

Elevation in circulating YKL-40 concentration in patients with cerebrovascular disease

Xue Xu^{1†}, Hongling Ma^{2†}, Jia Xu³, Haiwei Huang¹, Yan Xiong³, Hong Zhan^{3*}, Fan Huang^{1*}

¹Department of Neurology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China. ²Department of Neurology, Liaocheng People's Hospital, Liaocheng 252000, China. ³Department of Emergency Medicine, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China.

ABSTRACT

YKL-40 is a novel inflammatory protein. Elevated serum levels of YKL-40 have been reported in patients with atherosclerosis and other cardiovascular diseases, but the circulating profile of YKL-40 in patients with cerebrovascular disease has been less investigated. This prospective observational study aimed to determine serum levels of YKL-40 in patients with different subtypes and severities of cerebrovascular disease. Eighty patients with acute ischemic stroke, 30 patients with acute hemorrhagic stroke, 15 patients with transient ischemic attack (TIA) and 18 age- and gender-matched healthy control subjects were recruited. Blood was sampled. Serum levels of YKL-40 were measured by ELISA. In healthy control subjects, serum levels of YKL-40 were 45.09±31.41 ng/ml, significantly lower than those in patients with acute ischemic stroke (178.58±127.78 ng/ml), hemorrhagic stroke (105.32±87.35 ng/ml) and TIA (148.09±108 ng/ml) respectively ($p<0.05$). When the 80 acute ischemic stroke cases were stratified into four Oxfordshire Community Stroke subtypes, serum levels of YKL-40 were significantly higher in patients with total anterior (n=16), partial anterior (n=25) and posterior (n=12) circulation infarctions respectively than in those with lacunar (n=27) infarction ($p<0.05$). Moreover, 63 of 80 patients with acute ischemic stroke survived. Circulating levels of YKL-40 in these stroke survivors were associated with the United States National Institutes of Health Stroke Scale (NIHSS) scores of neurological deficit. In summary, serum levels of YKL-40 were elevated in patients with cerebrovascular disease in lesion subtype- and severity-dependent manners. These observations suggest a potential for YKL-40 as a diagnostic/prognostic biomarker for cerebrovascular disease.

KEY WORDS: YKL-40, cerebrovascular disease, stroke, infarction, diagnostic/prognostic biomarker

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INTRODUCTION

Cerebrovascular disease, often referred to as stroke, is a heterogeneous group of disorders in which areas of the brain are transiently or permanently affected by ischemia or bleeding. Stroke-associated burden and death rates vary considerably among countries, depending on the prevalence of cardiovascular disease risk factors and socioeconomic characteristics [1]. While stroke is the number one killer in middle-income countries, it is the 5th most common cause of

death in low-income countries [2]. In China, stroke claims 1.6 million lives and affects approximately 2.5 million new individuals annually [3], whereby posing a significant health problem.

Stroke is believed to be preventable and treatable through management of risk factors; an accurate and timely diagnosis is the key to optimizing the patient outcome [4]. Recently, serum biomarkers have been suggested to play an important role in the diagnosis of acute cerebral ischemia and in the subsequent implementation of timely reperfusion strategies [5]. Nevertheless, to date, no reliable biomarkers have been identified and clinically validated for cerebrovascular abnormalities.

YKL-40, named as a combination of the letter codes of its first three N-terminal amino acids, tyrosine (Y), lysine (K) and leucine (L), and its molecular weight (40 kD) [6], is a novel proinflammatory protein. Since its first identification in the media of human osteoblastic cell cultures [7], YKL-40 has been demonstrated to be produced in many other cell types as well [8,9]. Although YKL-40 is detectable in the peripheral

*Corresponding authors:

Hong Zhan,
Department of Emergency Medicine, First Affiliated Hospital,
Sun Yat-sen University, 58 Zhongshan Rd II, Guangzhou 510080, China
Phone: 86-20-8733-0202
E-mail: zhanhong81@126.com

Fan Huang,
Department of Neurology, First Affiliated Hospital, Sun Yat-sen University,
58 Zhongshan Rd II, Guangzhou 510080, China
Phone: 86-20-8237-9576
E-mail: huangfan.hf1@gmail.com

†Authors equally contributed to the work.

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blood of healthy individuals [10-12], its serum concentration is significantly increased in a number of disease conditions including cardiovascular disorders [13]. However, little information is available regarding blood levels of YKL-40 in Chinese patients with cerebrovascular disease. The present study was therefore conducted to assess changes in serum levels of YKL-40 in Chinese patients with cerebrovascular disease of different subtypes and severities, aiming at establishing the possible use of YKL-40 as a diagnostic/prognostic biomarker for cerebrovascular disease.

MATERIALS AND METHODS

Ethical Statement

The study was reviewed and approved by the Institutional Ethics Committee of Sun Yat-sen University in Guangzhou, China and conducted in compliance with the Helsinki Declaration. Written informed consent was obtained from all subjects.

Patients

A total of 125 patients with acute cerebral vascular disease who were referred to the Neurological Division in the Department of Emergency Medicine, the First Affiliated Hospital of Sun Yat-sen University consecutively between June 2010 and July 2011 were recruited. Patients with concomitant inflammatory disease such as infection and autoimmune disorder; liver or kidney disease, myocardial infarction or malignancies were excluded. Eighteen age- and gender-matched healthy volunteers were included as control subjects.

Diagnosis of Cerebrovascular Disease

All patients were diagnosed by a set of neurological examinations including cranial CT and/or MRI, while TIA that has been redefined recently as a brief (less than 24 h) episode of neurologic dysfunction caused by focal brain, spinal cord, or retinal ischemia by the American Heart Association and American Stroke Association without acute infarction [14] was determined based on the medical history of the event. In the 125 patients, 80 were diagnosed with acute ischemic stroke within 48 h of symptom onset, 30 with acute hemorrhagic stroke within 24 h of symptom onset, and the remaining 15 with TIA within 72 h of symptom onset. Based on the CT and/or MRI findings, the 80 acute ischemic stroke patients were stratified into four subtype groups: total anterior circulation infarcts (TACI, n=16), partial anterior circulation infarcts (PACI, n=25), posterior circulation infarcts (POCI, n=12) and lacunar infarcts (LACI, n=27) by the Oxfordshire Community Stroke Project (OCSP) criteria [15].

By day 14 of hospitalization, 17 out of 80 acute ischemic stroke patients died. The neurological deficit in the 63 ischemic stroke survivors was assessed using the United States National Institutes of Health Stroke Scale (NIHSS). Depending on the NIHSS score, the deficit was defined as mild (a score 1-4), moderate (a score 5-20) and severe (a score 21-42) respectively.

Measurement of Major Biochemical Variables and YKL-40 in the Blood

Blood was drawn by venipuncture into pyrogen-free tubes with no anticoagulant within 48 h after hospital admission. After clotting at room temperature for 3 h, the blood sample was centrifuged at 3,000×g for 5 min. Serum was collected and stored at -80°C until analysis.

Blood laboratory tests were performed using an autoanalyzer as instructed; white blood cell and platelet counts as well as serum concentrations of total cholesterol, triglycerides, HDL (high density lipoprotein) cholesterol and LDL (low density lipoprotein) cholesterol were measured. The measurement of serum levels of YKL-40 was performed with a commercial enzyme immunoassay (EIA) kit from Quidel Corporation (San Diego, California, USA) as previously described [16].

Statistical Analysis

Quantitative data were expressed as mean±standard deviation (SD) and analyzed by t-test or one way ANOVA together with Bonferroni test. Categorical data were expressed as percentages and analyzed by χ^2 -test. Differences were considered statistically significant when $p < 0.05$. All analyses were performed with the statistical software SPSS 13.0 (Chicago, IL, USA).

RESULTS

Basic Demographics

The median age of patients in the three cerebrovascular disease subgroups (i.e. acute ischemic stroke, n=80; acute hemorrhagic stroke, n=30; and TIA, n=15) was respectively 69.5 (39-92), 60 (36-84) and 66 (43-76) years, which were similar to that of the 18 healthy control subjects (56 years, rang 35-80 years). A similar male to female ratio was also observed in the three disease subgroups (46/34, 20/10 and 8/7, respectively) and control (11/7) group (Table 1).

The median age in the four subtypes of ischemic stroke (TACI, PACI, POCI and LACI) was respectively 74 (rang 55-92), 69 (rang 57-78), 67 (rang 50-86), and 67 (rang 39-83) years; there were no significant differences between these subtypes. In contrast, the male to female ratio was slightly different among TACI (7/9), PACI (10/15), POCI (9/3) and LACI (20/7) patients (Table 1).

Biochemical Parameters

Serum levels of glucose, total cholesterol, triglycerides, HDL and LDL cholesterol, white blood cell count and platelet counts were measured in the 125 patients. As shown in Table 1, no significant differences ($P>0.05$) in these variables were detected either between ischemic stroke, hemorrhagic stroke and TIA groups or between the four subgroups (TACI, PACI, POCI and LACI) of ischemic stroke patients.

Serum Levels of YKL-40

Presented in Figure 1 are the results of serum YKL-40 concentration measurements. The average serum levels of YKL-40 in 18 healthy volunteers were 45.09 ± 31.41 ng/ml. Compared to this baseline level, a significant elevation was observed in patients with acute ischemic stroke (178.58 ± 127.78 ng/ml), acute hemorrhagic stroke (148.7 ± 108 ng/ml) or acute TIA (105.32 ± 87.35 ng/ml) ($p<0.05$). Serum levels of YKL-40 were not significantly different between ischemic stroke, hemorrhagic stroke and TIA ($p>0.05$).

In the 80 ischemic stroke patients, stroke subtype-dependent changes in the average serum levels of YKL-40 were observed (Figure 2). LACI patients ($n=27$) had a level very close to the baseline level in normal control subjects. Compared to the level in LACI patients, significantly higher levels were detected in TACI ($P<0.01$), PACI ($p<0.05$) and POCI ($p<0.05$) patients.

Out of the 80 ischemic stroke patients, 63 survived and were subjected to the NIHSS assessment 14 days after admission. Presented in Table 2 are the results of this assessment together with the data on serum YKL-40 measured within 48 h after admission. Apparently, the more severe the neurologic deficit was, the higher the average serum level of YKL-40 in the acute ischemic stroke survivors.

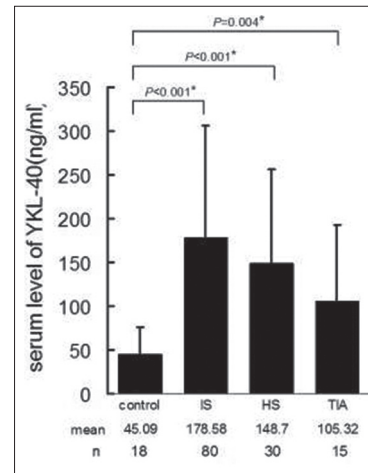


FIGURE 1. Average serum levels of YKL-40 in healthy control subjects and patients with acute ischemic stroke (IS), acute hemorrhagic stroke (HS) and transient ischemic attack (TIA) respectively. A single asterisk (*) indicates a significant difference from the control subjects at $\alpha=0.05$

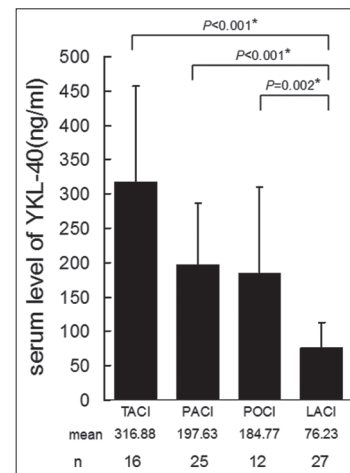


FIGURE 2. Average serum levels of YKL-40 in subtypes of ischemic stroke classified by the Oxfordshire Community Stroke Project criteria. TACI: total anterior circulation infarct ($n=16$); PACI: partial anterior circulation infarct ($n=25$); POCI: posterior circulation infarct ($n=12$); and LACI: lacunar infarct ($n=27$). A single asterisk (*) indicates a significant difference from the LACI group at $\alpha=0.05$

TABLE 1. Major biochemical variables in patients with different subtypes.

	Ischemic stroke					HS	TIA	p
	Overall	TACI	PACI	POCI	LACI			
Number	80	16	25	12	27	30	15	
Sex (M/F)	46/34	7/9	10/15	9/3	20/7	20/10	8/7	0.606
Age	69.5 (39-92)	74 (55-92)	69 (57-87)	67 (50-86)	67 (39-83)	60 (36-84)	66 (43-76)	0.076
Glucose*	6.2±2.7	8.2±4.6	6.3±2.4	5.3±1.2	5.7±1.8	6.5±3.0	5.9±2.0	0.697
Chol*	5.1±1.1	4.8±0.9	5.1±1.1	5.3±1.1	5.2±1.2	5.3±1.2	5.4±1.5	0.644
Triglycerides*	1.50±0.94	1.12±0.55	1.63±1.21	1.41±0.65	1.59±0.94	1.32±0.70	2.02±1.62	0.593
HDL Chol*	1.11±0.27	1.19±0.34	1.15±0.32	1.10±0.30	1.04±0.14	1.31±0.33	1.16±0.24	0.080
LDL Chol*	3.42±1.03	3.16±0.87	3.23±1.14	3.76±0.98	3.52±1.02	3.49±1.00	3.35±1.11	0.890
WBC count [§]	8.34±3.09	10.49±3.98	8.09±2.64	8.50±3.53	7.23±1.96	10.05±3.71	7.30±1.44	0.071
Platelet count [†]	224±87	219±105	203±83	242±56	235±94	207±75	211±35	0.782

(TACI: total anterior circulation infarct; PACI: partial anterior circulation infarct; POCI: posterior circulation infarct; and LACI: lacunar infarct) of ischemic stroke, hemorrhagic stroke (HS) or TIA. None of the variables tested showed significant difference between groups. Notes: Chol=cholesterol; * =mmol/L; [§] =10⁶/L; [†] =10⁹/L.

TABLE 2. Serum levels of YKL-40 in association with the United States National Institute of Health Stroke Scale (NIHSS) score of neurologic deficits in 63 patients who survived acute ischemic stroke at two weeks after hospital admission.

NIHSS score category	N	NIHSS score	YKL-40 (ng/ml)
Mild	14	2.76±1.12	77.10±49.52
Moderate	33	11.42±4.29*	160.49±89.17*
Severe	16	26.88±4.67**	338.73±92.72**

A single asterisk (*) and double asterisks (**) indicate significant differences from the mild score category at $p < 0.05$ and $p < 0.01$ respectively.

DISCUSSION

As a gene localized in a highly conserved region on human chromosome 1q31-q32 [17], YKL-40 is expressed in a relatively stable level across healthy human individuals of different races. In a Danish study involving a total of 245 healthy subjects (134 women and 111 men) ranging from 18 to 79 years in age, Johansen et al. [12] reported that the baseline serum levels of the YKL-40 protein in normal Scandinavians were 43 ng/ml, which increased with age ($r = 0.45$, $p < 0.0001$) but showed little diurnal rhythm-, physical activity-, and gender-associated variations. In this study, there were no significant differences in age and gender. Average serum levels of YKL-40 in the 18 healthy Chinese subjects in the present study were 45.09 ± 31.41 ng/ml, similar to the baseline levels detected in the Scandinavian population but lower than that (62.64 ng/ml) reported in the study of Zheng et al. [18] on 248 healthy Chinese subjects and higher than that (37.85 ± 14.12) reported in another Chinese study involving on 80 healthy subjects [19]. Apparently, the baseline levels of YKL-40 in the circulation needs to be further established in studies involving a larger sample size and perhaps more standardized assay protocols.

YKL-40 is a protein that shares a high degree of sequence similarity with chitinase 3 but lacks the classical chitinolytic activity. The physiological function of YKL-40 remains unclear. Nevertheless, recent studies have demonstrated that circulating levels of YKL-40 are elevated in numerous human diseases including coronary artery disease [20] and acute myocardial infarction [21], suggesting a diagnostic/prognostic role for this molecule as a biomarker of these diseases. In the present study, we demonstrated that the serum levels of YKL-40 were also elevated significantly ($p < 0.05$) in Chinese patients with cerebrovascular disease as compared with the levels in the healthy control subjects and that the elevation did not significantly differ between ischemic stroke, hemorrhagic stroke and TIA ($p > 0.05$). These observations support an association between circulating levels of YKL-40 and cerebrovascular disease subtypes and severities as previously reported [22].

We further assessed the relationship between the serum level of YKL-40 and the OCSF ischemic stroke subtypes. The results showed that the levels in LACI patients were similar

to the baseline levels in normal control subjects, but were significantly elevated in TACI ($P < 0.01$), PACI ($P < 0.05$) and POCI ($p < 0.05$) patients. Given a previous report that OCSF class-different patients are different significantly in mortality, morbidity, length of hospital stay, and complications (e.g. respiratory tract infection and seizures) [23], the association of serum levels of YKL-40 with the OCSF classification observed in the present study suggests that YKL-40 may be useful in predicting the severity of infarct lesions in acute ischemic stroke patients. A wide spectrum of acute and chronic infections as well as many exogenous and intrinsic sources of inflammation are associated with an increased risk for ischemic stroke and YKL-40 may play a role in tissue remodeling during inflammation [24]. These may partially explain the association between YKL-40 levels and cerebrovascular disease subtypes and severities, but the mechanism underlying the role for YKL-40 in the development of cerebrovascular lesions warrant further investigation.

Moreover, we observed that serum levels of YKL-40 were positively associated with the NIHSS score of neurological deficit. This finding is in agreement with the results of a recent study by Park and colleagues that the circulating YKL-40 level in 105 Korean patients with acute ischemic stroke was correlated with the severity of neurological deficit as assessed by the NIHSS [22].

At present, the cellular origin of increased circulatory YKL-40 in cerebrovascular disease remains unclear. Given that inflammation plays an important role in the secondary brain injury following ischemic and hemorrhagic strokes [25] and this inflammatory response involves immediate infiltration of blood components (e.g. red blood cells, leukocytes, and macrophages) as well as microglia and astrocyte activation at the injury site [26], it is likely that all these cells involved may contribute to the increased YKL-40 production in cerebrovascular disease. Nevertheless, this speculation warrants more intensive investigations.

CONCLUSION

In summary, we have demonstrated that circulating levels of YKL-40 are significantly elevated in patients with acute cerebral vascular disease including acute ischemic and hemorrhagic strokes and TIA. We have also demonstrated that YKL-40 in the circulation is associated with the severity of neurological deficit in acute ischemic stroke patients. These observations suggest that YKL-40 may potentially offer a novel diagnostic and/or prognostic biomarker for cerebrovascular disease.

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DECLARATION OF INTEREST

None of the authors have any commercial associations or sources of support that might pose a conflict of interest.

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