystatin C level and CST3 genotype with VCI, univariate logistic analysis was applied to calculate odds ratio (OR) and 95% confidence interval (CI).

Results

Comparisons of clinical characteristics between the VCI and CIND groups

Laboratory findings and clinical features of both groups were compared, as shown in Table 1. Patients in the VCI group had a lower MoCA score as compared to the CIND group ($p < 0.001$). VCI patients had a considerably higher serum cystatin C level than CIND patients (1.07 ± 0.28 vs 0.97 ± 0.27), and there was a significant difference for comparison ($p = 0.024$). Meanwhile, correlation analysis indicated that the serum cystatin C level was negatively correlated with the MoCA score ($r = -0.174$, $p = 0.032$). No significant differences in gender, age, clinical features, and other laboratory findings between these two groups were observed.

Phenotyping and analyses

The distributions of genotypes and allele frequencies of CST3 polymorphism in both groups were compatible with the Hardy-Weinberg equilibrium ($p > 0.05$). The results of RFPL genotyping were congruous with DNA sequencing analyses by GenBank. CST3 genotypes were listed as follows: CST3 AA (nucleotides C, T, and C at positions +73, −72, and −172), CST3 BB (nucleotides T, G, and G at these positions), and CST3 AB (nucleotides T/C, G, and G/T at these positions). There were significant differences in these three CST3 genotypes between the VCI group and CIND group ($\chi^2 = 6.075$, $p = 0.048$). We further found that significant differences in CST3 A allele frequency and B allele frequency existed between the VCI and CIND groups ($\chi^2 = 7.525$, $p = 0.006$) (Table 2).

Comparisons of the MoCA score and cystatin C in different genotypes

The total MoCA score was significantly different ($p = 0.048$) among groups with CST3 AA, CST3 BB, and CST3 AB genotypes. Notably, the lowest MoCA score was noticed in subjects with the CST3 BB genotype while it was the highest in the CST3 AA genotype. Patients with CST3 BB genotype had the highest serum levels of cystatin C in comparison with the lowest concentrations in the CST3 AA genotype. However, there was no statistically significant difference in serum cystatin C levels among these three genotypes ($p = 0.103$) (Table 3).

Correlations between cystatin C, CST3 genotypes, and VCI

Univariate logistic regression analysis was applied to examine the relationship between the independent variable serum cystatin C and the dependent variable VCI. As demonstrated, a high serum cystatin C level was associated with an increasing susceptibility to VCI (OR 3.837(1.176–12.520), $p = 0.026$). Similarly, when CST3 B allele was incorporated as an independent variable in the regression analysis, patients with this allelic frequency had significantly greater risk for VCI (OR 2.038 (1.048–3.963), $p = 0.036$) (Table 4).

Table 1 Comparisons of clinical characteristics between VCI and CIND groups

Characteristics	CIND ($n = 81$)	VCI ($n = 71$)	p value
Age (years)	71.40 ± 11.32	76.28 ± 15.16	0.440
Male	61 (62.96%)	45 (63.38%)	0.110
Hypertension	61 (62.96%)	61 (85.92%)	0.553
Diabetes mellitus	38 (46.91%)	30 (42.25%)	0.851
Smoking	29 (35.80%)	20 (28.17%)	0.718
HbA1c (%)	7.09 ± 1.90	7.10 ± 1.19	0.987
Cr (μmol/l)	86.58 ± 12.87	92.71 ± 22.74	0.510
UA (μmol/l)	328.80 ± 77.06	341.14 ± 61.90	0.689
TC (mmol/l)	4.83 ± 1.32	5.39 ± 0.97	0.290
TG (mmol/l)	1.37 ± 0.57	1.33 ± 0.40	0.847
HDL (mmol/l)	1.05 ± 0.28	1.00 ± 0.33	0.711
LDL (mmol/l)	3.20 ± 0.87	3.45 ± 0.77	0.487
Cystatin C (mg/l)	0.97 ± 0.27	1.07 ± 0.28	0.024
MoCA	28.01 ± 1.42	23.21 ± 1.19	< 0.001

Abbreviations: *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HbA1c* glycosylated hemoglobin, *Cr* serum creatinine, *BUN* blood urea nitrogen, *UA* uric acid, *HCY* homocysteine, *TC* total cholesterol, *TG* triglyceride, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *MoCA* Montreal Cognitive Assessment

Table 2 Cystatin C genotypes and allele frequencies in the VCI and CIND groups

Characteristics	CIND ($n = 71$)	VCI ($n = 81$)	p value
Genotype			
AA	61 (85.9%)	57 (70.4%)	0.048
AB	9 (12.7%)	18 (22.2%)	
BB	1 (1.4%)	6 (7.4%)	
Allele frequency			
A	131 (92.3%)	132 (81.5%)	0.006
B	11 (7.7%)	30 (18.5%)	

Table 3 Comparison of MoCA and cystatin C in different genotypes

Genotype	*N*	MoCA	Cystatin C
AA	118	27.57 ± 2.07	1.00 ± 0.28
AB	27	26.44 ± 2.61	1.09 ± 0.26
BB	7	25.46 ± 2.79	1.18 ± 0.29
P		0.048	0.103

Discussion

VCI is a major but severe complication resulted from acute cerebral infarction. To date, far too little attention has been paid to the potential reliable and point-of-care screening markers for VCI. In our case-control study, we revealed that significant correlations were present between a high serum cystatin C level and CST3 B allele, and VCI after cerebral infarction. Hence, it is possibly of great clinical value to evaluate the serum cystatin level and CST3 gene polymorphism for early diagnosis of VCI.

Cystatin C has been known for its essential clinical value in early detection of renal and some cardiovascular diseases [20]. In recent decades, many investigators have attempted to figure out the associations between cystatin C and neurologic disorders. Cystatin C is responsible for certain regulatory mechanisms involving atherosclerosis, amyloid (Aβ) deposition [21], neural regeneration and degeneration [22], antiapoptosis [23], responses against oxidative stress [24], and protective effect on cognitive impairment. Conversely, results derived from a fundamental study [25] noticed that high cystatin C is correlated with cognitive impairment instead of protection. In terms of epidemiologic studies, debate about the association of cognitive impairment and serum cystatin C continues. Studies found that cystatin C was negatively [7, 10, 26], positively [11–14, 27], or not [28] correlated with cognitive impairment. Among clinical studies, evidence on this correlation of cystatin C with VCI has grown up around the focus of AD. However, there have been few studies exploring this relation in patients with cerebral infarction. In the current case-control study, the serum cystatin C level was significantly higher in the VCI group than that in the control group. In addition, the level was positively associated with the increasing severity of cognitive impairment and negatively with the MoCA score. Overall, as indicated by these logistic regression analyses, a high serum level of cystatin C might be a risk factor for VCI after cerebral infarction.

Table 4 Single factor logistic regression analysis of VCI against risk factors

Risk factors	*B*	SE	Wald	*p*	OR	95%CI
Cystatin C	1.345	0.603	4.965	0.026	3.837	1.176–12.520
B allele carrying	0.712	0.339	4.399	0.036	2.038	1.048–3.963

Previous studies investigating the association between cystatin C and cognitive impairment were not consistent. There may be two main considerations accounting for the diversity in this association. Firstly, it could be related to different stages of certain neurodegenerative diseases including AD. This is in agreement with a study by Zerovnik E [29] who assessed AD patients. In the early stage of AD, the combination of cystatin C and Aβ [30] could help eliminate redundant soluble Aβ. Therefore, nerves could be protected as a result of degradation and export of Aβ from neurons. In the later stages of AD, however, increased demand of autophagolysomes for cleaning up Aβ may diminish the protective effect of cystatin C. In our previous observational study, we figured out that the cystatin C level rose rapidly at the beginning, but dropped gradually after 1 to 2 weeks post cerebral infarction [2]. This finding is in part similar to the theoretical hypothesis by Zerovnik and coworkers [29]. Secondly, the serum cystatin C level may not be equivalent to the cystatin C level in tissues or cells. Tobin et al. [31] found that hypercystatinemia was related to metabolic disturbance characterized by a high cystatin C level with concomitant low cystatin C mRNA levels in peripheral monocytes. In the present study, patients with acute cerebral infarction within 3 days after onset in our study were actually in the early stage of VCI, and our data revealed a significant association of high cystatin C with VCI. Curiously, a concern was raised on why cystatin C appeared not to play a role in neuroprotection even in the early stage of VCI based upon aforementioned hypotheses. At this point, we speculate that a high serum cystatin C level was thought to be negatively correlated with a low tissue or cellular cystatin C level. And it may be the latter that is more relevant to the neurodegenerative consequences such as VCI after cerebral infarction. And this will be further investigated in our future studies that shed light on the cystatin C level in cerebrospinal fluid (CSF).

Cystatin C is coded by CST3. And up to now, CST3 B has been widely recognized as the allele that carries risk for susceptibility to AD [32, 33], and genotype CST3 BB has been examined to be associated with a decrease of cystatin C secretion. In contrast, Crawford and coworkers [34] showed that CST3 AA is a novel risk factor for late-onset AD in Caucasian patients in their eighties. In spite of that, no evidence on the correlation between CST3 polymorphism and AD has been shown in the population of Japan, Germany, and Italy [35–37]. Also, little attention has been paid to whether CST3 gene polymorphism is associated with VCI after cerebral infarction. We noticed that patients with allele B possessed a risk of VCI that were three times higher than those without allele B. The MoCA score was also evaluated, and it turned out to be significantly different among three groups with various CST3 genotypes. The MoCA score was the

lowest in the CST3 BB group, while it was the highest in the CST3 AA group. And thereby it could be proposed that CST3 B allele is a risky gene for the decline in cognitive function after cerebral infarction; it may also indicate that CST3 B allele is one of the risks for VCI.

CST3 B allele is associated with low expression of cystatin C. There are totally three Ksp I restricted polymorphisms (CCGCGG) in the 5′-upstream region of CST3. Two of them are located in the 5′ flanking promoter region while another one is in the signal peptide region of the exon 1. As a result of linkage disequilibrium, three CST3 polymorphism sites only generate two common haplotypes, including CST3 A and CST3 B. Studies have shown that alanine located at the penultimate position of the signal peptide could be substituted by threonine due to nucleotides G/A mutation at position +73. Such a substitution may alter the secretory and modification processes of cystatin C through underlying mechanisms such as interfering the transport of cystatin C towards endoplasmic reticulum and Golgi complex or disrupting the cleavage of the signal peptide and the mature protein. Accordingly, the secretory amount of cystatin and its activity were reduced [38]. In our findings, interestingly, serum cystatin C concentration in patients with CST3 B allele was higher than that in those with CST3 A. This further supports our hypothesis that the cystatin C level in serum differs from that in the tissues or cells. Possible explanations are elucidated as follows. An increase in concentration of cystatin C reflects the enhanced inhibitory activity of the protease [39], and on the contrary, a decrease in cellular cystatin C level indicated by CST3 B may reduce the inhibitory effect of matrix degradation. These phenomena altogether promote the development of atherosclerosis [40]. Systemic or regional inflammation is also an essential factor of atherosclerosis, which may cause the disturbance of cystatin C metabolism, and thus different levels of intracellular and extracellular cystatin C may be examined. What is more, as cystatin C and Aβ combine and aggregate surround the cerebral arteriolar walls causing amyloid deposition; the number of vascular smooth muscle cells would decrease progressively in the presence of ongoing buildup of amyloid deposits. These may contribute to the microangiopathy, cerebral hemorrhage, and other cerebrovascular diseases. Under such circumstances, cystatin C could be released from the central nervous system into the blood stream, which may also lead to a discrepancy between the serum and tissue levels of cystatin C.

Conclusions

A high serum cystatin C level and CST3 B allele may be risk factors for VCI after acute cerebral infarction. It is likely that the measurement of the serum cystatin C level and detection of CST3 polymorphism are of great clinical value in early diagnosis of cognitive impairment in older patients, but more robust evidence is needed.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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