

## Clinical study

# YKL-40 levels in the cerebrospinal fluid and serum of patients with aneurysmal subarachnoid hemorrhage: Preliminary results

Mehmet Yasar Kaynar<sup>1</sup> MD, Taner Tanriverdi<sup>1</sup> MD, Ali Metin Kafadar<sup>1</sup> MD, Tibet Kacira<sup>1</sup> MD, Fatma Yurdakul<sup>2</sup> MD, Hafize Uzun<sup>3</sup> PHD, Koray Gumustas<sup>3</sup> PHD

<sup>1</sup>Department of Neurosurgery, <sup>2</sup>Department of Anaesthesiology, <sup>3</sup>Department of Biochemistry; Istanbul University, Cerrahpasa Medical Faculty, Istanbul, Turkey

**Summary** YKL-40 is a newly discovered matrix protein thought to be secreted during the acute stages of inflammation. Clinical studies have revealed that YKL-40 has growth factor and potent migration factor activity for cells involved in inflammation and tissue remodeling processes. It has recently been speculated that YKL-40 may serve as a specific serologic marker of neutrophil function at the site of acute tissue inflammation. We aimed to quantify the levels of YKL-40 in both cerebrospinal fluid and serum of ten consecutive patients with aneurysmal subarachnoid hemorrhage and to speculate on the origin of this glycoprotein. The levels were also compared with ten control patients with hydrocephalus. We found that patients with aneurysmal subarachnoid hemorrhage had significantly higher YKL-40 levels in both cerebrospinal fluid and serum than controls. The authors believe that YKL-40 is expressed in cerebrospinal fluid due to stress on neural structures while a damaged blood-brain barrier allows entry of neutrophils and macrophages from the systemic circulation.

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## INTRODUCTION

A matrix protein, YKL-40, is found in the specific granules of neutrophils isolated from the blood of healthy people.<sup>1</sup> It is a member of the 18-glycosylhydrolase family, which also induces chitinase and chitinase-related proteins.<sup>2</sup> It has been named YKL-40 from its molecular mass (40 kDa) and the one letter code for its first three N-terminal amino acids.<sup>3</sup>

The exact physiological function of YKL-40 has yet to be defined. However, the pattern of its expression in normal and pathological states suggests that it plays a role in the inflammatory process and may have a function in tissue remodeling.<sup>4–6</sup> YKL-40 is also synthesized by activated macrophages<sup>7</sup> and by chondrocyte–synovial cells in patients with rheumatoid arthritis.<sup>8</sup> A positive correlation between the severity of the disease state and the levels of YKL-40 in either cerebrospinal fluid (CSF) or serum of patients with a variety of pathological conditions has been demonstrated in recent clinical studies, the majority of which speculated that YKL-40 might be a valuable biochemical marker of inflammation, similar to C-reactive protein (CRP).<sup>4–6</sup>

As a putative role for inflammation in vasospasm following aneurysmal subarachnoid hemorrhage (SAH) has been postulated in the last decade,<sup>9–11</sup> we aimed to evaluate levels of YKL-40 in both the CSF and serum of patients with aneurysmal SAH.

## PATIENTS AND METHODS

### Patients

Ethical approval for this study was obtained from the Human Investigations Committee at Istanbul University and all patients, or the

next of kin if the patient was unconscious, provided informed consent. We studied patients referred to our neurosurgical unit from January to June 2003 within three days of SAH established by CT. We excluded patients with any kind of infection in which YKL-40 may be elevated at the time of CSF and serum collection.

### Demographic characteristics of patients and controls

This study included 10 consecutive patients with aneurysmal SAH and 10 control patients, seven of whom had normotensive hydrocephalus and three with hydrocephalus secondary to aqueduct stenosis without any other known central nervous system disease. The average age was  $54.7 \pm 13.7$  and  $50.0 \pm 24.4$  years for patients with SAH and the control group, respectively. A summary of demographic data of the SAH patients is provided in Table 1.

### Specimen handling

For each patient, serial blood (venepuncture) and CSF (lumbar puncture) samples taken concurrently were collected within the first 3 days, and on the 7<sup>th</sup> day following SAH. From the control group, blood samples were collected via venepuncture, and CSF samples were obtained during the insertion of a ventriculoperitoneal shunt. Samples from the control group were obtained once. As soon as possible, each 10 ml CSF and blood specimen was centrifuged at 10,000 rpm for 15 minutes and the supernatant stored at  $-70$  °C until assayed.

### YKL-40 measurement

CSF and serum YKL-40 levels were quantitatively measured in nanograms per milliliter (ng/ml). YKL-40 was assayed by a sandwich immunoassay in a microtitre stripwell format (Chondrex, Metra Biosystems Inc, Mountainview, CA). The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin binds to streptavidin on the strip and captures YKL-40. A conjugated

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Correspondence to: Taner Tanriverdi MD, P.K: 4, Cerrahpasa, 34303, Istanbul, Turkey. Tel./Fax: +90 212 414 34 27; E-mail: tanerato2000@yahoo.com

**Table 1** Clinical characteristics of patients with SAH

Patient	Age/Sex	H-H	FG	S-VS	Aneurysm	GOS (6 months)
1	65/F	III	2	Yes	PCoA	Death
2	67/F	II	3	No	ACoA	Good recovery
3	58/M	III	3	Yes	ICA	Severe disability
4	71/F	V	3	Yes	MCA	Death
5	67/F	II	3	Yes	PCoA	Death
6	56/F	III	3	Yes	OA	Severe disability
7	36/M	II	3	No	ACoA, MCA	Good recovery
8	52/F	II	2	No	MCA	Good recovery
9	43/F	II	2	No	PCoA	Good recovery
10	32/M	III	3	No	ACoA	Death

ACoA: anterior communicating artery; F: female; FG: Fisher grade; GOS: Glasgow outcome scale; H-H: Hunt-Hess grade; ICA: internal Carotid artery; M: male; MCA: middle cerebral artery; OA: ophthalmic artery; PCoA: posterior communicating artery; S-VS: symptomatic vasospasm.

polyclonal anti-YKL-40 antibody conjugated with alkaline phosphatase binds to the captured YKL-40. Bound enzyme activity is detected using p-nitrophenyl phosphate as a substrate. The intra-assay coefficient of variation was less than 7%.

### Statistical analysis

Data were analyzed by using the SPSS statistical program (SPSS, Chicago, IL). Due to the number of samples in this study, a non-parametric test, the Mann-Whitney U test, was chosen to evaluate significant differences between patient and control group. Differences within each group were tested by a "Student t" test. A probability value of less than 0.05 was considered statistically significant.

## RESULTS

Twenty CSF and 20 serum samples from the patients with SAH and 10 CSF and 10 serum samples from the control group were obtained for this prospective clinical study. The samples were

tested for YKL-40. The results from the patient and control groups are provided in Table 2. There was no statistically significant difference in age or sex between the two groups ( $p > 0.05$ ).

### Levels of YKL-40 in CSF

Levels of YKL-40 were markedly different in patients with SAH and controls. In the control group, the mean concentration was  $151.5 \pm 42.4$  ng/ml. In contrast, the average level in SAH patients was  $636.3 \pm 203.9$  ng/ml within the first 3 days, and  $436.0 \pm 114.6$  ng/ml on day 7 after SAH. These differences were highly significant ( $p = 0.00001$ ). There was a significant decrease in concentration over time post-SAH ("t" test;  $t = 5.1$ ,  $p = 0.006$ ).

### Levels of YKL-40 in serum

The average serum YKL-40 level in the control group was  $117.1 \pm 25.9$  ng/ml while the average level in SAH patients was  $405.2 \pm 145.1$  ng/ml within the first 3 days and  $296.3 \pm 93.1$  ng/ml on day 7 after SAH. These differences were also highly

**Table 2** Levels of YKL-40 (ng/ml) in CSF and serum of patients with SAH and control

Patient	First 3 days		Day 7	
	CSF	CSF	Serum	Serum
1	435	372	249	175
2	398	306	376	217
3	477	391	215	196
4	523	405	326	232
5	456	322	278	325
6	798	476	546	431
7	861	518	429	357
8	697	543	576	305
9	975	671	642	438
10	743	356	415	287
Mean $\pm$ SD	$636.3 \pm 203.9$		$436.0 \pm 114.6$	
			$405.2 \pm 145.1$	
			$296.3 \pm 93.1$	
Control	CSF		Serum	
1	77		98	
2	157		127	
3	121		75	
4	175		146	
5	162		135	
6	134		107	
7	106		86	
8	199		119	
9	216		157	
10	168		121	
Mean $\pm$ SD	$151.5 \pm 42.4$		$117.1 \pm 25.9$	
p value	0.00001 <sup>a</sup>		0.00001 <sup>a</sup>	

significant ( $p = 0.00001$ ). There was a significant decrease in concentration over time post-SAH, as was the case for CSF (“ $t$ ” test;  $t = 3.8$ ,  $p = 0.004$ ).

## DISCUSSION

As in other inflammatory reactions, a complex cascade including recruitment of leukocytes and soluble mediators from the blood stream occurs after aneurysmal SAH. Hence, studies of soluble molecules within the subarachnoid space after SAH are required for a comprehensive understanding of the pathophysiological mechanisms behind the inflammatory process leading to vasospasm.

Recently, it has been shown that YKL-40 is expressed by monocytes/macrophages in peripheral blood and synovial tissue.<sup>12,13</sup> Although the exact physiological function of YKL-40 is unknown, the results of clinical studies including in community-acquired pneumonia,<sup>4</sup> active rheumatoid arthritis,<sup>14</sup> hepatic fibrosis,<sup>15</sup> breast cancer,<sup>16</sup> colorectal cancer,<sup>17</sup> and human glioma<sup>18</sup> have disclosed that serum levels of YKL-40 are correlated with the severity of disease. Moreover, high levels of YKL-40 in serum and CSF were recently found to be an independent predictor of death in patients with pneumococcal bacteremia<sup>4</sup> and purulent meningitis,<sup>6</sup> respectively. These studies suggest that YKL-40 may have an important role in inflammation and be used as a serological marker for such inflammatory diseases. In addition, the CSF YKL-40 concentrations were found to be high in patients with spinal disorders causing spinal stenosis.<sup>19</sup>

To the best of our knowledge, this study is the first to investigate CSF and serum YKL-40 concentration during the acute stage of aneurysmal SAH. We found that YKL-40 levels in both serum and CSF samples within the first 3 days and on day 7 of SAH were significantly higher in SAH patients than in controls. The levels in both serum and CSF showed significant decrease over time, suggesting that the acute burden of inflammation occurring within the first 3 days, during which time vasospasm is rarely encountered, is located within the local (brain) and systemic circulation. A cellular source of YKL-40 production cannot be defined by this study in a limited number of patients; however, a possible candidate is the central nervous system macrophage. An alternative source of YKL-40 in the CSF may be that of systemic inflammatory cells entering the CSF through a damaged blood-brain barrier. It has been speculated that patients with encephalitis, who have a high degree of parenchymal involvement but little CSF infiltrate, had very high levels of YKL-40 in their CSF,<sup>6</sup> supporting the hypothesis that YKL-40 is most likely produced by resident macrophages rather than by cells that infiltrate the CSF. Conflicting data in the literature relating to the source of YKL-40 suggest the need for immunohistochemical analyses of the brain or CSF in patients with SAH.

In the present study, serum and CSF YKL-40 levels in patients with SAH were consistently higher than control values but there was a significant decrease towards post-SAH day 7 (by which time the risk of vasospasm reaches its peak), suggesting the degree of inflammation after SAH progressively decreased.

A key question derived from our results is: “if YKL-40 is correlated with the degree of inflammation, why does it decrease towards day 7 of SAH, when we know that as inflammatory processes increase, vasospasm is more likely to occur?”

Recently, it has been demonstrated that YKL-40 is a growth factor for connective tissues and a potent migration factor for endothelial cells.<sup>20,21</sup> Thus YKL-40 may function in tissue remodeling after SAH. As YKL-40 is also produced and released by exocytosis of specific neutrophil granules,<sup>1</sup> neutrophils may have

an important function in the acute phases of SAH and in the development of vasospasm.

Regardless of the source, the function of YKL-40 remains unknown. Nonetheless, the pattern of its expression in normal and diseased states suggests that it plays an important role in facilitating cell migration through the extracellular matrix and tissue remodeling at sites of inflammation.<sup>21</sup> In addition, treatment with antibiotics in patients with infections including bacterial pneumonia, and bacterial meningitis has led to normalization of serum YKL-40 within 1 week.<sup>4,5</sup>

## Limitations of this study

This preliminary study included a rather limited number of patients with aneurysmal SAH. Further studies with a large patient population should be designed in order to determine the source of YKL-40 in the CSF and serum more accurately. Furthermore, it would be important to obtain CSF and serum samples on consecutive days during the first 14 days of SAH in order better to establish whether there is a correlation between vasospasm and YKL-40 levels. It would be also possible then to speculate about the relationship between clinical features and the trend of YKL-40 levels.

## CONCLUSION

Although there are some important limitations in our study, it is clear that serum and CSF YKL-40 concentrations were markedly higher following aneurysmal SAH than in the control group. The parallel courses of serum and CSF YKL-40 indicate that in SAH, inflammation occurs in the brain and systemic circulation. Further clinical studies with a large number of patients will lead us to speculate about the source and biological function of YKL-40 and it may be used as an acute phase marker for SAH in future.

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