

Elevated interictal serum HSP-70 levels as an indicator of neurodegeneration for chronic migraine

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Abstract

Objective: To investigate whether there is a relationship between chronic migraine and heat shock protein-70.

Methods: The case-control progressive study was conducted at Ankara Numune Teaching and Research Hospital, Ankara, Turkey, from January to June 2013, and comprised patients over 18 years of age who were diagnosed with chronic migraine and did not have any other known neurological illness. Age and gender-matched volunteers with no history of headache or neurological illness were included as controls. In order to exclude other central nervous system diseases, computed tomography and/or magnetic resonance imaging was carried out. Blood samples to evaluate serum heat shock protein-70 levels were obtained from the patients during headache-free periods and from the controls following 8 hours of fasting. The samples were interpreted using the enzyme-linked immunosorbent assay reader.

Results: There were 40 controls and an equal number of cases in the study. Mean heat shock protein-70 levels were higher in the cases 2.37 ± 1.91 ng/dl compared to the controls 1.81 ± 1.30 ng/dl, but the difference was not statistically significant ($p=0.12$). Serum heat shock protein-70 levels were also compared in terms of the duration of migraine disease, frequency of migraine attacks, Visual Analogue Scale score, migraine attack duration and the presence of aura, but no statistically significant difference was found ($p=0.13$, $p=0.17$, $p=0.90$, $p=0.68$, $p=0.95$ respectively).

Conclusion: Heat shock protein-70 was not a reliable chronic migraine biomarker.

Keywords: HSP-70, Chronic migraine, Neuro-degeneration. (JPMA 66: 677; 2016)

Introduction

Migraine is thought to be a neurovascular disease, which is activated due to changes in the modulating nociceptive inputs from the raphe and locus coeruleus nuclei in the brainstem or a cortical spreading depression. Chronic migraine (CM) is defined as 15 or more headache days per month for at least 3 months, with 8 or more days per month fulfilling migraine criteria, in the absence of medication overuse, according to the International Headache Society (IHS).¹ Many structural and functional changes take place in several cerebral areas involved in pain processing of patients with CM. Neuroimaging studies using advanced techniques showed abnormalities in the brainstem and in the lateral and medial pain pathways.² It is also known that neuronal and/or glial damage takes place at the cellular level in migraine. In one study, serum S100B and neuron specific enolase (NSE)

levels, which are markers of neuronal and/or glial damage in migraine, were shown to be elevated in migraine patients during the ictal and interictal states, proving that there is also an insidious and slow damaging process in the brain. In the light of these findings, the study concluded that migraine could be associated with neuro-degeneration.³

Heat shock proteins (HSPs) are a large family of proteins which are present in all organisms and in many tissues.⁴ HSPs are molecular chaperones that have functions including refolding of misfolded and aggregated proteins, prevention of protein aggregation, and formation of polypeptides. HSPs also perform many cyto-protective functions.^{5,6} They are also involved in protein translocation through membrane and signal transduction proteins, and are secreted during heat, oxygen stress, and infections.⁷⁻⁹ In addition to their tasks as chaperones, they inhibit the apoptosis cascade.¹⁰ Heat shock protein-70 (HSP-70) is the form which is most studied among all HSPs, and it is reported in several studies as it is foremost stimulated due to stress.¹¹ Its relationship with many neurological and systemic diseases has been studied. The HSP-70 molecule increases in concentration both in brain tissue, including glia and endothelial cells, and serum in certain chronic neurological diseases, including cerebral ischaemia,

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epilepsy, trauma and also in neuro-degenerative diseases.¹²

The current study was planned to investigate whether there is a relationship between HSP-70 and CM, which could be associated with neuro-degeneration.

Patients and Methods

The case-control progressive study was conducted at Ankara Numune Teaching and Research Hospital, Ankara, Turkey, from January to June 2013, and comprised patients diagnosed with CM according to the International Classification of Headache Disorders II (ICHD-II) 2003,¹³ and did not have any other known neurological illness. Age and gender-matched volunteers with no history of headache or neurological illness were included as controls. The volunteers were medical students, nurses, residents and physicians, and were taking no medications. The patients were aged between 18 and 59 years, had not received prophylactic drug treatment for migraine in the preceding 3 months, had no history of neurological disease and normal cranial imaging and were not under a migraine attack during the assessment phase of the study. Pregnant or breastfeeding women, mentally retarded subjects, and those under prophylactic treatment or having a medical history of a neurological disease other than migraine were excluded.

All patients and healthy volunteers were informed verbally about the study, they all read and signed the Helsinki Declaration of 1975 and their written consents were obtained after approval of the study was granted by the institutional ethics committee.

Medical and family history of all patients and controls were noted. Physical and neurological examinations were then carried out. In order to exclude other pathologies related to the central nervous system (CNS), computed tomography (CT) and/or magnetic resonance imaging (MRI) were performed. Migraine characteristics, duration of disease, and socio-demographic characteristics of the patients were recorded. The severity of pain was assessed with a visual analogue scale (VAS). According to the VAS, the severity of headache was evaluated in three groups defined as light for 1 to 3, medium for 4 to 7, and severe for 8 to 10. Blood samples to evaluate serum HSP-70 levels were taken from the patients during headache-free periods and from volunteers after 8-hour fasting. After being left for clotting for 30 minutes, the samples were centrifuged at 1000G for 15 minutes. The serum samples were then transferred to a separate biochemical tube and were stored at -80° degrees Celsius until the day of evaluation. Serum HSP-70 level was determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (USCN Life Science Inc., Wuhan, China) strictly

following the manufacturer's instructions. Serum HSP-70 levels were measured in the ELISA reader after being dissolved and brought to room temperature on the day of evaluation.

Statistical analysis of the data was made using SPSS 21.0. Numeric variables were summarised by mean $\pm$ standard deviation (SD) and median [min-max], and categorical variables were summarised by frequency and percentage. Before the groups were compared in terms of numerical variables, parametric test assumptions (normality and homogeneity of variances) were checked.

Whether there is a significant difference between two independent groups in terms of numerical variables was determined by t test in case of fulfilment of parametric test assumptions. In case of failure to fulfil parametric test assumptions, Mann-Whitney U test was used. Kruskal Wallis test was used for comparison among more than two groups. Whether there were any differences between the groups with respect to categorical variables was examined by chi-square test or Fisher's exact test. The relationship between numerical variables was given with the Spearman correlation coefficient. The level of significance was taken as $p < 0.05$.

Results

There were 40 subjects in each of the two groups. Family history and characteristics of both the groups were noted (Table-1). HSP-70 levels were higher in the patients 2.37 ± 1.91 ng/dl compared to the controls 1.81 ± 1.30 ng/dl, but this was not statistically significant ($p = 0.12$).

Among the patients, the duration of complaints revealed that 2(5%) patients had a migraine history less than one year, and mean serum HSP-70 level was 3.52 ± 2.49 ng/dl. Besides, 10(25%) patients reported a migraine history of 1 to 5 years, and their mean serum HSP-70 level was 1.27 ± 1.18 ng/dl. Further, 21(52.5%) patients with a migraine history of 6 to 10 years had mean serum HSP-70 level of 2.90 ± 2.03 ng/dl. A migraine history of over 10 years was reported by 7(17.5%) patients and their mean serum HSP-70 level was 2.06 ± 1.78 ng/dl. A comparison of serum HSP-70 levels with regard to the duration of migraine complaints did not indicate a statistically significant difference ($p = 0.13$) (Table-2).

Examination of patients for usage of symptomatic triptane revealed that whereas 6(15%) patients had a history of triptane usage (mean serum HSP-70 level: 2.78 ± 1.23 ng/dl), 34(85%) patients did not use triptane (serum HSP-70 level: 2.30 ± 1.67 ng/dl). Serum HSP-70 levels of patients who used triptane in the symptomatic period were higher than those who did not use triptane,

Table-1: Demographic features of patients and controls.

		Patient (n=40)	Control (n=40)	P-value
Age (Years)		39.4±10.5	39.4±10.5	0.99
Serum HSP-70 (ng/dl)		2.37±1.91	1.81±1.30	0.12
Gender (Female/Male) (n,%)		35/5 (87.5/12.5)	35/5 (87.5/12.5)	1.00
Family history of migraine	No (n,%)	32 (80.0)	34 (85.0)	0.76
	Yes (n,%)	8 (20.0)	6 (15.0)	
Presence of comorbidities	No (n,%)	29 (72.5)	25 (62.5)	0.47
	Yes (n,%)	11 (27.5)	15 (37.5)	
Comorbidities	Hypertension (n,%)	3 (7.5)	7 (17.5)	-
	Type 2 Diabetes Mellitus (n,%)	1 (2.5)	5 (12.5)	
	Hypothyroidism (n,%)	3 (7.5)	5 (12.5)	
	Lumbar and/or cervical discopathy (n,%)	3 (7.5)	2 (5.0)	
	Hyperlipidaemia (n,%)	1 (2.5)	3 (7.5)	
	Major Depression (n,%)	2 (5.0)	1	
	Rheumatoid Arthritis (n,%)	1 (2.5)	-	

Table-2: Correlation between the characteristics of attack and HSP-70 levels.

		Mean±SD	Median [Min-Max]	P-value
Duration of migrainous complaints (year)	<1 year (n=2)	3.52±2.49	3.52 (1.8 - 5.3)	0.13
	1-5 years (n=10)	1.27±1.18	0.90 (0.1 - 3.9)	
	6-10 years (n=21)	2.90±2.03	3.26 (0 - 8.3)	
	>10 years (n=7)	2.06±1.78	1.76 (0 - 4.4)	0.17
Migraine attack frequency (month)	5-6 attacks (n=20)	2.83±1.76	3.29 (0 - 5.4)	
	7-8 attacks (n=14)	2.38±2.19	1.95 (0 - 8.3)	
	9-10 attacks (n=2)	0.63±0.21	0.63 (0.5 - 0.8)	
	>10 attacks (n=4)	0.95±0.75	0.68 (0.4 - 2.0)	
Migraine attack duration(hour)	<24 hours (n=5)	2.22±2.00	2.13 (0.4 - 5.3)	0.68
	24-48 hours (n=24)	2.07±1.63	1.78 (0 - 5.4)	
	48-72 hours (n=10)	2.99±2.45	3.31 (0 - 8.3)	
	>72 hours (n=1)*	4.34±	4.34 (4.3 - 4.3)	
Aura	No (n=29)	2.41±2.02	1.80 (0 - 8.3)	0.95
	Yes (n=11)	2.27±1.65	2.90 (0 - 4.4)	
VAS	4-7 (n=14)	2.6±2.3	2.5 (0 - 8.3)	0.90
	8-10 (n=26)	2.2±1.7	1.8 (0 - 5.4)	
Symptomatic triptane usage	No (n=34)	2.30±1.67	1.78 (0 - 8.3)	0.42
	Yes (n=6)	2.78±1.23	3.22 (0.7 - 4.2)	
Symptomatic NSAID usage	No (n=23)	2.28±1.69	2.10 (0 - 5.4)	1.00
	Yes (n=17)	2.50±2.21	1.8 (0.3 - 8.3)	

HSP-70: Heat shock protein-70

SD: Standard Deviation

VAS: Visual Analogue Scale

NSAID: Non-steroidal anti-inflammatory drugs.

but this difference was also not statistically significant ($p=0.13$).

Discussion

HSP-70 is elevated in both blood and brain tissues in a variety of chronic neurological diseases, including neuro-degenerative ones. Besides, to our knowledge, there has

been no study which has investigated a relationship between chronic migraine and HSP-70 serum levels. In our study, the reason for serum HSP-70 levels being not statistically significant in CM patients compared to the controls could be due to its physiology. HSP-70 is synthesised as free soluble proteins or detergent-soluble membrane vesicles in neurons, glia and the endothelial

cells of the brain under stress conditions, such as ischaemia.¹⁴ Transportation of HSP-70 to extracellular space and consequently to blood is probably due to plasma membrane-related transport proteins or cellular damage resulting in necrosis.¹⁵ Under normal conditions, HSP-70 is expressed in very low concentrations in healthy brain tissue. It may also be measured at low levels in serum as it cannot pass the blood-brain barrier because it is not lipid-soluble. Second, the low serum concentrations could be due to the blood samples not being taken from central veins, as it is known to be diluted in peripheral venous blood.

There is as yet no published study about the half-life of human serum HSP-70. In a study in which mouse embryonic fibroblasts were used, the cytosolic HSP-70 half-life was found to be approximately 30 minutes.¹⁶ In our study we waited about 30 minutes before centrifuging in order for blood to coagulate after being taken from patients. Therefore another reason for serum HSP-70 levels not being statistically high in CM patients in our study could be due to a short serum half-life of HSP-70.

We inquired before we conducted our study whether structural and functional changes in cranial imaging of migraine patients could be related to an neurodegenerative course. It is thought that the physiopathological mechanism of formation of white matter changes in migraine patients can be explained via microvascular ischaemic damage. These white matter changes are interpreted as indirect symptoms of focal cerebral hypoperfusion which is triggered during migraine attacks.¹⁷⁻¹⁹ Cortical spreading depression (CSD), which is accepted in the physiopathogenesis of all migraine phenotypes, indirectly changes the permeability of the blood-brain barrier through matrix metalloproteinase-9 dependent cascade activation. CSD may also cause white matter cellular damage via glutamatergic excitotoxicity and intracellular calcium-mediated apoptosis.^{20,21} Local secretion of vasoactive neuropeptides can cause more advanced changes in cerebral haemodynamics.²² Migraine may also be related to oxidative stress caused by endothelial dysfunction and increased levels of nitric oxide during the attacks. If endothelial changes are accompanied by thrombocyte aggregation, this process may cause microvascular brain damage.^{23,24}

Triptane and non-steroidal anti-inflammatory drugs (NSAIDs) prevent migraine attack and suppress neurogenic inflammation, though with different mechanisms. Triptanes are very effective in preventing migraine attack and reducing pain. When triptane is taken

during migraine attack, it prevents inflammatory substances from being released from nerve endings by preventing central sensitisation. It also blocks nociceptive signal transmission in the trigeminal system and causes vasoconstriction. NSAIDs, which inhibit prostaglandin synthesis, are also very effective drugs in migraine attack treatment. It has been shown that NSAIDs block neurogenic dural plasma extravasation and calcitonin gene-related peptide (CGRP)-related dural vasodilatation-induced trigeminal sensitisation. Using triptane or NSAIDs prevents acute stress response and HSP-70 synthesis by inhibiting inflammatory substance release.²⁵ There is as yet no study which has examined the relationship between serum HSP-70 levels and triptane or NSAID usage in CM. In our study we wanted to research whether there is a significant decrease in serum HSP-70 levels of CM patients using triptane or NSAIDs during an attack. We found no statistically significant difference. We infer that it is because any high serum HSP-70 levels last only for a very short time in acute attack periods or that the blood samples of chronic migraine patients are taken at headache-free periods in the study.

A variety of clinical comorbidities in our study did not correlate with a change in serum HSP-70 levels. In order to prevent bias we searched for data analysing the interaction between serum HSP-70 levels and comorbidities in our study (hypertension, type 2 diabetes mellitus, hyperlipidaemia, hypothyroidism, lumbar/cervical discopathy, major depression, and rheumatoid arthritis) among the published studies to date, but we found no data confirming that they might affect serum HSP-70 levels.

Our study has some limitations. First, a limited number of patients and healthy controls were included in the study. Second, we considered it would be more appropriate to evaluate serum HSP-70 levels in headache-free periods to rule out probable high levels of serum HSP-70 caused by CSD-based neurovascular hypothesis when blood is taken during a migraine attack. So we took blood samples only during headache-free periods. To show with greater certainty whether there is a relation between serum HSP-70 levels and migraine, blood should also be taken during migraine attacks. Keeping these limitations in mind, our data does not support the hypothesis that a neurodegenerative course takes place in the brain of CM patients. It would be more probable and logical to explain structural and functional changes in the brain with a neurovascular etiopathogenesis that is followed by cerebral vascular changes.

Conclusion

Our study could not reach a definite conclusion that CM is

a neuro-degenerative disease. Finding that there was no significant increase in serum HSP-70 levels made us think of a few possible reasons. First, HSP-70 may not be interpreted as a chronic stress biomarker because of a short half-life and as blood samples were taken during headache-free periods. Also, as HSP-70 molecules are not lipid-soluble and expressed in very low concentrations in the brain, comparing samples taken from cerebro-spinal fluid (CSF) or blood samples taken from bigger veins which should be also closer to brain, can give us more accurate results in future studies. On the other hand, it may be wrong to associate structural and functional brain changes with neuro-degeneration. Since it is a vascular disease, these structural and functional brain changes may be explained with generally accepted CSD-mediated micro-vascular ischaemic changes triggered during a migraine attack. However, in order to confirm our assumptions, we need future studies to provide guidance from more reliable and valid data.

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Conflict of Interest: None.

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