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Review Article

Heat Shock Protein70 in Epilepsy- @

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ABSTRACT

Objective: Heat Shock Proteins (HSP) are a group of proteins that are produced in all living cells in response to a variety of stress stimuli. Cell damage is induced in nervous system diseases such as stroke, neurodegenerative disease, epilepsy and trauma. Since seizures cause stress for our organism, Hsp70 elevation as stress protein can be expected. The aim of this study is to investigate the level of Hsp70 in epilepsy patients either resistant to drug or remission.

Methods: Serum Hsp70 levels of refractory epilepsy patient group (n = 78), remission epilepsy group (n = 39) and control group (n = 30) were investigated by ELISA method.

Results: The mean Hsp70 levels in the refractory epilepsy group was $0,08 \pm 0,04$ ng/ml, in the remission epilepsy group was $0,1 \pm 0,1$ ng/ml and in the control group was $0,07 \pm 0,03$ ng/ ml. Statistical analysis showed no difference between the groups. However high levels of Hsp70 were found 4 in the resistant epilepsy group and 3 in the remission epilepsy group and none in the control group.

Conclusion: Although there was no statistical difference of Hsp70 between the 3 groups the presence of high levels 7 patients in the epilepsy group was striking. High Hsp70 can be found in patients with epilepsy, So Hsp70 may be useful in determining the prognosis of the patient. This result may pave a way to future studies.

INTRODUCTION

Heat Shock Proteins (HSP) are members of a protein family which have various functions, and the common features are produced when they are exposed environmental factors such as sudden temperature changes of the cells, changes in anoxia, reactive oxygen metabolites and glucose levels. It was found that Heat Shock Proteins (HSP) in the central nervous system are produced in many cell types comprising neuronal, glial, and endothelial cells in various pathological conditions such as cerebral ischemia, neurodegenerative diseases, epilepsy and trauma [1]. They exist in both extracellular and intracellular compartments. They protect cells from inflammation in apoptosis and cerebral ischemic injury in Parkinson's disease. They take auxiliary role in antigen presentation in the emergence of immune response in multiple sclerosis [2]. It is thought that they arise as a stress marker in epilepsy [3].

Hsp70, which is the most induced member of the heat shock protein family, is an anti-apoptotic protein chaperone. Hsp70 is over expressed in malignant human tumors with different origin, and the expression in normal cells is basically a stress indicator. The function of the Hsp70 protein under normal conditions is to function as chaperones that assist the folding of newly synthesized polypeptide and is to transport proteins to the opposite sides of membranes. Although Hsp70 is a stress marker for brain damage and takes role to prevent it, the place in the pathogenesis of epileptic seizures has not been clarified yet. It has been suggested that it takes neuroprotective role by preventing apoptosis. Hsp70 can be passed from cerebrospinal fluid-brain barrier and plasma membrane of neurons. It has been demonstrated that exogenous Hsp70 can be penetrated the brain areas such as cortex, hippocampus, thalamus, hypothalamus and pontine reticular formation in start and spread of generalized tonic-clonic seizures. Thus, it is indicative of the presence of HSP 70 as a stress marker for epilepsy [4]. On the other hand it might act as a neuroprotective chaperon on epilepsy. Seizures are triggered by hyper excitability that is caused by the excessive calcium influx into the cells. Hsp70 has an influence of the decrease of the calcium influx to the neurons thus prevents brain cells from seizure-induced apoptosis [5]. Consequently, Hsp70 might be involved in endogenous cellular preservation during seizures.

The aim of our study is to investigate the presence of Hsp70 in serum as a stress marker in Refractory Epilepsy (RE) and Epilepsy Remission (ER) groups and discuss the usefulness of serum Hsp70 for epilepsy patients.

METHODS

78 patients using multiple antiepileptic in accordance with the ILAE's definition of refractory epilepsy and 39 patients in remission in accordance with the ILAE's definition of remission epilepsy and 30 healthy individuals were included to this study among patients being monitored in the epilepsy clinic [6-9]. Resistant epilepsy is defined as the continuing of seizures despite the combination therapies of at least two antiepileptic drugs in appropriately selected doses that can be tolerated. Remission epilepsy is reported if the patient has remained seizure-free for at least one year with the treatment or if the seizure recurred within more than three times the previous longest seizure duration. According to the study's exclusion criteria, patients with Alzheimer's disease, Parkinson's disease, stroke, spastic paraparesis, multiple sclerosis, inflammatory and neurodegenerative neurological diseases such as motor neuron diseases or cystic fibrosis, sarcoidosis, systemic infections or sepsis, autoimmune disease, diabetes mellitus or another endocrine disease, heart disease, fever, cancer or alcoholism except epilepsy were excluded from the study. Ethics Committee Approval was taken (protocol number: 203/2012). Patients were informed about the study protocol. A proclamation form prepared in accordance with the Helsinki Declaration was signed before the work.

Accordingly 55 of 78 patients who had been diagnosed with Epilepsy Remission [ER] and 39 patients with Refractory Epilepsy [RE] group were included into the Hsp70 study. 10 cc of blood was taken and stored for Hsp70 study for routine examinations according to the protocol specified in patients and 30 healthy control subjects.

Detailed medical history was taken from each patient; physical and neurological examination were performed; antiepileptic drug blood levels, Electroencephalogram [EEG], cranial Magnetic Resonance Imaging [MRI] were studied to support the diagnosis of epilepsy in remission and refractory epilepsy. During sampling all of the patients were in the interictal period.

Hsp70 analysis

Blood samples for study of Hsp70 were stored as frozen at -20°C after centrifugation until completion of the study. Collection period of blood samples was 5 months.

In serum samples, Hsp70 levels were measured with Hsp70 high sensitivity enzyme immunoassay kit used for human serum. Serum samples were tested according to the manufacturer's instructions

with ELISA method (Enzo Life Sciences - Lausen/ Switzerland). The concentration of the patient sample was determined by evaluating the resulting absorbance values based on the standard curve plotted.

Statistical analysis

Statistical analysis was carried out with IBM-SPSS 19 package program. Chi-square or Fisher's exact test were used for comparisons between categorical variables and Student's t-test, One way Anova tests were used for comparison between the groups. All hypothesis testing was performed in 0,05 Type I error level ($p < 0.05$ was considered significant).

RESULTS

Thirty (54.5%) patients were male, 25 (45.5%) were female of 55 patients in refractory epilepsy group. nineteen (48.7%) were male, 20 (51.3%) were female patients in the remission group. Fifteen were male (50%) and 15 were female (50%) in the control group. The age of patients in refractory epilepsy group ranged between 20-68 and the average was 36. Whereas the age of patients in the remission group ranged between 17-69 (average 36,2). The average age of the control group was 37 (20-65). Refractory Epilepsy [RE] and Epilepsy Remission [ER] and control groups were similar in terms of gender and age in statistical comparison ($p = 0.071$ and $p = 0.082$).

In the imaging studies, findings of gliosis and encephalomalacia due to different causes were found in 21 (27%) patients, Mesial Temporal Sclerosis (MTS) was found in 7 (9%) patients and neuronal migration anomaly was seen in 2 (3%) patients. The other findings of epilepsy were arachnoid cyst, cerebral cortical atrophy, basal ganglion degeneration, hydrocephalus, porencephaly, glial tm in 9 (11%) patients. Imaging examination was normal in 39 (50%) patients. In the remission group findings of gliosis and encephalomalacia due to different causes were found in 1 patient, MTS in 1 patient, neuronal migration anomaly in 7 patients. 4 patients have other causes as arachnoid cyst, cerebral cortical atrophy, basal ganglion degeneration, hydrocephalus, porencephaly, glial tm. Twenty six of the patients' imaging examination was normal.

Fifty nine patients in the resistant epilepsy group were using two (76%), 16 patients were using three (20%) and 3 patients (4%) were using four antiepileptic drugs. Thirty two patients (82%) in the remission group were using one antiepileptic drug. Seven of the patients (18%) were not on medication. In the refractory epilepsy group 42 had focal epilepsy, 38 had jeneralized epilepsy. In the remission epilepsy group 13 were focal epilepsy and 26 were generalized epilepsy (generalized tonic clonic, myoclonic, absence epilepsy) (Table 1).

The mean Hsp70 values of the 3 three groups were as follows 0,08 \pm 0,04ng/ml in the Refractory Epilepsy [RE] group 0,1 \pm 0,1 in the Epilepsy Remission [ER] group and 0,07 \pm 0,03 the control group. High levels of Hsp70 were found in 4 Epilepsy Remission [ER] and 3 Refractory Epilepsy [RE] patients and none in the control group. There no statistical difference between the groups. Hsp 70 levels and p values are shown in (Table 2). High levels of Hsp 70 were found in 4 patients in the refractory epilepsy group. Two of them were primary generalized epilepsy (Juvenile myoclonic epilepsy and generalized tonic clonic epilepsy). One was using valproate and lamotrigine combination the other was using valproate and Zonisamide for seizure control. The other two patients were focal epilepsy, one of them was using evetiracetam and lamotrigine combination, the other was using Carbamazepine, Lamotrigine, and Topiramate. In

Table 1: Features of the patients

Patients	Refractory epilepsy	Epilepsy in remission	Healthy individuals
Number	78	39	30
Age (years)	36	36,2	37
Hsp70 (ng/ml)	0.1 \pm 0.1	0.1 \pm 0.1	0.07 \pm 0.032
More than 1 seizure type	51.3%	27%	
Pathological EEG	60%	23%	
Polypharmacy	100%	none	
Status epilepticus	16.6%	none	

Table 2: Comparison of two groups according to the serum Hsp70 values

	n	Hsp70 (ng/ ml) Average \pm SD	p
Refractory epilepsy	78	0.08 \pm 0.04	
Control	30	0.07 \pm 0.03	0.233
Remission epilepsy	39	0.1 \pm 0.1	
Control	30	0.07 \pm 0.032	0.12
Refractory epilepsy	78	0.08 \pm 0.04	
Remission epilepsy	39	0.1 \pm 0.10	0.17

the remission epilepsy group 3 patients had high Hsp70 levels. One of the focal epilepsy and was using Carbamazepine, 2 of them were generalized epilepsy (juvenile myoclonic epilepsy, juvenile absence epilepsy). One of them was using Lamotrigine, the other was using Valproate.

DISCUSSION

Hsp70 serum levels were not statistically significant among Epilepsy Remission [ER] and Refractory Epilepsy [RE] groups and healthy controls in our study. However presence of high levels of Hsp70 in the epilepsy group and none in the control group was striking. This result was exciting to show the presence of high Hsp levels can be a biomarker in the epilepsy patients. The similarity of the mean Hsp70 levels in the 3 groups may be that the epilepsy patients groups were not etiological homogeneous groups. In the studies made so far, HSPs were evaluated as markers that active in the cells of the microenvironment and occurring in response to various stress factors. These factors include sudden changes in temperature, changes in anoxia, reactive oxygen metabolites and glucose levels. It is also known that heat shock proteins had neuroprotective properties, to act as antigen-presenting in the formation of immune response. However, it has been reported that all of these effects occur in the acute phase with said agent [10]. In this context, Ce et al. have been studied Hsp27 levels in serum in attack and remission periods in multiple sclerosis patients, and found that said values are significantly higher in patients during attack than in normal subjects [2].

A recent study is showed that Hsp70 levels began to increase within the first hour of the epileptic seizures and it attained maximum at 24 hours [8]. Similar results are demonstrated with Hsp70 increase in kainic acid-induced seizures in experiments with mouse. Yang et al. demonstrated that expression of Hsp70 increased in CA3 region of the hippocampus after seizure in a kainic acid-induced mouse model [10]. This increase decreased in the next days of seizures and returned to normal after 5 days. Therefore, researchers is thought that Hsp70 increase is not protective from neuronal damage, it is a consequence of the stress caused by the seizure. High levels of heat shock protein, only occurs at the beginning of stress. Even if cell continues to

exposure to stress, heat shock protein levels start to decrease after peaking at 48 hours and returns to levels close normal at day 5 [11,12].

Mesial Temporal Sclerosis (MTS) is the most known cause of intractable epilepsy that can be treated by hippocampal resection. In the surgery material of human Mesial Temporal Sclerosis (MTS) cases, Kandratavicius et al. showed increased expression of HSP70 in the hippocampal formation. After successful surgery, they saw decreased levels of HSP70 and HSP90. Surgical excision of the hippocampus with more HSP expression showed poorer outcome compared with the hippocampus with less HSP expression [13]. In our study, we have not found an increase of Hsp70 in blood samples taken during the interictal period. This may be due to that Hsp70 increase may be associated with the short-term stress caused by seizures rather than seizure frequency. Difference of our study from other studies conducted in this area is that it is performed independently of the time of the seizure that we have identified as stress factor for Hsp70 measurements. Thus, RE is considered as a chronic disease and is intended to interpret the significance of serum Hsp70 levels identified in this process.

As well as Hsp70 is able to cause autoimmune disease as in the other subgroup families of heat shock proteins, it is known that extracellular Hsp70 takes a protective role on the cells, has neuroprotective effect on nervous system diseases such as especially epilepsy, ALS [14]. Therefore, high Hsp levels are considered to be a possible target for the treatment approaches such as stimulation of cellular defense mechanisms against diseases, gene therapy and chaperones regulatory reagents [15-17].

According to the results of our study, serum high Hsp70 levels was found in either resistant epilepsy or remission epilepsy patients. Three of them were generalized, 4 of them were focal epilepsy. This finding may pave the way of future studies that may be held in more epilepsy patients especially in etiological homogenous patients.

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