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Cerebrospinal Fluid Cystatin C Levels Following Treatment for Aneurysmal Subarachnoid Hemorrhage

Anevrizmal Subaraknoid Kanama Tedavisi Sonrası Beyin Omurilik Sıvısı Sistatin C Düzeyleri

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ABSTRACT

AIM: To investigate the changes of cerebrospinal fluid (CSF) cystatin C (CC) levels associated with the postoperative ischemic conditions and prognostic outcome in patients with aneurysmal subarachnoid hemorrhage (SAH).

MATERIAL and METHODS: The study group consisted of 40 patients with microsurgically clipped intracranial aneurysms (IA's) and 22 control CSF samples. In patients, CSF samples were taken from the lumbar intrathecal catheter for CC measurement, at the beginning of operation, immediately after the operation (early postoperative), and the first postoperative day (late postoperative).

RESULTS: CC levels in three periods were significantly higher in patients with Hunt-Hess scores of 4, 5 than 1, 2, 3. There was a significant difference between the CC concentrations on the first postoperative day and controls. In patients who developed focal cerebral ischemia, CC levels at early and late postoperative periods were significantly higher than the group without ischemia. In addition, patients with poor prognostic outcome (GOS score of 1, 2, 3) had significantly higher levels of CC in all three periods than that of patients with good outcome (GOS score of 4, 5).

CONCLUSION: The raised CSF CC concentrations appear to be associated with the severity of bleeding, intraoperative ischemic events and poor prognostic outcome in patients with aneurysmal SAH.

KEYWORDS: Cystatin C, Focal cerebral ischemia, Aneurysmal subarachnoid hemorrhage

ÖZ

AMAÇ: Anevrizmal subaraknoid kanamalı hastalarda (SAK), postoperatif iskemik durumlar ve prognozla ilişkili olarak beyin omurilik sıvısı (BOS) sistatin C (SC) düzeylerindeki değişiklikleri araştırmaktır.

YÖNTEM ve GEREÇLER: Çalışma grubu, mikrocerrahi olarak klibe edilmiş intrakranial anevrizmalı (İA) 40 hasta ile 22 kontrol BOS örneğini içermekteydi. Hastalarda BOS örnekleri lomber intratekal katater yoluyla ameliyatın başında, hemen sonrasında (erken postoperatif) ve ameliyat sonrası birinci günde (geç postoperatif) sağlandı.

BULGULAR: Üç dönemdeki CC düzeyleri de Hunt-Hess skoru 4, 5 hastalarda, 1, 2 ve 3 olanlara göre anlamlı olarak yüksekti. Postoperatif birinci gün SC düzeyleri ile kontrol grubu arasındaki fark istatistiksel olarak anlamlı idi. Fokal serebral iskemi gelişen hastalarda erken ve geç postoperatif dönemdeki SC düzeyleri, iskemi gelişmeyen gruba göre anlamlı olarak yüksekti. Ayrıca, kötü prognoz gösteren (GOS 1, 2, 3) hastalar, iyi prognoz gösterenlere göre (GOS 4, 5) her üç dönemde de anlamlı olarak yüksek SC konsantrasyonlarına sahipti.

SONUÇ: Anevrizmal SAK'lı hastalarda artmış BOS SC düzeyleri, kanamanın ciddiyeti, intraoperatif iskemik olaylar ve kötü prognozla ilişkili görünmektedir.

ANAHTAR SÖZCÜKLER: Sistatin C, Fokal serebral iskemi, Anevrizmal subaraknoid kanama

INTRODUCTION

Intracranial aneurysms (IA's) are the most common cause of spontaneous subarachnoid hemorrhage (SAH). SAH is the most catastrophic stroke type leading to a mortality rate over 50 % (3). Focal cerebral ischemia can occur after aneurysm surgery due to intraoperative aneurysm rupture, prolonged temporary vessel occlusion, parent artery occlusion or postoperative vasospasm. Although cytoprotective strategies are routinely used during surgery, clinical or radiographic evidence of stroke is sometimes inevitable.

Cystatin C (CC) as an inhibitor of cysteine proteases is known to play important biological roles in numerous cellular systems particularly growth promoting activity, anti-inflammatory and anti-microbial properties (16). Furthermore it is involved in many neurological diseases such as Alzheimer's disease (AD), Icelandic variant of hereditary cerebellar hemorrhage with amyloidosis (HCHWA), sporadic cerebral amyloid angiopathy (CAA), Guillain-Barre syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP), multiple sclerosis (MS) and ischemic cerebrovascular diseases (CVDs) (2, 9, 18, 19, 26, 30). Its functional importance as a neuroprotectant or mediator of neurodegeneration has also been discussed in previous experimental cerebral ischemia models (12, 21, 22).

The aim of this study was to assess the changes of cerebrospinal fluid (CSF) CC levels associated with the postoperative ischemic conditions and prognostic outcome in patients who underwent microsurgical clipping due to IA's.

MATERIAL and METHODS

Patient Population and Preoperative Evaluation

40 consecutive patients suffering from aneurysmal subarachnoid hemorrhage (SAH) were included in the study. All patients underwent a standardized intake assessment including medical history, physical examination and suitable imaging procedures. Neurological condition was assessed using validated standard grading system of Hunt and Hess scale (11). The severity of SAH in computerized tomography (CT) was recorded according to Fisher CT score (8). All patients were followed by daily transcranial Doppler (TCD) examination in terms of cerebral vasospasm. Severity of vasospasm was scored in a range of 0-3 according to Lindegaard ratio of < 3, 3-4, 4-6 and > 6 respectively (17). Pre and postoperative mean scores were revealed with the calculation of daily results. To reveal aneurysms, routine cerebral four vessel digital subtraction angiography (DSA) was performed preoperatively. Furthermore, we obtained 22 control CSF samples from the subjects in whom lumbar puncture was performed for diagnostic purposes but no hemorrhagic, infectious, neoplastic or degenerative pathology could be defined. This study protocol was approved by the local Ethics Committee at Akdeniz University School of Medicine.

Intraoperative Management

After anaesthesia procedures, a lumbar intrathecal catheter was placed into the L4-5 space. Initially, 2 cc CSF samples were taken for CC measurement. During the operation, if required, intermittent CSF drainage had been obtained in order to decrease brain retraction. Standard pterional craniotomy and microsurgical clipping were used for all the patients. In case of a needed temporary vessel occlusion, for the purpose of cerebral protection, 3 mg/kg pentothal was administered intravenously as bolus and 1.25 g/kg mannitol was immediately started as infusion before the temporary occlusion and continued during the temporary occlusion period. The patients were kept under normothermia and normotension.

Postoperative Evaluation

After surgery, a standardized medical management was carried out, including administration of calcium antagonists, volume expansion, hemodilution, maintenance of optimal general hemodynamic and medical status. CT scans were obtained from the patients at the day of operation and the first postoperative day. Daily TCD follow-up was continued in the postoperative period. Complete four-vessel angiography was performed within 2 weeks postoperatively. The outcome of each patient was assessed at hospital discharge according to the Glasgow Outcome Scale (GOS) score (1). To quantify the level of recovery patients have achieved GOS scores between 4 and 5 assigned as "good" and GOS score < 3 as "poor".

Cystatin C Measurements

2 cc CSF samples were taken from the intrathechal catheter at initial, immediately after the operation (early postoperative) and the first postoperative day (late postoperative) periods. Samples were preserved in a cold storage at-20 °C temperature. The CSF samples were separated in a cooled centrifuge at 4 °C with 3000 g and subsequently frozen at-80 °C for analysis. CC measurements were performed with a latex-enhanced reagent (N Latex Cystatin C, Dade Behring, Marburg, Germany) using a Behring BN II analyzer (Dade Behring). The interassay coefficients of variation were 4.8 % at 0.56 mg/L and 3.7 % at 2.85 mg/L.

Statistical Analysis

All data were presented as the mean ± standard deviation. Comparisons between data groups were computed from SPSS 20.0 for windows. Normality analyses was performed by Kolmogorov-Smirnov test. Nonparametric Mann-Whitney U tests were used for independent samples. In addition, nonparametric Freidman test was applied to compare with three or more dependent samples. Statistical significance was defined as a probability value of less than 0.05.

RESULTS

We studied 40 patients consecutively with microsurgically clipped IA's for a one year period. All patients were presented with SAH. The mean age was 47.35 ± 10.12. 22 patients were female and 18 were male. In addition, control CSF samples were obtained from 22 subjects (12 female, 10 male, mean age 47.13 ± 15.71). Aneurysms were located on the anterior cerebral artery (ACA) in 17, middle cerebral artery (MCA) in 16 and internal carotid artery (ICA) in seven patients. In 17 patients, cerebral vasospasm was detected by TCD with a mean score of 1.58 ± 0.77. All patients were operated with the standard pterional craniotomy and microsurgical clipping in 2-13th day of hemorrhage. In 29 patients, temporary vessel occlusion was applied with a mean time of 8.13 ± 7.6 minutes. Postoperative TCD follow-up revealed vasospasm findings in 23 patients with a mean score of 1.91 ± 0.94. 16 (40%) patients showed focal ischemia on the postoperative CT scan. Focal ischemia occurred with reasons such as intraoperative rupture, prolonged temporary occlusion, obliteration of

aneurysm with trapping procedure, perforator occlusion or poor preoperative grade in our patients. Beside the clinical findings, ischemic territory was also diagnosed with later CT scan controls. 12 of 40 (% 30) patients had a poor outcome (GOS score; 1, 2 or 3) whereas 28 (% 70) patients had a good outcome (GOS score; 4 and 5). The demographic and clinical data of all the patients were shown in Table I. The mean CSF CC levels at initial, early and late postoperative periods were 1.3 ± 0.7, 2.17 ± 1.88 and 1.96 ± 1.55 mg/L respectively. There is no significant difference between three values (Friedman test, p=0.082). Box-plot diagram shows the distributions for CC levels of controls and initial, early and late postoperative periods (Figure 1). The CSF CC levels for all patients were demonstrated in Table II. The mean CC levels of 22 control CSF samples were 1.05 ± 0.43. Late postoperative CC levels were significantly higher than that of control group (Mann-Whitney U test, $p < 0.05$). CC levels in three periods were significantly higher in patients with Hunt-Hess scores of 4, 5 than 1, 2, 3 (Mann-Whitney U test, $p < 0.05$). There was no significant difference for CC levels between the groups of Fischer scores 1, 2 and 3, 4. In patients who showed focal ischemia on later CT scan, CC levels on early and late postoperative periods were significantly higher than the group without ischemia. (Mann-Whitney U test, $p < 0.05$). There was no significant difference between CSF CC levels and presence of vasospasm on TCD. Temporary vessel occlusion times were 8.12 ± 10.75 and 4.41 ± 3.5 minutes in patients with focal ischemia positive and negative groups respectively. This difference was not found significantly. In addition, patients with poor prognostic outcome (GOS score of 1, 2, 3) had significantly higher levels of CC in all three periods than that of patients with good outcome (GOS score of 4, 5) (Mann-Whitney U test, $p < 0.05$).

DISCUSSION

Multiple physiological and pathophysiological processes in the brain are modulated by a delicate balance of protease activities and inhibitors (22). CC is a potent endogenous inhibitor of cysteine protease. It is synthesized by the choroid plexus in the central nervous system (CNS), and its concentration in the CSF is 5.5-fold of that in the serum (4). In addition, CC is also involved in immune system modulation and neuronal stem cell proliferation (15, 27, 31).

As found in many experimental studies, proteolytic activity contributes to post-ischemic neuronal degeneration (10, 14, 23-25, 29, 33, 34). During cerebral ischemia, with the rise of intracellular calcium, proteolytic enzymes are released from the lysosomes and cause degradation of cellular components (32). Among lysosomal proteases, cathepsin B can also activate the caspases which play an essential role during ischemic period by inducing inflammation and apoptosis (6, 7). The increase in CC concentration occurs in various

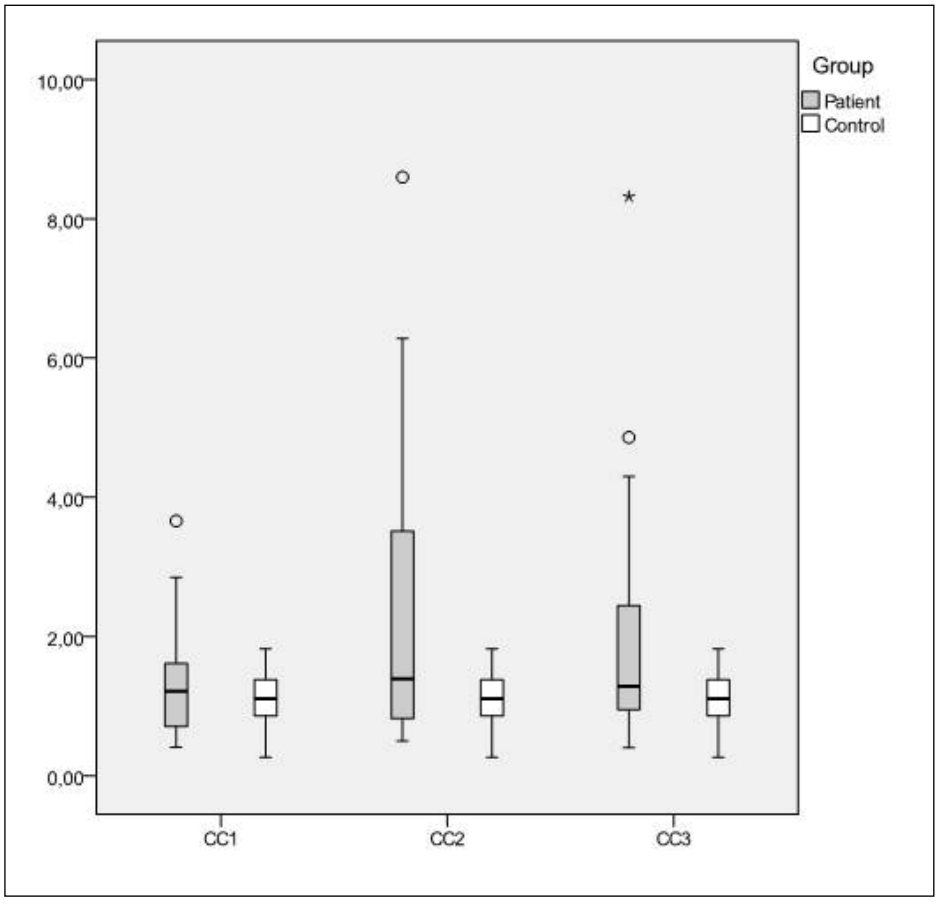

Figure 1: Box-plot diagram shows the distributions for CC levels of controls and initial (CC1), early (CC2) and late postoperative periods (CC3).

Table I: Demographic and Clinical Data of all the Patients

Patient No	Age	Sex	Hunt Hess Grade	Fisher CT Grade	Aneurysm Location	Preop. TCD Score	Temp. Occlusion Vessel/ Time (min)	Postop. TCD Score	Focal ischemia on Postop. CT	GOS Score
1	50	M	2	4	MCA	1	M_1 / 7	1	-	5
2	37	F	2	2	MCA	3	M_1 / 5	3	-	5
3	32	M	1	3	MCA	1	M_1 / 40	3	+	3
4	55	F	2	3	MCA	0	M_1 / 5	0	-	5
5	44	F	2	4	MCA	2	M_1 / 3	1	-	5
6	62	M	2	3	AComA	1	Both A_1 / 2	1	-	5
7	45	F	2	2	ICA - PComA	1	-	0	-	5
8	63	F	3	4	AComA	2	Both A_1 / 4	3	+	2
9	50	M	5	4	AComA	1	Both A_1 / 5	0	+	1
10	53	F	3	4	AComA	0	Both A_1 / 8	0	-	5
11	55	F	1	1	AComA	0	Both A_1 / 8	0	+	2
12	49	M	1	3	MCA	0	M_1 / 2	2	-	5
13	54	M	2	3	AComA	0	Both A_1 / 25	3	+	2
14	41	M	3	2	MCA	0	-	0	-	5
15	41	M	3	4	AComA	0	-	0	-	4
16	32	M	2	2	AComA	0	Both A_1 / 5	0	-	5
17	67	M	1	2	MCA	0	M_1 / 9	0	-	5
18	48	F	3	2	MCA	1	M_1 / 4	1	-	5
19	45	F	1	3	AComA	1	-	2	+	3
20	60	F	1	1	ACA-A1	0	-	0	+	1
21	36	F	2	2	AComA	0	Both A_1 / 4,5	0	-	5
22	52	M	5	4	AComA	3	Both A_1 / 3	3	+	1
23	49	F	1	1	AComA	0	-	0	-	5
24	55	F	5	4	MCA	3	-	3	+	1
25	59	F	2	4	AComA	2	Both A_1 / 6,5	3	+	1
26	36	M	2	2	MCA	0	M_1 / 12	0	-	5
27	47	F	2	4	AComA	1	Both A_1 / 13	1	+	4
28	59	F	1	1	MCA	0	M_1 / 7	1	-	5
29	39	M	1	2	AComA	0	Both A_1 / 8	1	+	5
30	52	M	1	1	MCA	0	M_1 / 12	1	+	5
31	31	M	1	2	AComA	0	Both A_1 / 7	0	-	5
32	43	F	2	4	MCA	0	M_1 / 6	0	+	5
33	31	F	2	3	MCA	0	M_1 / 9,5	2	-	5
34	42	M	1	1	MCA	0	M_1 / 8	0	-	5
35	29	F	1	1	ICA-Paraclinoid	0	-	0	-	5
36	48	F	1	1	ICA-Paraclinoid	0	ICA / 4	3	-	5
37	55	F	2	2	ICA-Paraclinoid	1	-	1	-	5
38	37	M	3	3	ICA – PComA	2	-	3	+	3
39	46	F	1	3	ICA – PComA	1	ICA / 4	1	-	5
40	65	M	1	2	ICA – PComA	0	-	1	+	3

***M:** Male, **F:** Female, **CT:** Computed Tomography, **Preop:** Preoperative, **TCD:** Transcranial Doppler, **Temp:** Temporary, **Postop:** Postoperative, **GOS:** Glasgow Outcome Scale, **MCA:** Middle Cerebral Artery, **AComA:** Anterior Communicating Artery, **ICA:** Internal Carotid Artery, **PComA;** Posterior Communicating Artery.*

Table II: Cerebrospinal Fluid Cystatin C Levels (mg/L) at Initial, Early and Late Postoperative Periods in all of the Patients

Patient No	CSF Cystatin C levels (mg/L)		
	Initial	Early Postop.	Late Postop.
1	1.35	2.16	2.46
2	1.18	0.81	1.07
3	0.78	3.75	1.24
4	1.44	1.59	1.51
5	1.16	0.77	0.89
6	1.13	1.3	1.16
7	1.11	0.83	0.88
8	2.38	3.77	2.07
9	1.72	8.6	8.32
10	0.42	0.83	1.51
11	0.66	1.79	1.38
12	1.87	3.09	4.01
13	1.90	1.89	2.05
14	1.51	0.94	0.96
15	0.53	0.63	0.74
16	1.35	0.90	1.26
17	1.12	1.08	1.18
18	1.48	2.10	0.94
19	0.42	1.48	1.10
20	0.92	1.55	1.82
21	0.59	0.55	0.59
22	2.21	4.72	3.41
23	0.65	1.1	0.87
24	3.66	5.16	4.86
25	2.85	4.08	3.5
26	0.41	1.02	0.4
27	2.35	3.87	3.1
28	1.09	1.16	0.98
29	1.22	3.28	2.43
30	0.48	4.9	2.25
31	1.31	0.62	1.22
32	0.69	0.66	1.14
33	0.72	0.5	0.65
34	1.41	1.05	1.3
35	1.28	0.55	0.76
36	1.73	1.86	1.75
37	1.21	0.55	0.85
38	1.93	4.56	3.9
39	0.69	0.73	3.66
40	1.33	6.28	4.3

CSF: *Cerebrospinal fluid,* ***Postop:*** *Postoperative.*

injured and/or degenerating animal brain models (16). This overexpression in response to injury represents a critical role of CC in regulating protease activity in pathological process. In a study of Palm et al., CC was upregulated in hippocampus CA1 pyramidal cells following transient forebrain ischemia in rats (22). These findings suggested that CC possibly provided an endogenous mechanism of neuroprotection to limit cysteine proteolytic activity in degenerating neurons after ischemia. The inhibition of cathepsins B and L by CC administration was also demonstrated to protect CA1 cells after global ischemia in primates (34). In contrast, intrahippocampal application of CC in rats caused neuronal death (20). CC was manifested to induce autophagy as a cell survival mechanism in cell cultures exposed the cytotoxic challenges (28). This evidence may be respected the protective role of CC independent of its protease inhibition. Olsson et al. studied the neuroprotective effect of endogeneous CC during focal and global cerebral ischemia in mice with CC gene knocked out (21). Their results indicate that gene deletion of CC causes the absence of endogeneous protease inhibition and aggravates brain damage following focal ischemia with increased proteolytic activity. But on the other hand, following global ischemia, because of the absence of its unfavorable properties such as, amyloidogenesis, immunomodulatory effects and selective effects on neuronal structures overcome the antiproteolytic activity and diminish the destruction. Contradictory reports point out the importance of maintaining delicate balance between proteases and their inhibitors in order to obtain effective neuroprotection.

Although given many experimental studies, we found only two clinical reports in which CSF CC levels were studied in ischemic CVD. In one of these reports, Umegae et al. found the low levels of CSF CC in ischemic white matter lesions and they explained this decrease with the reduced number of astrocytes that secrete cystatin C in response to the stimuli of proteases and inflammatory cytokines (30). In another study of Kálmán et al., CSF CC levels were measured in patients with ischemic type of vascular dementia and found in the normal range (13). Our study demonstrates some interesting clinical results, compared to levels of CSF CC levels in patients with aneurysmal SAH. Firstly, high levels of CSF CC levels appeared to be associated with the clinical severity of SAH. Secondly, in patients who developed focal cerebral ischemia, an increase in CC concentrations was observed beginning from the early postoperative period. Finally, high levels of CSF CC were also associated with the poor prognostic outcome. The origin of raised CSF CC concentrations may be explained with the increased synthesis and secretion from astrocytes or choroid plexus cells in response to injury (5). With the increased CC levels, a possible neuroprotective mechanism may be processed in order to maintain the cell survival during brain injury. In addition, CC could be interpreted as an early biomarker to predict the development of focal cerebral ischemia and prognostic outcome. This study, as the first clinical trial to investigate the changes of CSF CC levels in such a patient group may lead to future studies.

Further experimental and clinical investigations in larger series should be conducted to determine whether CC plays a neuroprotective role or can be a predictive biomarker in ischemic cascade.

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