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Original Research

The Predictive Role of Heat Shock Proteins 27 and 60 in Pediatric Patients with Ataxia Telangiectasia

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ABSTRACT

Objective: This study aimed to measure the levels of heat shock proteins (HSP) 27 and 60 as mediators for hypoxia and tissue injury in pediatric patients with ataxia telangiectasia (AT). Another aim was to determine the prognostic role of HSP 27 and 60 in AT.

Methods: This study analyzed the data of AT patients (n=15) aged 4-16 years and of age-matched healthy controls (n=20). HSP 27 and 60 levels were measured from serum samples of AT patients using an enzyme-linked immunosorbent assay and compared with those of healthy controls.

Results: Serum levels of HSP 27 and 60 were higher in patients with AT than in age-matched healthy controls (p=0.010 and p=0.000, respectively).

Conclusion: In AT patients, levels of HSP 27 and 60 are increased to prevent tissue injury; therefore, treatment targeting HSPs may prevent disease progression and development of secondary malignancy in this patient population.

Keywords: Ataxia telangiectasia, heat shock protein 27, heat shock protein 60, chaperone, children

INTRODUCTION

Ataxia telangiectasia (AT) or Louis–Bar syndrome (OMIM 208900) is a pediatric autosomal recessive disorder characterized by progressive neuromotor dysfunction, variable immunodeficiency, genomic instability, hypersensitivity to ionizing radiation, and predisposition to malignancies (1, 2).

Most AT patients die of recurrent pulmonary infection due to severe immune deficiency or cancer development following chromosome rearrangements. An early symptom of AT is ataxia, which is the lack of movement coordination and the inability to control body posture. Ataxia is caused by neurodegeneration and, in particular, by death of Purkinje cells (3).

The ataxia telangiectasia mutated (ATM) gene, also referred to as ATM serine/threonine kinase that is located on the long arm (q) of chromosome 11 between positions 22 and 23, controls several aspects of cell cycle and promotes repair of double-strand breaks. As a result, clinical manifestations lead to the absence of ATM. Previous studies have linked this defect to cancer, sterility, radiosensitivity, and neurodegeneration (1). Identification of the molecular mechanism of ATM gene function in neural tissues provides insight into the mechanisms of neurodegeneration.

Heat shock protein 27 (HSP 27) is a molecular chaperone expressed in cells under various stress conditions and offers cytoprotection from various deleterious molecular events (4). HSP 60 influences the development of autoimmune diseases, including rheumatoid arthritis in humans, systemic sclerosis, Parkinson's disease, psoriasis, Kawasaki disease, Behcet's disease, and early-onset atherosclerosis (5). The important role of HSP 60 in neuron maintenance has been supported by the finding that homozygosity for a missense mutation in the HSPD1 gene (p.Asp29Gly) is associated with a fatal hypomyelinating leukodystrophy (Mit-CHAP60 disease) (6).

In this study, we aimed to measure the levels of HSP 27 and 60 as mediators of hypoxia and tissue injury in pediatric patients with AT and determine their value as prognostic indicators of AT.

METHODS

This study included 15 patients (7 boys and 8 girls) aged 4–16 years who were diagnosed with AT (AT group) and treated and followed up at Gaziantep University Faculty of Medicine Pediatric Neurology Department between March 2014 and April 2015. Twenty (9 boys and 11 girls) healthy age-matched subjects served as controls (control group). Patients who had symptoms or diseases other than AT syndrome (e.g., eating disorders, endocrine, metabolic, hepatic, renal diseases, etc.) were excluded from the study. The diagnosis of AT syndrome was based on history, neurologic examination, and magnetic resonance imaging data. Upon physical examination, ataxic gait and telangiectasia

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were determined. To rule out ataxia associated with oculomotor apraxia, patients were examined for oculomotor apraxia, and the absence of sensory motor neuropathy findings in electromyography and the presence of normal deep tendon reflexes were documented. Levels of creatinine kinase were within normal ranges. Genetic mapping of the ATM gene was not performed due to technical impossibilities. The International Cooperative Ataxia Scale (ICARS) (7) was used for evaluating ataxic score, and the results associated with ICARS and HSP 27 and 60 were evaluated by statistical analyses.

Blood samples were collected from the patients confirmed to have no fever and/or signs of infection. To measure HSP levels in the AT group and control group, 5 mL of blood was collected in gel separator tubes. After centrifugation (4,000 rpm for 10 min), serum was separated and stored at – 80°C. HSP 27 and 60 (AssayPro, USA) levels in the serum samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) method.

To measure HSP 27 and 60 levels, 50 µL of samples from each specimen was added to the wells, followed by 2-h incubation. After incubation, 50 µL of biotinylated HSP 27 and 60 primary antibodies were added to the wells and incubated for 2 h. Thereafter, the wells were washed, and 50 µL of streptavidin-peroxidase conjugate was added, followed by the addition of a stop solution. The mixture was analyzed with an ELISA reader (Biotech Instruments, USA) at 450 nm. The relationship between the levels of HSP 27 and 60 with alpha fetoprotein, ataxia score, age, and sex in the AT group was examined.

Gaziantep University's ethical committee approved the study, and informed consent was given by all patients and families of the healthy controls.

Statistical Analysis

All analyses were performed using SPSS 13.0 (SPSS Inc.; Chicago, IL, USA) statistical software package. Descriptive results were expressed as mean±standard deviation. The significance of the differences between repeated measurements in each patient was evaluated using the Wilcoxon matched-pairs test. The significance of the differences in terms of seizure type and sex was compared using the Mann–Whitney U test. Correlations between parameters were evaluated using the Spearman correlation test. For all tests, a p-value less than 0.05 indicated a statistically significant difference. The diagnostic validity analysis included receiver operating characteristics (ROC) curve analysis, with specific values for the area under the curve, the significance of the area, and confidence intervals. Sensitivity (SS), specificity (SP), positive and negative predictive values, and positive and negative likelihood ratios are also expressed in the analysis. The calculation of the overall accuracy (ACC) of the prediction was based on previous parameters. The cut-off point for measurement the calculation of which is based on the coordinates of the ROC curve is also presented in the analysis. The positive likelihood ratio is calculated as sensitivity/1 - specificity. The negative likelihood ratio is calculated as 1- specificity/sensitivity. The overall accuracy is calculated as the sum of the positive and negative predictive values.

Main Points:

- HSPs provide protection for normal cellular functions, including stabilizing cellular cycle and inhibiting apoptosis due to unknown mechanisms.
- HSP 27 and 60 levels were found to be significantly higher in patients with ataxia telangiectasia compared to controls.
- Development of therapies targeting HSPs could prevent disease progression and the development of secondary malignancies in patients with AT.

RESULTS

The mean age of the AT group and control group was 10.2±3.48 (4–16) years and 10.1±3.56 years, respectively. No statistically significant difference was found in the mean age (p=0.987) or sex (p>0.05).

The mean serum HSP 27 levels in the AT group and control group were 3.238±3.566 ng/mL and 0.537±0.253 ng/mL, respectively. This result indicates that the HSP 27 levels in the AT group were statistically different from those in the control group (p=0.010) (Table 1).

The mean serum HSP 60 levels in the AT group and control group were 2.142±1.514 ng/mL and 0.357±0.284 ng/mL, respectively. A statistically significant difference was found between the groups (p=0.000) (Table 1).

In the AT group, serum HSP 27 and 60 levels was not statistically significantly associated with alpha fetoprotein levels and ataxia score (ICARS) (7) (p>0.05). In addition, serum HSP 27 and 60 levels were not associated with patient age and sex (p>0.05).

Receiver operating characteristic (ROC) curve analysis of serum HSP 27 and 60 levels:

As depicted in Figure 1, the ROC curve analysis revealed an area under the curve (AUC) of 0.758 and 0.953 for HSP 27 and 60, re-

Figure 1. ROC curve for serum HSP 27 compared HSP 60. HSP 60 shows higher AUC than HSP 27

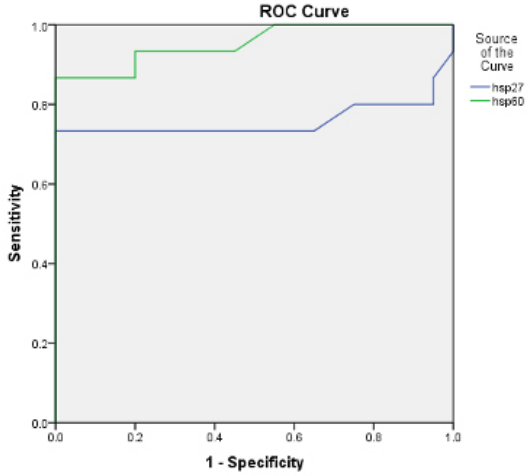

Table 1. Mean±SD values of the AT group and control group

	AT group	Control group	p
HSP 27	3.238±3.566 ng/mL (min–max/0.04–12.2)	0.537±0.253 ng/mL (min–max/0.08–0.93)	p=0.010
HSP 60	2.142±1.514 ng/mL (min–max/0.35–6.68)	0.357±0.284 ng/mL (min–max/0.03–0.91)	p=0.000

spectively. Serum HSP 60 showed higher AUC than HSP 27 as well as higher sensitivity to stressful conditions implicated in the pathogenesis of AT, indicating the potential of HSP 60 serum levels to serve as an early diagnostic marker.

DISCUSSION

HSPs were first identified as increased levels of polypeptides in response to excessive heat in *Drosophila melanogaster*. HSPs provide protection for normal cellular functions, including stabilizing cellular cycle and inhibiting apoptosis due to unknown mechanisms. HSP may be divided into the following groups according to their molecular sizes: high-molecular mass HSPs (110, 90, 70–72, and 55–60 kDa) and small HSPs (HSP 27, ubiquitin, αB-crystallin) (4). HSPs are expressed at low levels in most eukaryotic cells but are induced by cellular stress such as increased temperature, radiation, chemical exposure, oxidative stress, and physiological and pathological stimuli (8, 9).

HSP 27 is expressed in various tissues such as in cancer and in the brain, and it is responsible for resistance to cell damage and development of tumors. Increased HSP 27 levels can prevent apoptotic mechanisms. Recent studies have shown that this protein has protective effects against neurodegenerative diseases, including Huntington disease, amyotrophic lateral sclerosis, and Alzheimer's disease (5, 10). The protective effects and expression of HSP 27 and 60 are potential targetable proteins for therapy in AT pathology.

The HSP family can provide cellular protection for maturation under normal conditions and may enhance the progression of tumor cells through the cell's life cycle. In addition, new drugs for each HSP may affect the mechanisms of the cell's life cycle. Therefore, HSPs may be a promising and important target for the development of new therapies, especially in cancer. A recent study (11) revealed that HSPs were upregulated in the hippocampus, inferior parietal lobe, and cerebellum in patients with mild cognitive impairment. The authors suggested that an alteration in the chaperone protein function contributed to the pathogenesis and progression of Alzheimer's disease. Targeting HSPs could be a therapeutic approach to delay disease progression.

Hyperthermia enhances X-ray killing in cells derived from both normal and AT individuals. However, normal cells that were allowed to recover at 37°C between heat and X-ray treatments do not exhibit heightened radiosensitivity, whereas AT cells remain sensitive (12). Thus, heat, or HSPs induced by heating, may modulate ATM protein function and affect cell survival. Heat and inducible HSPs can change ATM gene functions and tend to be radiosensitive.

Akbar et al. (13) reported that HSP 27 expression particularly decreased neuronal cell death in the hippocampal CA3 region, resulting in significant reductions in kainate-induced seizure severity and mortality rate. In HSP 27 transgenic animals, modulation of caspase-3 induction and apoptotic features were responsible for the reduction of seizure severity in HSP 27. These studies indicate that HSP 27 playsa major neuroprotective role in the central nervous system.

HSP 60 is a molecular chaperone that ensures correct folding of mitochondrial proteins in mitochondria. It is a predominant mitochondrial protein with important homeostatic functions. Induction of HSP 60 has been demonstrated in cerebral ischemia models, possibly reflecting mitochondrial stress. Similarly, increased HSP 60 levels were found in the cerebrospinal fluid (CSF) of children with traumatic brain injury (TBI), and this increase was independent of the severity of trauma (14). Increased HSP 60 levels in the CSF were suggested to reflect the severity of mitochondrial stress or damage after TBI. A study involving patients who had acute cerebral infarction and transient ischemic attack showed that serum HSP 60 levels were higher in these patients than in controls and that these levels increased as a response to neuronal injury (15).

According to Xi et al. (16), HSP 27 may have protective role against the intractable epilepsy. HSP BAP1 (heat shock 27-kDa-associated protein 1), a protein inhibiting the function of HSP 27, is abnormally expressed in the neocortex of patients with intractable epilepsy and may have inhibit the protective role of HSP 27 in epilepsy. A similar study established that overexpression of HSP 27 was used as a marker, because seizures increased in the cortical regions due to stress. They reported that HSP 27 levels in intractable epileptic patients undergoing temporal lobe surgery were higher than HSP 27 levels in autopsied brain tissue from the control group without known neurologic disease (5). In addition, Chen et al. (17) found massive expression of HSP 27 in the frontal cortex of AT patients compared with controls in postmortem autopsy. They did not find a difference in the levels of other stress proteins (HSP 70, αB-crystallin, and prohibitin) in cortical and cerebellar tissues. Because of an increase in HSP levels in the frontal cortex of AT patients, they claimed that HSP 27 prevents increased oxidative stress.

As one limitation of this study, patients could not be tested for the ATM gene mutation. However, our patients were diagnosed with AT clinically based on examination and radiological findings, and they were followed up by our department. We could not find a significant correlation between HSP levels and ataxia scores. However, HSP 27 and 60 levels were analyzed in a small number of patients. None of the enrolled patients had secondary malignancy. Thus, these proteins should be investigated in a larger group of AT patients with or without malignancy.

CONCLUSION

Despite the limited number of patients, levels of HSP 27 and 60 were significantly higher in pediatric patients with AT than in healthy controls. The increased levels and their preventive role against tissue damage or as predictors of cancer risk should be investigated in future studies. This would help in the determination of follow-up and treatment options. As there is growing knowledge on extracellular chaperones, chaperone networks, and therapeutic use of chaperones (i.e., chaperonotherapy), awareness of chaperonology will most likely be widespread in the near future for AT patients. Since the analyzed patients did not have malignancy, we thought that increased HSP levels may prevent tissue injury. We believe that the development of treatments and target HSPs may prevent disease progression and development of secondary malignancies in AT patients. HSPs have promising potential as diagnostic markers and as prognostic indicators due to their ability to improve cell performance during stress. For this reason, HSPs may be a plausible target in AT.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University of School of Medicine (2015/92).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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