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Cerebrospinal Fluid and Serum Chitotriosidase Levels in Patients with Aneurysmal Subarachnoid Haemorrhage: Preliminary Results

Anevrizmalı Subaraknoid Kanamalı Hastalarda Beyin Omurilik Sıvısı ve Serum Kitotirosidaz Seviyeleri: Öncü Sonuçlar

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ABSTRACT

AIM: The purpose of this study was to investigate the time course(s) of the cerebrospinal fluid and serum chitotriosidase changes in patients with aneurysmal subarachnoid hemorrhage and to show whether cerebrospinal fluid and/or serum chitotriosidase levels might be used as a specific marker for disease severity.

MATERIAL and METHODS: Chitotriosidase in the cerebrospinal fluid and serum was measured within the first 3 days, at Day 5 and at Day 7 after aneurysmal subarachnoid hemorrhage in 20 patients, and the results were compared to 8 patients with normotensive hydrocephalus.

RESULTS: Mean cerebrospinal fluid chitotriosidase levels were found to be higher on days 5 and 7 of subarachnoid hemorrhage and the serum levels were always higher than controls at all times in subarachnoid hemorrhage patients. However, no relationship was found between elevated chitotriosidase levels and the clinical parameters including symptomatic vasospasm and outcome at 6 months.

CONCLUSION: Results indicate that chitotriosidase is elevated in the acute stages of subarachnoid hemorrhage but is not a specific marker of subarachnoid hemorrhage severity.

KEY WORDS: Aneurysm; Chitotriosidase; Inflammation, Macrophage, Subarachnoid hemorrhage

ÖZ

AMAÇ: Bu çalışmada, anevrizmaya bağlı subaraknoid kanamalı hastalarda beyin omurilik sıvısı ve serum kitotirosidaz seviyelerinin zaman içerisindeki değişimlerinin değerlendirilmesi ve eğer varsa beyin omurilik sıvısı veya serum kitotirosidaz seviyeleri ile hastalık ciddiyeti arasındaki ilişkinin ortaya konması amaçlanmıştır.

YÖNTEM ve GEREÇLER: Beyin omurilik sıvısı ve serum kitotirosidaz seviyeleri, anevrizmaya bağlı subaraknoid kanamalı 20 hastada subaraknoid kanamanın ilk 3 günü, 5. ve 7. günlerinde ölçülmüş ve sonuçlar normal basınçlı hidrosefali nedeniyle ameliyat edilen 8 hastanın sonuçlarıyla karşılaştırılmıştır.

BULGULAR: Kontrol seviyelerle karşılaştırıldığında, ortalama beyin omurilik sıvısı kitotirosidaz seviyeleri subaraknoid kanamanın 5. ve 7. günlerinde yüksek çıkmıştır. Serum seviyeleri ise ölçülen tüm zamanlarda hastalarda yüksek bulunmuştur. Bununla birlikte, artmış kitotirosidaz seviyeleri ile klinik parametreler (semptomatik vazospazm, 6. ay klinik sonuçlar) arasında herhangi bir bağlantı bulunamamıştır.

Sonuç: Sonuçlar kitotirosidazo seviyelerinin subaraknoid kanamanın akut döneminde artığını fakat subaraknoid kanama derecesiyle ilgili özel bir molekül olmadığını göstermiştir.

ANAHTAR SÖZCÜKLER: Anevrizma; Enflamasyon; Kitotirosidaz; Makrofaj, Subaraknoid kanama

INTRODUCTION

Subarachnoid hemorrhage (SAH) following an intracranial aneurysm rupture is significant health care problem and delayed ischemic neurological deterioration due to cerebral vasospasm (VS) still remains a challenging problem. Although great advances in the treatment of SAH and VS have occurred over the past three decades, the outcome still remains poor. The molecular mechanisms participating in the development of VS have not clearly been elucidated yet. However; it is strongly believed abnormal brutal contact of the extraluminal wall of the arteries with all components of blood, such as erythrocytes, leukocytes, platelets and plasma components occurs after SAH, Oxyhemoglobin from the erythrocytes after SAH initiates a cascade of complex reactions of inflammation on the vascular endothelium that generates new diffusible agents including enzymes that cause morphological and functional modifications in cerebral arteries. Further improvements in both the treatment and prevention of VS will probably result from advances in our understanding of its pathophysiological mechanisms propagated mainly by inflammation. The pivotal role of inflammation as a contributing factor in VS has been discussed very well in some review articles (14,17,21).

Chitotriosidase (Chito) is a human chitinase member of family 18 glycosyl hydrolases and is selectively and predominantly secreted by activated macrophages(18). Recent studies have demonstrated that activated macrophages massively express Chito in several pathological conditions and serum Chito activity has been correlated with the severity of various neurological diseases (3,9,11,13, 20,24,26) and also with atherosclerosis (2,7). Thus, it has been suggested that Chito activity is not only a biochemical marker of macrophage activation in several diseases but can also be regarded as an important player in inflammation.

In this prospective clinical study, we aimed to measure the time course(s) of Chito in the cerebrospinal fluid (CSF) and serum of patients with aneurysmal SAH as Chito is regarded as an important agent of inflammation, and has been shown to play a key role in the development of VS after SAH. Our hypothesis is that if Chito correlates with the severity of some neurological diseases, it would be important to measure Chito levels in either CSF or serum of patients with aneurysmal SAH and the levels might correlate with symptomatic vasospasm (S-VS). The results of SAH patients and controls, which included the patients who underwent ventriculo-peritoneal shunting due to normal pressure hydrocephalus, were also compared.

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 the patients referred to our neurosurgical unit from February to December 2004 where SAH was established by computerized tomography. We excluded patients who had any kind of infection at the time of CSF and serum collection, in which Chito may play a part. The sole inclusion criterion was the admission of the patients to our unit within the first 3 days of SAH during which no VS is expected to occur.

Demographics of patient and control groups

For this study, we included 20 patients with aneurysmal SAH while 8 patients with normal pressure hydrocephalus without any other known central nervous system (CNS) disease served as the subjects. (Table I) demonstrates the demographic and clinical data of SAH patients and controls. The average age of the SAH patients was 46.30±13.20 years (range 21-65 years). The Glasgow Outcome Scale (GOS) scores at hospital discharge showed good recovery in eight, moderate disability in two and severe disability in two patients. Six patients died before discharge and, two demonstrated a persistent vegetative state. GOS at 6 months was dichotomized into favorable or good outcome (moderate disability) and unfavorable or bad outcome (severe disability, persistent vegetative state and death).

The average age of the control group was 66.0±9.82 years (ranged 32-65 years). All had normotensive hydrocephalus and underwent ventriculo-peritoneal shunting.

Specimen handling

Sixty-eight CSF and 68 serum samples were assayed for Chito. For each patient, serial blood and CSF samples at the same time were collected within

Table I: Clinical data of the SAH patients and the control group.

Patient	Age/Sex	HH	FG	VS	Aneurysm	Treatment	GOS
1	65/F	II	2	Yes	ACoA	Clip	1
2	45/M	III	3	Yes	ACoA	Clip	3
3	35/M	II	2	No	ICA	Clip	5
4	65/F	V	4	Yes	ACoA	Coil	1
5	62/M	III	4	Yes	PCoA	Clip	1
6	40/F	II	4	Yes	OphA	Clip	4
7	32/M	II	2	No	ACoA	Coil	5
8	48/M	III	2	Yes	PCoA	Clip	5
9	37/F	II	1	Yes	ACoA	Coil	5
10	32/M	III	2	No	ACoA	Clip	1
11	56/F	II	2	No	OphA	Clip	5
12	23/F	II	2	No	ICA	Clip	5
13	57/M	III	3	Yes	ACoA	Clip	3
14	54/M	II	3	Yes	OphA	Clip	2
15	44/M	III	1	Yes	PCoA	Clip	4
16	52/F	II	1	No	ACoA	Coil	5
17	57/M	II	3	Yes	ICA	Clip	2
18	56/F	III	3	Yes	ACoA	Coil	1
19	45/F	III	3	Yes	ACoA	Coil	1
20	21/M	I	1	Yes	BA	Coil	5

Control	Age/Sex	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

ACoA: anterior communicating artery; BA: basilar artery; FG: Fisher grade; HH: Hunt-Hess grade; ICA: internal carotid artery; NPH: normal pressure hydrocephalus; OphA: ophthalmic artery; PCoA: posterior communicating artery; VS: symptomatic vasospasm.
GOS (Glasgow Outcome Scale) at 6 months: (1) death, (2) persistent vegetative state, (3) severe disability, (4) moderate disability, and (5) good recovery.

3 days (day 1 to 3), 5th and 7th day of SAH. Blood and CSF samples were collected via venipuncture and lumbar puncture, respectively. From the control group, blood samples were collected via venipuncture, and CSF samples were obtained while the ventriculo-peritoneal shunting was performed. The 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 was stored at -70 °C until assayed.

Measurement of Chito

Chito activity was measured by incubating 5 μL serum with 100 μL of 22 μmol/L 4-methylumbelliferyl-β-D-N,N′,N′′.triacetylchitotriose (MU-(β-GlcNAc)3; Sigma Chemical Co., St. Louis, MO) as substrate in Mellvain's phosphate-citrate buffer, pH 5.2 for 1 h at 37°C, modified from Hollak et al. (18). The reaction was terminated by adding 120 μL 0.5 mol/L Na2CO3-NaHCO3 buffer, pH 10.7, and the fluorescence of 4-methylumbelliferone was measured with a fluorimeter (Titertek; excitation

355nm, emission 460 nm). Chito activity was expressed as mmol/mL/h.

Statistical Analysis

We used a commercially available statistical software package (SPSS version 13.0; Inc., Chicago, IL) for all statistical analysis. The mean ± standard deviations (±SD) were calculated for each Chito value. The nonparametric Mann-Whitney U test was used as a statistical method for all comparisons. The nonparametric Spearman's correlation test was used for correlations. Differences were considered statistically significant if the probability value was less than 0.05.

RESULTS

(Table II) provides a summary of the statistical data.

Levels of Chito in CSF

The CSF levels of Chito did not markedly differ between in the SAH and control groups. In the control group, the mean concentration of this enzyme was 7.05±9.76 mmol/mL/h. In SAH patients, the mean values were 5.77±5.92 mmol/mL/h within the first 3 days, 9.85±11.79 mmol/mL/h on Day 5 and 7.46 ± 7.0 mmol/mL/h on Day 7. These differences between the groups were not statistically significant (p = 0.50, 0.28, 0.11, respectively). The mean elevated level of Chito peaked on Day 5 post-SAH. (Figure 1A) demonstrates the time courses of Chito in the CSF in SAH and control patients. Comparing post-SAH days to each other did not reveal sa tatistically significant difference (p>0.05).

Levels of Chito in serum

The serum Chito levels in SAH patients and controls were not also markedly different. The mean concentration of Chito in the control patients was 48.06±25.72 mmol/mL/h compared to 65.47±57.27, 60.55±38.17, and 69.55±57.51 mmol/mL/h in the SAH group within the first three days and on the 5th and 7th days after the hemorrhage, respectively. The differences in concentration between the two groups of patients were not statistically significant (p=0.79, 0.54, 0.47, respectively). The mean elevated levels of serum Chito peaked on Day 7 after SAH. The serum levels measured at the three time points after SAH were always higher than those of the CSF (Figure 1B). Comparing the elevated levels between the days demonstrated no statistically significant difference (p>0.05).

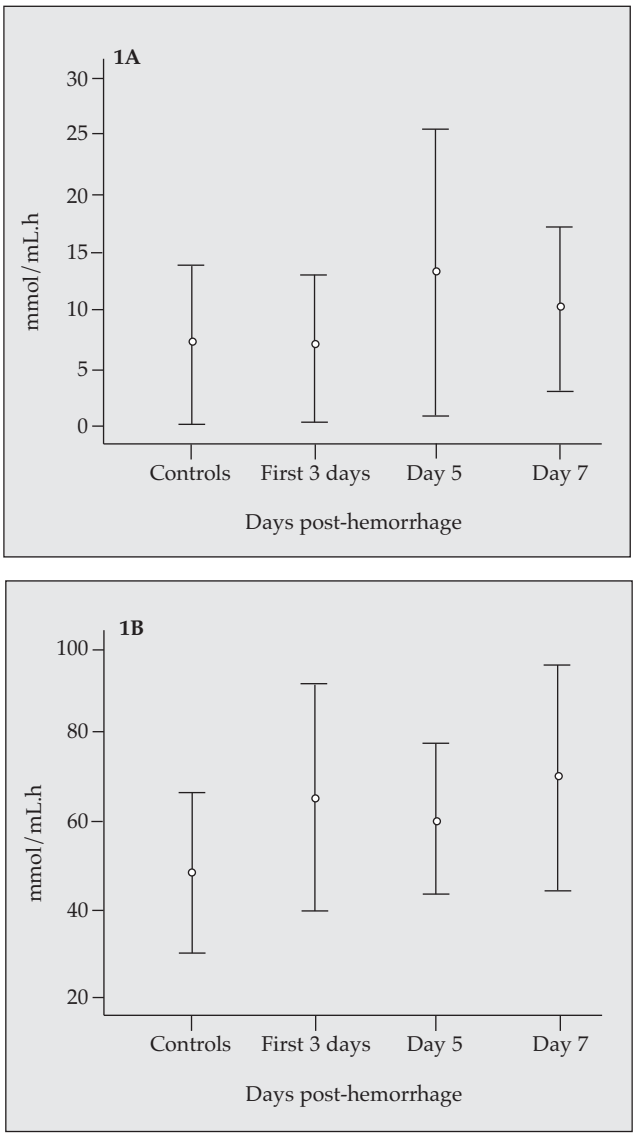

Figure 1: Graph showing a comparison of levels of Chito in the CSF (**A**) and serum (**B**) of patients with SAH and controls. Squares represent the means ± standard errors of the means and bars denoted the range of values. The differences in the levels of this molecule between the two groups of patients were insignificant (for the CSF; p=0.50, 0.28 and 0.11; for serum; p = 0.79, 0.54 and 0.47 within the first 3 days, 5th and 7th days of SAH, respectively).

Chito and symptomatic vasospasm

In this study, 14 patients with SAH experienced S-VS during the hospital stay. We compared the mean CSF and/or serum levels of Chito for each related post-SAH day between the patients with S-VS and those without in order to see whether the levels of this molecule change as S-VS occurs. We found no statistically significant difference between the two groups regarding S-VS (p>0.05).

Table II: The levels of chitotriosidase in the CSF and serum of the SAH patients and controls*.

Patient	First 3 days†	Day 5†	Day 7†	First 3 days§	Day 5§	Day 7§
1	1.92	3.47	1.80	70.0	103.0	48.0
2	1.27	20.56	15.11	60.0	45.50	98.50
3	2.20	1.92	2.50	57.0	50.50	92.0
4	2.92	2.02	3.58	22.50	22.0	23.0
5	4.08	0.12	5.0	26.0	38.50	40.0
6	0.23	0.25	23.0	5.50	2.0	4.50
7	26.0	29.13	27.18	132.50	126.50	129.0
8	14.52	47.67	3.0	235.50	138.0	258.50
9	4.20	13.0	5.20	74.0	56.50	60.0
10	7.60	4.47	4.95	32.50	88.50	74.0
11	1.40	19.87	9.57	80.0	69.50	100.0
12	8.18	10.95	4.07	116.0	71.50	15.0
13	2.75	1.90	3.74	28.0	27.0	25.0
14	6.78	5.78	7.0	39.0	45.0	67.0
15	5.85	7.60	4.03	152.50	108.50	102.0
16	3.78	11.12	11.16	27.50	33.50	39.50
17	0.95	0.62	0.55	2.0	4.50	4.0
18	3.45	7.38	3.70	86.0	76.0	79.50
19	9.35	5.23	10.0	29.50	31.0	33.0
20	8.12	3.97	4.23	33.50	73.50	98.50
Mean ± SD	***5.77±5.92***	***9.85±11.79***	***7.46±7.0***	***65.47±57.27***	***60.55±38.17***	***69.55±57.51***
Control						
1	17.59			50.50		
2	2.13			30.50		
3	2.90			98.50		
4	0.18			6.50		
5	26.96			52.0		
6	1.88			45.0		
7	1.47			49.0		
8	3.35			52.50		
Mean±SD	***7.05±9.76***			***48.06±25.72***		
"P"	**NS**			**NS**		

Chitotriosidase levels in the CSF† and serum§ in the SAH patients and controls.
* Levels are presented as mmol/mL/h. NS: Non-significant.

Chito and outcome at 6 months

Six-month follow-up showed 10 patients had a favorable outcome. When we compared the mean CSF or serum levels between the patients with favorable and unfavorable outcomes, no difference was found (p>0.05).

Correlations

The elevated CSF and serum levels of Chito correlated within the first 3 days post-SAH (Spearman's correlation; p=0.01). The Fisher grade showed correlation with elevated serum and CSF Chito on Days 3 (p=0.01) and 7 (p=0.01) and on Day 5 (p=0.02), respectively.

Other factors

We were interested in whether age and the sex affected Chito levels and compared the CSF and serum Chito levels by age and sex in both SAH and control patients. In this study, the SAH group consisted of eleven male and nine female patients with a mean age of 46.3 ± 13.20 years and the control group included four male and four female subjects with a mean age of 66.0 ± 9.82 years. There was a statistically significant difference between the groups with respect to age (p=0.001). In the SAH group, the mean age in the females and males was 48.77±13.85 and 44.27±12.94 years, respectively and there was no statistically significant difference (p=0.44). When considering CSF Chito levels, we found no significant difference at any time after SAH between female and male patients (p>0.05). The same situation was true for the serum levels (p>0.05). Interestingly, male patients always had higher CSF and serum Chito levels than those of female patients. In the control group, the mean ages were 66.25 ± 13.93 and 65.75 ± 5.56 years in female and male controls, respectively. No significant difference was found regarding the age in controls (p=0.77). However; marked differences were found in both CSF and serum Chito levels in female and male controls. The mean CSF Chito levels were 12.70 ± 11.70 and 1.41 ± 0.86 mmol/mL/h in female and male controls, respectively. The difference was significant (p = 0.02). Serum levels were also markedly different between female and male controls. The mean serum Chito levels were 63.37 ± 23.43 and 32.75 ± 19.22 mmol/mL/h in female and male controls, respectively. The difference was significant (p = 0.02). In contrast to SAH patients, female controls had higher CSF and serum Chito levels than those of male controls.

DISCUSSION

Main findings

We have demonstrated that Chito, which is a parameter of activated macrophages, is elevated in both CSF and serum in acute stages of aneurysmal SAH. The CSF and mainly the serum levels in SAH patients were always higher than those of controls. In contrast to previous studies demonstrating a correlation between the disease severity and serum or CSF Chito levels in several neurological diseases including Alzheimer's disease (9,13), cerebrovascular dementia (13), and ischemic stroke (3, 20), we could not demonstrate similar correlation between elevated CSF or serum Chito levels and the severity of SAH, namely S-VS and bad outcome, suggesting Chito levels either in the CSF or in serum may not be used as a specific biochemical marker for SAH or we may speculate that this discrepancy between our results and the others may be due to less number of SAH patients included in this study. Furthermore, in contrast to previously published studies (16), we found that age and sex had no effect on the elevated levels of Chito.

The strength and the limitations of this study

Chito can be considered an inflammatory protein since it is solely secreted by the activated macrophages (8, 18). It has been shown that its production occurs after at least 1 week of cell culture and increases with time; thus, it does not behave as an acute protein but rather as a chronic inflammatory marker (5). Based on this information, the strength of this study comes mainly from two points: first, this is a clinical prospective preliminary study in which Chito levels were determined both in the CSF and serum of patients after aneurysmal SAH for the first time and second, the time course of its elevation was monitored during the acute stages of SAH although no statistically significant differences were found.

This study has a significant limitation as a preliminary study. It is necessary to include a larger population of aneurysmal SAH patients and to compare it with a normal healthy population that should be age-matched. We would like to emphasize that we did not compare SAH patients with age-matched controls, which, in this study, was composed of patients with normal pressure hydrocephalus, an entity commonly encountered in the elderly. The ideal control group should consist of

healthy subjects since serum samples may be obtained easily. We could not include healthy volunteers due to ethical obstacles since obtaining the CSF samples from healthy subjects is not ethical. We selected patients with normal pressure hydrocephalus as collecting the CSF samples was easy during ventriculo-peritoneal shunting and gave us an opportunity to compare both CSF and serum levels. Furthermore, adjustment of serum or CSF Chito activity according to the Chito genotype is important because of the high frequency of the defective allele in the general population. The literature states that there is a large range of serum Chito activity in control subjects, even those sharing the same genotype (6), and an overlap therefore exists between serum Chito values from controls and affected subjects.

Relation to the current literature

As in other inflammatory reactions, a complex network including recruitment of leukocytes and soluble mediators from the blood stream occurs after aneurysmal SAH. Thus, studies of soluble molecules within the subarachnoid space after SAH are still required for a comprehensive understanding of the pathophysiological mechanisms remained behind the inflammatory process of VS. Recent clinical studies have reported that Chito, an enzyme secreted only from the activated macrophages (18), is expressed in pathological conditions and has been evaluated as a serum marker in several neurological disorders (11). Furthermore, some recent studies have shown that Chito levels may be used as an important predictor of the disease severity in atherosclerosis (2, 7), some infectious diseases (1, 22, 23), sphingolipidoses such as Nieman Pick (26), GM1-gangliosidosis (10, 16), Krabbe (16), Gaucher (12, 25, 26) and Fabry diseases (24), Alzheimer disease (13), cerebrovascular dementia (13), sarcoidosis (15), acute ischemic stroke (3, 20) and bronchial asthma (4). The pattern of its elevation in normal and disease states suggest that Chito plays an important role in inflammatory process and in the defense against microorganisms with chitin in their extracellular matrix. Thus, it seems that Chito will be used as a marker for the stage of inflammation in routine clinical practice in the near future.

The CSF and serum Chito levels in this study showed a trend to decrease and increase, respectively over time. These results suggest that the acute burden of inflammation occurring within first 3 days (no vasospasm is expected to occur) is located within the local (brain) and systemic circulation. Since Chito is only secreted from the activated macrophages and since VS is strongly associated with inflammation, we would expect either CSF or serum Chito levels to peak toward day 7 in which the possibility of occurrence of VS is expected to reach its maximum level. We found a decrease in the CSF and an increase in the serum toward Day 7 of SAH, supporting our hypothesis that Chito may have a function only in early phases of SAH. Patients with S-VS did not show significantly higher Chito in either CSF or serum compared to those of the patients who had no S-VS, suggesting that Chito may not be used as a prognosticator in aneurysmal SAH. The cellular source of Chito production cannot be excluded from this study, however; highly possible candidates might be the CNS macrophages and systemic macrophages, which enter the CNS through the damaged blood-brain-barrier since serum Chito levels were found to be always higher than the levels in the CSF. Based on our data, a key question remained unanswered: "if Chito is correlated with the degree of inflammation, why does it decrease in the CSF towards day 7 of SAH, when we know that as inflammatory processes increase, VS is more likely to occur?" Similar results have been found in one of our previous study demonstrating levels of YKL-40 (19), another chitinase member secreted by the activated macrophages, in both the CSF and serum of patients after aneurysmal SAH.

CONCLUSION

In conclusion, it is clear that serum and CSF Chito concentrations were found to be higher following aneurysmal SAH than in the controls. However, elevated CSF and serum Chito levels are not associated with S-VS and bad outcome in SAH. Thus, it cannot be used as a CSF and/or serum marker for inflammation in patients with aneurysmal SAH. Further clinical studies with a larger population of SAH patients will lead us to speculate about the source and biological function of Chito in the CNS more accurately.

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REFERENCES

1. Aguilera B, Ghauharali-van der Vlught K, Helmond MT, et al. Transglycosidase activity of chitotriosidase; improved enzymatic assay for the human macrophage chitinase. J Biol Chem 2003; 278: 40911-917.
2. Artieda M, Cenarro A, Ganan A, et al. Serum chitotriosidase activity is increased in subjects with atherosclerosis disease. Arterioscler Thromb Vasc Biol 2003; 23: 1645-52.
3. Barone SS, Barone R, Zanda B, et al. Chitotriosidase in patients with acute ischemic stroke. Eur Neurol 2005; 54: 149-153.
4. Bierbaum S, Superti-Furga A, Heinzmann A. Genetic polymorphisms of chitotriosidase in Caucasian children with bronchial asthma. Int J Immunogenetics 2006; 33: 201-4.
5. Boot RG, Renkema GH, Strijland A, et al. Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. J Biol Chem 1995; 270: 26252-56.
6. Boot RG, Renkema GH, Verhoek M, et al. The human chitotriosidase gene: nature of inherited enzyme deficiency. J Biol Chem 1998; 273: 25680-85.
7. Boot RG, van Achterberg TAE, van Aken BE, et al. Strong induction of members of the chitinase family of proteins in atherosclerosis: Chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol 1999; 19: 687-94.
8. Bouzas L, Guinarte JC, Tutor JC. Chitotriosidase activity in plasma and mononuclear and polymorphonuclear leukocyte populations. J Clin Lab Analysis 2003; 17: 271-75.
9. Casal JA, Robles A, Tutor JC. Serum markers of monocyte/macrophage in patients with Alzheimer's disease and other types of dementia. Clinical Biochemistry 2003; 36: 553-56.
10. Civallero G, Michelin K, de Mari J, et al. Twelve different enzyme assays on dried-blood filter paper samples for detection of patients with selected inherited lysosomal storage diseases. Clinica Chimica Acta 2006; 372: 98-102.
11. Czartoryska B, Fiszer U, Lugowska A. Chitotriosidase activity in cerebrospinal fluid as a parameter of inflammatory processes in neurological diseases. J Lab Med 2001; 25: 77-81.
12. Deegan PB, Moran MT, McFarlane I, et al. Clinical evaluation of chemokine and enzymatic biomarkers of Gaucher disease. Blood Cells, Molecules, and Diseases 2005; 35: 259-67.
13. Di Rosa M, Dell'Ombra N, Zambito AM, et al. Chitotriosidase and inflammatory mediator levels in Alzheimer's disease and cerebrovascular dementia. Eur J Neurosci 2006; 23: 2648-56.
14. Grasso G. An overview of pharmacological treatments for cerebrovascular dysfunction after experimental subarachnoid hemorrhage. Brain Research Reviews 2004; 44: 49-63.
15. Grosso S, Margollicci MA, Bargagli E, et al. Serum levels of chitotriosidase as a marker of disease activity and clinical stage in sarcoidosis. Scand J Clin Lab Invest 2004; 64: 57-62.
16. Guo Y, He W, Boer AM, et al. Elevated plasma chitotriosidase activity in various lysosomal storage disorders. J Inherit Metab Dis 1995; 18: 717-22.
17. Harrod CG, Bendok BR, Batjer HH. Prediction of cerebral vasospasm in patients presenting with subarachnoid hemorrhage: A review. Neurosurgery 2005; 56: 633-54.
18. Hollak CEM, van Weely S, van Oers MHJ, et al. Marked elevation of plasma chitotriosidase activity: a novel hallmark of Gaucher disease. J Clin Invest 1994; 93: 1288-92.
19. Kaynar MY, Tanriverdi T, Kafadar AM, et al. YKL-40 Levels in the cerebrospinal fluid and serum of patients with aneurysmal subarachnoid hemorrhage: Preliminary Results. J Clin Neurosci 2005; 12: 754-57.
20. Palasik W, Fiszer U, Lechowicz W, et al. Assessment of relations between clinical outcome of ischemic stroke and activity of inflammatory processes in the acute phase based on examination of selected parameters. Eur Neurol 2005; 53: 188-193.
21. Pluta RM. Delayed cerebral vasospasm and nitric oxide: review, new hypothesis, and proposed treatment. Pharmacology and Therapeutics 2005; 105: 23-56.
22. Tharanathan RN and Kittur FS. Chitin-the undisputed biomolecule of great potential. Crit Rev Food Sci Nutr 2003; 43: 61-7.
23. van Eijik M, van Roomen CPAA, Renkema GH, et al. Characterization of human phagocyte-derived chitotriosidase, a component of innate immunity. Int Immunol 2005; 17: 1505-12.
24. Vedder AC, Cox-Brinkman J, Hollak CEM, et al. Plasma chitotriosidase in male Fabry patients: A marker for monitoring lipid-laden macrophages and their correction by enzyme replacement therapy. Molecular Genetics and Metabolism 2006; 26: doi: 10.1016/j.ymgme.2006.04.013.
25. Vellodi A, Foo Y, Cole TJ. Evaluation of three biochemical markers in the monitoring of Gaucher disease. J Inherit Metab 2005; 28: 585-592
26. Wajner A, Michelin K, Burin MG, et al. Biochemical characterization of chitotriosidase enzyme: comparison between normal individuals and patients with Gaucher and with Niemann-Pick diseases. Clinical Biochemistry 2004; 37: 893-97.