Serum hyaluranidase levels in patients with aneurysmal subarachnoid haemorrhage

Isman F, Kucur M, Tanriverdi T, Kacira T, Sanus G Z, Kemerdere R, Hanimoglu H, Kaynar M Y

ABSTRACT

Introduction: The purpose of this study was to investigate the time course(s) of the serum hyaluronidase levels in patients with aneurysmal subarachnoid haemorrhage and to show whether there is a correlation between symptomatic vasospasm and serum levels of hyaluronidase.

Methods: This prospective, open, non-randomised clinical study consisted of 20 patients with aneurysmal subarachnoid haemorrhage, and eight patients with normotensive hydrocephalus who served as the control group. Serum hyaluronidase levels were detected within the first three days, days five and seven after aneurysmal subarachnoid haemorrhage, and the results were compared with those from the control group. The results were also compared with those of the clinical parameters, including the patient's outcome at six months and symptomatic vasospasm.

Results: Mean serum hyaluronidase levels were higher on days five and seven, and comparisons with either day five (p-value is 0.001) and/or day seven (p-value is 0.00001) showed a statistical difference between subarachnoid haemorrhage and controls. However, no relationship was found between elevated serum hyaluronidase levels and the clinical parameters including symptomatic vasospasm (p-value is greater than 0.05) and outcome at sixth months (p-value is greater than 0.05).

Conclusion: Our results indicate that serum hyaluronidase is elevated in the acute stage(s) of subarachnoid haemorrhage; however, no difference was found between serum hyaluronidase levels and subarachnoid haemorrhage severity. Clinical studies with larger population of patients with aneurysmal subarachnoid haemorrhage are required.

Keywords: aneurysm, extracellular matrix, hyaluronidase, subarachnoid haemorrhage, vasospasm

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INTRODUCTION

Subarachnoid haemorrhage (SAH) is a leading cause of death and severe disability in the world, and usually occurs as a result of an aneurysm rupture, resulting in profound inflammatory reactions on the endothelium of the ruptured vessel wall, which ultimately may cause vasospasm (VS), a deleterious consequence of SAH. The survival of the neurons, particularly in peri-infarcted regions due to VS, determines the extent of the patient's recovery. Many efforts have been made to understand the pathophysiological mechanism(s) behind the inflammatory processes of VS seen after SAH and encouraging results have been demonstrated.[1-3] It has been suggested that vascular endothelium is the most important structure where inflammation after SAH takes place.[1] Future improvements in the treatment or the prevention of VS after aneurysmal SAH will probably come from the advances in our understanding of its pathophysiological mechanisms. The pivotal role of inflammation as a contributing factor to VS has been discussed extensively in some review articles.[2-4]

Hyaluronidase (Hyal) is a catabolic enzyme that degrades hyaluronan (HA). HA is a highly charged, high-molecular mass polyanion of repeating disaccharides units found in the extracellular matrix (ECM) of soft tissues.[5] There are six Hyal-like sequences in the human genome, Hyal-1 being the only such enzyme in the human circulation.[6] Increases in HA production and turnover are often associated with increases in Hyal levels. Several recent studies have shown that elevated activity of Hyal in tissues is considered to be implicated in the regeneration and restoration processes in tissues, in the increased vascularisation[7-10] and in the uncontrolled cellular growth rate of tumours.[11-15] However, Hyal levels have not been studied previously in patients with SAH, although only three reports have evaluated HA and Hyal in patients with

Department of Biochemistry, Taksim Teaching and Research Hospital, Istanbul, Turkey

Isman F, MD
Biochemist

Fikret Biyal Central Research Laboratory, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey

Kucur M, MD
Biochemist

Department of Neurosurgery

Tanriverdi T, MD
Neurosurgeon

Kacira T, MD
Neurosurgeon

Sanus G Z, MD
Neurosurgeon

Kemerdere R, MD
Resident

Hanimoglu H, MD
Resident

Kaynar MY, MD
Professor

Correspondence to:
Dr Taner Tanriverdi,
105 Rue Milton Ouest,
Apt # 309,
Montreal,
Quebec H2X 1V4
Canada
Tel: (90) 533 3100 753
Fax: (90) 212 4143 400
Email: tanerato2000@yahoo.com

Table I. Clinical characteristics of patients with subarachnoid haemorrhage.

Patient no.	Age (years) /gender	Hunt -Hess	Fisher grade	Aneurysm location	Symptomatic vasospasm	Treatment	GOS at six months
1	65/F	II	2	ACoA	Yes	Clip	1
2	45/M	III	3	ACoA	Yes	Clip	3
3	35/M	II	2	ICA	No	Clip	5
4	65/F	V	4	ACoA	Yes	Coil	1
5	62/M	III	4	PCoA	Yes	Clip	1
6	40/F	II	4	OphA	Yes	Clip	4
7	32/M	II	2	ACoA	No	Coil	5
8	48/M	III	2	PCoA	Yes	Clip	5
9	37/F	II	1	ACoA	Yes	Coil	5
10	32/M	III	2	ACoA	No	Clip	1
11	56/F	II	2	OphA	No	Clip	5
12	23/F	II	2	ICA	No	Clip	5
13	57/M	III	3	ACoA	Yes	Clip	3
14	54/M	II	3	OphA	Yes	Clip	2
15	44/M	III	1	PCoA	Yes	Clip	4
16	52/F	II	1	ACoA	No	Coil	5
17	57/M	II	3	ICA	Yes	Clip	2
18	56/F	III	3	ACoA	Yes	Coil	1
19	45/F	III	3	ACoA	Yes	Coil	1
20	21/M	I	1	BA	Yes	Coil	5

ACoA: anterior communicating artery; BA: basillar artery; ICA: internal carotid artery; OphA: ophthalmic artery; PCoA: posterior communicating artery.
GOS at six months: (1) death; (2) persistent vegetative state; (3) severe disability; (4) moderate disability; and (5) good recovery

ischaemic stroke.[16-18] Thus, we hypothesised that Hyal turnover increases in brain tissue after SAH, and our aim in the present study was to investigate the serum levels of Hyal during the various time points in patients after aneurysmal SAH. Furthermore, we tried to show whether symptomatic vasospasm (S-VS) had any effect on serum Hyal levels, and on outcome at six months after the ictus.

METHODS

Ethical approval for this prospective, non-randomised, open study was obtained from the Human Investigations Committee at Istanbul University, and informed consent was sought from all patients, or the next-of-kin, if the patient was unconscious. We studied the patients referred to our neurosurgical unit from February to December 2004 with SAH established by computed tomography (CT) (Somatom Plus CT, Siemens, Berlin, Germany). We excluded patients who had any kind of infection in which Hyal had been involved at the time of serum collection. Patients who showed S-VS three days after the onset of haemorrhage were transferred to the intensive care unit and serial blood samples were obtained for the possibility of infection.

In this study, S-VS was defined as delayed neurological deterioration that could not be attributed to rebleeding, hydrocephalus, intracerebral haematoma, electrolyte abnormalities, or toxic and metabolic factors. It usually follows a typical course, with gradual blunting of the level of consciousness starting 3–10 days after SAH, followed within hours by focal neurological deficits. At the first suggestion of S-VS, a central venous catheter was introduced; a pulmonary artery catheter (Swan-Ganz catheter) was used if the patient had pre-existing cardiac or pulmonary disease. Simultaneously, necessary diagnostic tests were conducted to rule out other causes of delayed neurological deterioration. Once the diagnosis of S-VS was established, hypervolaemic haemodilution was initiated under the guidance of a consulting cardiologist. No digital cerebral angiography was carried out in this study, but an S-VS was considered to have occurred based on the patient's clinical deterioration and the low density areas (new infarct) on CT. The sensitivity and/or specificity of clinical assessment predicting S-VS has been documented clearly in the literature.[1,3,4,19-21] The sole inclusion criterion was admission of the patients to our unit within the first three days post-SAH (no VS was expected to occur during this time).

This study included 20 patients with aneurysmal SAH and eight control patients with normal pressure hydrocephalus without any other known central nervous system diseases. Among the patients with SAH, ten had anterior communicating artery (ACoA) aneurysms, three had posterior communicating artery (PCoA) aneurysms, three had internal carotid artery (ICA) aneurysms, three had ophthalmic artery (OphA) aneurysms, and one had basilar artery (BA) aneurysm. The average age of the patients with SAH was 46.30 ± 13.20 (range 21–65) years. Glasgow Outcome Scale (GOS)[19] at hospital discharge showed good recovery in eight, moderate disability in two, and severe disability in two patients. Six patients

Table II. Clinical characteristics of control patients.

Control case no.	Age (years)/gender	Diagnosis
1	67/F	NPH
2	72/M	NPH
3	71/F	NPH
4	68/M	NPH
5	47/F	NPH
6	64/M	NPH
7	59/M	NPH
8	80/F	NPH

NPH: normal pressure hydrocephalus

died before discharge and two demonstrated a persistent vegetative state. GOS at six months was dichotomised into favourable or good (good outcome, moderate disability) and unfavourable or bad (severe disability, persistent vegetative state and death) outcomes.[22]

The average age of the control group was 66.0 ± 9.82 (range 59–80) years. All had normotensive hydrocephalus. Summary of demographical data of the patients with SAH and controls are provided in Tables I and II, respectively. 68 serum samples were assayed for Hyal. For each patient, serial blood samples were collected within the first three days, on day five and day seven post-SAH. Blood samples were collected via venipuncture, while routine blood analysis was performed in the preoperative period. The samples from the control group were obtained once. As soon as possible, each 10 ml blood specimen was centrifuged (Allegra X-22, catalog no: BK392184, Northbrook, IL, USA) at 10,000 rpm for 15 minutes and the supernatant was stored at minus 70°C until assayed (Labrebco, NU-9483gc, Plymouth, MN, USA).

The method of Natowicz and Wang[23] was used. Briefly, 10 ml serum was incubated with 250 ml of buffered substrate solution (0.10 mol/L sodium formate, pH 3.9 containing 0.1 mol/L sodium chloride, 250 mg/L HA and 1.5 mmol/L saccharic acid 1, 4-lactone) for four hours at 37°C. The enzyme reaction was specifically terminated by the addition of 50 ml of 0.8 mol/L potassium tetraborate at pH 9.1 to each sample. The tubes were heated for three minutes in a boiling water bath and cooled in tap water. The p-dimethylaminobenzaldehyde reagent (1.5 ml), prepared as described by Natowicz and Wang, was added to each sample. These samples vortexed, heated at 37°C for 20 min, briefly centrifuged and read at 585 nm. Consequently, the reaction product "reducing N-acetylglucosamine termini" was determined. Blanks for the reaction consisted of tubes in which the buffered substrate was incubated for four hours at 37°C in the absence of serum and which subsequently received potassium tetraborate, then serum and then treated as mentioned above. A standard curve was formed by using known concentrations of N-acetylglucosamine. In this method, a unit of Hyal activity was defined as the production of a micromole of reaction product (reducing terminal N-acetylglucosamine) per min

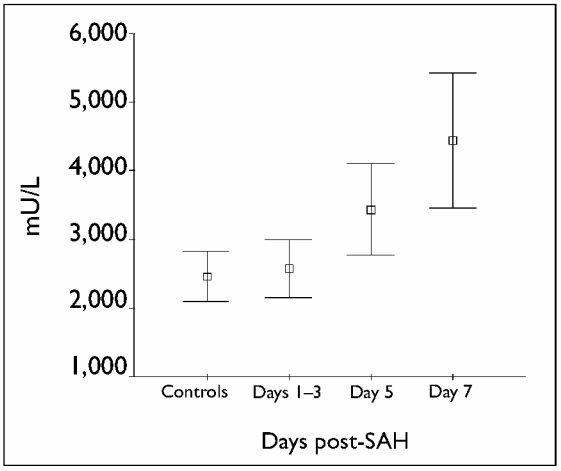

Fig. 1 Graph shows the comparison of levels of hyaluronidase in the serum of patients with subarachnoid haemorrhage and controls. Squares represent the means ± standard errors of the means, and bars denote the range of values. The differences in the levels of this enzyme between the two groups of patients were significantly different on days five ($p = 0.001$) and seven ($p = 0.00001$) post-SAH, but no significance was found within the first three days ($p = 0.26$). Comparisons of the elevated levels between three time points after SAH showed marked difference ($p < 0.002$).

at 37°C. The results were expressed as mU/L. Data were analysed using the Statistical Package for Social Sciences version 11.0 (SPSS Inc, Chicago, IL, USA). Statistical analysis was performed using the nonparametric Mann-Whitney U test. A probability value of less than 0.05 was considered to be statistically significant.

RESULTS

60 serum samples from the patients with SAH and eight serum samples from the control group were obtained for this prospective clinical study. The samples were tested for Hyal. The summary of the results in the SAH and control groups are provided in Table III. The serum levels of Hyal markedly differed between the SAH and control groups. In the control group, the mean concentration of this enzyme was 2,450.7 ± 517.7 mU/L. In contrast, serum samples from all patients with SAH had higher Hyal levels during the first three days and day five and day seven post-SAH. The mean values in this group were 2,772.2 ± 664.2 mU/L within first three days, 3,651.9 ± 774.4 mU/L on day five and 4,786.6 ± 1,259.4 mU/L on day seven. The difference between the two groups was statistically significant on days five and seven post-SAH ($p = 0.001$ and 0.00001, respectively). The mean elevated levels of Hyal measured within the first three days of SAH and controls did not reach statistically significant difference ($p = 0.26$). The levels showed a trend of constant increase toward day seven post-SAH (by which time the risk of VS reached its peak). Furthermore, when comparing each elevated levels obtained in three time points after SAH, marked statistically significant difference was found ($p < 0.003$). The time courses of Hyal in the serum of patients with SAH and controls are shown in Fig. 1.

Table III. Mean (± standard deviation) serum concentrations of hyaluronidase in patients with subarachnoid haemorrhage and controls.

Parameter	Hyal (mU/L)	†p	S-VS$^+$	S-VS$^-$	‡p	GO	BO	§p
Controls	2,450.7 ± 517.7							
Patients								
Day ≤ 3	2,772.2 ± 664.2	0.26	-	-	-	2,774.3 ± 435.6	27,70.1 ± 861.1	0.70
Day 5	3,651.9 ± 774.4	0.001	3,671.07 ± 887.4	3,607.3 ± 477.6	0.80	3,801.3 ± 619.9	3,502.6 ± 912.2	0.38
Day 7	4,786.6 ± 1,259.4	0.00001	4,646.2 ± 1,228.4	5,114.0 ± 1,385.6	0.62	4,934.3 ± 920.5	4,638.9 ± 1,566.1	0.54

Hyal: hyaluronidase; S-VS: symptomatic vasospasm; GO: good outcome (at six months); BO: bad outcome (at six months)
† Patients vs. controls
‡ Patients with S-VS vs. those without
§ Patients with GO vs. BO

In this study, 14 patients with SAH experienced S-VS during the hospital stay. We compared the mean serum levels of Hyal for each related day post-haemorrhage between the patients who had S-VS and those who had no S-VS, in order to see whether if S-VS had any effect on serum levels of Hyal. We found that there was no statistical difference between two groups regarding S-VS. Table III shows the summary of statistical results. Ten patients showed a favourable (good) outcome at six month follow-up. When we compared the mean serum levels between the patients with favourable and unfavourable outcomes, no marked difference was found ($p > 0.05$) (Table III). In this study, the SAH group consisted of 11 male and nine female patients with a mean age of 46.3 ± 13.20 years, and the control group included four males and four females with a mean age of 66.0 ± 9.82 years. There was a statistically significant difference between each group with regard to age ($p = 0.001$). Age, gender, Fisher grade on CT, Hunt-Hess grade at presentation had no effect on elevated levels of Hyal in SAH patients ($p > 0.05$).

DISCUSSION

The main finding that can be drawn from this clinical study is that Hyal activity is elevated in the early period, and this elevation is striking on day seven of the aneurysmal SAH, by which time the risk of VS reaches its corresponding peak.[1-3] There have been limited numbers of studies regarding ECM proteins and/or enzymes release after cerebrovascular accidents. Searching the literature showed only three reports which evaluated the levels and/or role of either HA or Hyal activity in ischaemic stroke.[16-18] Yasuda et al investigated whether Hyal administration could alter the size, morbidity, and mortality of experimental acute cerebral infarctions induced by the left common carotid artery occlusion in a large population of Mongolian gerbils. They found significantly decreased infarct size and mortality rate in Hyal-treated gerbils compared to controls.[18] Al Qteishat et al evaluated HA expression and its degradation by Hyal in ischaemic brain tissue in a rat model of cerebral ischaemia induced by middle cerebral artery occlusion. The authors showed up-regulation of Hyal-1 and -2 between one hour and 21 days after stroke. Furthermore, the hyaladherins, a HA receptor, also increased after stroke. By using immunohistochemistry, they showed association of Hyal-1 and -2 and hyaladherins with neurons in the infarcted and peri-infarcted regions and Hyal-1 with microvessels. They suggested that HA synthesis and degradation in the stroke hemisphere might have an impact on neuronal survival, angiogenesis and general tissue remodeling after stroke.[17]

In a recent study, Al Qteishat et al demonstrated changes in HA production and metabolism following ischaemic stroke in human subjects.[16] They measured serum levels of HA, Hyal and hyaluronic acid synthase (HAS), an enzyme maintaining the serum levels of HA in the body, on admission, and on days three, seven and 14 after stroke, in 54 patients, and compared the results with 24 healthy, age-matched control serum samples. They found that the concentrations of all molecules were increased in the serum after stroke. Hyal activity reached its peak on day three after stroke and was still elevated compared with controls even after 14 days. Furthermore, Hyal was also up-regulated in stroke-affected tissues together with HA and HAS. They suggested that oligodendrocytes could be a potential source of Hyal secretion after stroke and concluded that significant elevation in the enzymes responsible for HA synthesis and degradation, together with up-regulation of HA receptors reflect tissue remodelling after stroke and be partially responsible for increased angiogenesis and neuronal migration. Increased HA and Hyal production and HA degradation have also been demonstrated in injured endothelium of aorta in rats.[7,8] In these studies, it has been stated that HA is synthesised by the arterial cells of the injured aorta in a high molecular mass form and then degraded by Hyal to smaller forms, namely HA-oligosaccharides (O-HA). O-HAs have been found to stimulate angiogenesis *in vivo* and proliferation of endothelial cells in culture but this effect was not shown with native HA.[24]

Overall, studies suggest that Hyal is also expressed in the endothelium of injured vessels and may have a role in remodelling. Rapid increases in circulating levels of HA and Hyal have been demonstrated in patients with rheumatoid arthritis,[25] liver disease[9] as well as in cancer

patients.[4,26] Elevated HA levels have been shown to be an early hallmark of rheumatoid arthritis[25] and toxic liver injury.[9] In this context, it might be possible to use Hyal levels in serum as a serum marker for VS in SAH. The answer to this question requires further clinical studies, including larger population of SAH patients. There are limitations of this preliminary study. Firstly, the study should also have evaluated HA levels. Secondly, the study should have included a larger population of patients to investigate whether ECM proteins were associated with VS. Finally, the control group should have been chosen from healthy, age-matched subjects. In conclusion, serum Hyal activity is increased in the early period of aneurysmal SAH and reaches its peak level on day seven post-SAH (by which time the risk of VS reaches its peak). Determination of serum Hyal or HA levels may provide clinical parameters for assessing disease status, as well as shed new insights into the pathophysiological mechanisms of VS. Further experimental and/or clinical studies are required in order to ascertain the role of ECM proteins in patients with SAH.

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