

A novel marker in patients with alopecia areata

Abstract

Introduction: Alopecia areata is a common autoimmune disease involving hair follicles and nails. This study aimed to compare the serum level of HSP70 (as an inflammatory marker) in patients with alopecia areata and control group.

Methods: In this case control study, 50 individuals with alopecia areata and 50 healthy individuals were recruited. The two groups were matched for age and sex. After entering the study, the demographic characteristics were determined by questioning them and recorded in the information form. Then the serum levels of HSP70 were measured. The data were analyzed using SPSS software.

Results: The Mean HSP70 plasma level were significantly higher in patients with alopecia areata than healthy subjects (61.79 ± 3.3 vs. 46.37 ± 2.8 , $P=0.001$) which was statistically significant. The mean serum level of HSP70 in patients with alopecia areata was 64.57% in the acute phase and 59.21% in the chronic phase. This difference was not statistically significant ($P=0.560$). The mean serum level of HSP70 in the Localized form was 45.93 and in the diffused form was 85.58, which was statistically significant ($P=0.001$). The mean serum level of HSP70 in the mild, moderate and severe forms was 45.93, 87.66, 81.72 which was statistically significant ($P<0.001$).

Conclusion: Due to findings of this study and the role of HSP70 in inflammation, the effect of this protein can be useful in the diagnosis and treatment of alopecia areata disease. HSP70 can also be a new Biomarker in the active form of the disease. Perhaps in the future, it will be used as a target for treatment, which further studies are suggested.

Keywords: alopecia areata, hsp70, serum level, biomarker, hypoxia, acidosis, ophiasis, eosinophils, ulcerative colitis, diabetes mellitus, down syndrome, antithyroglobulin, anti-microsomal antibodies, hormonal therapy, nitric oxide compounds, heavy metal

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Introduction

Alopecia areata is a chronic inflammatory disease causing hair loss on the scalp, face and sometimes on other areas of the body. The main manifestation of the disease is usually a rapid onset of hair loss in a specific area and usually round.¹ People of all ages, both sexes and all ethnic groups can develop alopecia areata. The prevalence of Alopecia areata is 1.7%.^{2,3} The skin of the affected area is flat or slightly red and may have short hair pieces.⁴ Hair loss may be completely reversible, become chronic or cause loss of whole head hair or whole body hair.³⁻⁵

1. Alopecia areata patchy — The most common form, with one or more coin-sized hairless patches on the scalp or other areas of the body
2. Alopecia totalis — Total loss of the hair on the scalp
3. Alopecia universalis — Complete loss of hair on the scalp, face and body.^{6,7}

The presence of eosinophils in biopsy is a useful diagnostic symptom in Alopecia areata. Measurements of triiodothyronine, thyroxine, thyroid stimulating hormone, antithyroglobulin and anti-microsomal antibodies, especially for children, should be performed.⁸

Alopecia areata may be associated with thyroid disease, severe anemia, Addison disease, vitiligo, lupus erythematosus, ulcerative colitis, diabetes mellitus and other autoimmune diseases.^{8,9} (HSP) Heat shock protein was first introduced in 1964 by Ritossa showed that due to heat, a rearrangement of the fruit insect's chromatin called *Drosophila busckii* is created and concluded that its synthesizing

genes are activated by heat, so it is called heat shock protein.¹⁰ These proteins include about 1-2% of total protein in normal conditions and 4 to 6% in stress conditions in all eukaryotic cells.¹¹ Based on the molecular weight, they are divided into five families: the heat shock protein family 104, the heat shock protein 90, the heat shock protein 70, the heat shock protein 60, and the small heat shock proteins such as the heat shock protein 27.¹² Heat shock proteins exist in normal cells and prevent the creation of inappropriate spatial structures caused by inappropriate protein gathering but, due to biological stress and increased toxic and inflammatory chemicals, it is useful in protecting cells from stress.^{13,14} Oxidative stress indicates an imbalance between the appearance of free radicals of oxygen and the ability of the biological system to detoxify or repair their destructive effects.¹⁵ The HSP70 family is the most sensitive group of these proteins and has the most protected structure. HSP70 is a protein that binds to ATP and is found in 60-80% of the eukaryotic cells.¹⁶ The HSP70 is vital to both cell function and survival after stress. In addition to the thermal shock, several stimuli, including hypoxia, acidosis, active oxygen lines, active nitrogen classes, viral infections, malignancies, autoimmune disease, and induced transcription.¹⁷ Since inflammatory and immune factors play a role in the pathogenesis of alopecia areata, we decided to study the association between the serum level of HSP70 (as a systemic inflammatory marker) and the pathogenesis of alopecia areata.

Methods

In this study, people who were clinically diagnosed with alopecia areata by a dermatologist and had following conditions were entered:

- Non- existence of non-epithelial ovarian cancer, endometrial cancer, breast, lung, pancreas cancer
- Lack of diseases such as endometriosis, lymphoma, pelvic inflammatory disease, infections, cirrhosis.
- Non-use of antibiotics, hormonal therapy, nitric oxide compounds and heavy metal
- Non- existence of Cardiovascular or brain ischemic heart disease

50 people diagnosed with alopecia areata by a dermatologist. 50 people as control group among those who had the above inclusion criteria but was not affected by Alopecia areata disease, they were selected according to the age of patients with less than two months of duration of the disease were categorized to the acute group, and those with a duration of illness of more than two months were categorized in the chronic group. Then all of these people are referred to the Lab for testing HSP70. These people should be fasting at least 4 hours before the test. In the laboratory, five ccs of blood are taken from them and thrown into the test tube and were centrifuged within 1 hour, and the serum of patients and the control group were transferred to the 20°C freezer. After collecting all the control samples and samples, in one day all the samples were extracted from the freezer and de-freeze and homogenized and prepared for measurement. The HSP70 kit does a measurement of HSP70 by ELISA. After determining the mean serum level of HSP70, the data from the two groups were compared together, and the results were analyzed statistically. SPSS20 software is used to analyze the data. At first, the data were considered for the normal distribution using the Kolmogorov-Smirnov test. Due to lack of normal distribution at the level of error of the first one, 0.05, the Chi-square test and the non-parametric Man-Whitney test was used for comparison.

Results

This study was performed on 50 patients with alopecia areata and 50 healthy non-Alopecia areata patients. The mean age of the case group was 29.78 ± 1.69 , and the mean age of the control group was 27.44 ± 1.84 . 64% of the cases were males, and 36% were females. In the control group, 32% were males, and 68% were females. There was a statistically significant difference in the frequency of gender between the two groups. Therefore, the two groups were not homogeneous in gender (Table 1). Of the total 50 cases studied, 30 were in a limited form of the disease (one or more lesions) and 20 in the form of a diffuse (total hair loss of head, face and body) (Table 2). Of the 50 patients studied, 30 had mild involvement severity (one or more lesions), 13 had moderate involvement severity (total involvement of head and face hair), and 7 had severe involvement (total body hair involvement) (Table 2).

Table 1 Comparison of frequency distribution of gender in case and control groups

Gender	Group		Chi-Square Test
	Case	Control	
Male	32 (64.0%)	16 (32.0%)	p=0.001
Female	18 (36.2%)	34 (68.0%)	
Total	50 (100.0%)	50 (100.0%)	

In the study of HSP70 levels, the result was that the mean serum level of HSP70 in the case group was 61.79 and in the control group was 46.37. This difference was statistically significant ($P=0.001$)

(Table 3). Regarding the fact that the frequency distribution of gender was not homogeneous between case and control groups, analysis in each of the two sexes was performed to eliminate the gender segregation effect on Table 4. The result of the Mann-Whitney test showed that the mean serum level of HSP70 was significantly higher in the form of the diffuse disease than the limited form of the disease ($P<0.001$) (Table 4). To compare the mean serum HSP70 level in patients, Kruskal-Wallis test was used based on the severity of involvement. The result of Kruskal-Wallis test showed that this difference was statistically significant ($P<0.001$). Tukey's post hoc test showed that the mean HSP70 in the mild group with the other two groups has a statistically significant difference ($p<0.001$), While the mean of the moderate group was not significantly different with the severe group ($P=0.485$) (Table 4).

Table 2 Comparison of frequency distribution of disease state and severity in case group

Type	Frequency (%)
limited	30 (60%)
Diffuse	20 (40%)
Mild	30 (60%)
Moderate	13 (26%)
Severe	7 (14%)

Table 3 Comparison of mean serum level of HSP70 in case and control groups

Group	Mean±SD	Z	Mann-Whitney test
Case	61.79±3.38	-4.1	0.001> P
Control	46.37±2.87		

Table 4 Mean HSP70 score in study population

Variable	Group*	Group*		Z	Mann-Whitney test
		Case	Control		
Gender	Male	62.67±4.30	42.58±4.89	-3.3	P=0.001
	Female	60.22±5.62	48.16±3.48	-2.4	P=0.014
State	Limited	45.93±1.08	---	-5.4	P<0.001
	Diffuse	85.58±4.63	---		
Severity	Mild	45.93±1.08	---	0.62	P<0.001
	Moderate	87.66±6.02	---		
	Severe	81.72±7.47	---		

*Mean±SD

Discussion

This case-control study was conducted to evaluate the serum level of HSP70 in patients with alopecia areata and compare it with the control group. In this study, 50 patients with Alopecia areata and 50 subjects were selected as a control group, 64% of cases were males, and 36% were females. In the control group, 32% were males, and 68% were females. The mean of total age in the sample was 28.61 ± 1.25 (63-4 years), which was 27.44 ± 1.84 in the control group and 29.78 ± 1.69 in the case group. Our findings showed that plasma levels of HSP70 were significantly different in patients with alopecia areata and healthy people so that plasma levels of HSP70

were significantly higher in patients with alopecia compared with healthy subjects ($P < 0.001$). In this study, serum levels of HSP 70 were measured in different forms of the disease. Our findings in this regard showed that the serum level of HSP70 in the limited form of the disease was 45.93 and in the form of diffuse was 85.58, which was statistically significant. Also, the mean serum level of HSP70 in mild, moderate and severe forms was 45.93, 87.66, and 81.72, respectively. This difference was statistically significant. Since the end of our efforts with the keywords of the HSP70 serum level and alopecia areata in various databases such as Up To Date, JoVE, Pubmed, Elsevier, Ovid, Google scholar and other scientific sources, an article similar to that of our dissertation not found, Therefore, comparing the results of this study with the same article was not possible, so the following results are presented in several relatively similar articles:

- i. A review was conducted in Poland in 2015, the results of which showed that HSP70 is present in colorectal epithelium and patients with inflammatory bowel disease, its expression in the active phase of the disease increases.¹⁸
- ii. In a study conducted in Tehran in 2014, the serum level of HSP70 was evaluated in 76 patients with rheumatoid arthritis and 36 healthy subjects; It is concluded that the serum level of HSP70 is significantly higher in patients with rheumatoid arthritis ($P < 0.05$).¹⁹ From this study, it can be concluded that the expression of HSP70 in inflammatory phase increases.
- iii. In a 2014 study in Turkey, total antioxidant and total oxidant levels in alopecia areata patients were evaluated in 46 patients with alopecia areata and 36 healthy subjects. The level of these markers in patients was higher than healthy subjects, but there was no significant relationship ($P > 0.05$).²⁰

In a study conducted in Egypt in 2013, the serum level of HSP70 was evaluated in 32 patients with vitiligo and 10 healthy skin patients, HSP70 is significantly higher in the skin of patients with vitiligo than in healthy subjects ($P < 0.001$).²¹ Given the similar inflammatory mechanisms involved in the pathogenesis of vitiligo and alopecia areata, it can be concluded that HSP70 increases in the active phase of alopecia areata. In a study in Korea in 2013, the association of the HSP70 gene with the alopecia areata was investigated. 228 patients with alopecia areata and 236 healthy subjects were chosen, The HSP70 synthesis gene was associated with a disease attack ($P = 0.002$).²² In a study in 2010 in Miami, 35 mice were exposed to thermal stress, and 8 out of 35 mice suffered from alopecia areata at the back, abdomen, and head.²³ As mentioned, the expression of HSP70 increases under conditions of thermal stress, so it can be concluded from this study that the expression of HSP70 increases in the active phase of the disease. Recently, in this disease, a new prognostic biomarker called "I hair" has been found.²⁴ In a study in Turkey in 2014, the serum level of oxidative stress markers (SOD, GSH-Px) was significantly higher in patients with alopecia areata than in control subjects ($P < 0.001$). There was no significant relationship in MDA level between two groups and also between the levels of these markers with severity, duration, recurrence and form of the disease ($P > 0.05$).²⁵ In a study in Tehran in 2007, the average level of heat shock protein 70 in the articular fluid in patients with rheumatoid arthritis was significantly higher in patients with osteoarthritis.²⁶ Given the fact that HSP70 has an antioxidant and anti-inflammatory effect and protects the core of the cells and lipid membrane against damage,²⁷ and it is found in renal and peripheral vascular diseases, atherosclerosis, type 1 and 2 diabetes and after surgery in the serum

and released from tumor cells, peripheral blood mononuclear cells, B and T lymphocytes, amniotic fluid cells and macrophages,²⁸ Because the expression of HSP70 increases by many stimulants (including hypoxia, active oxygen classes, infections, UV rays, heat stress, etc.)¹⁷ And since T-lymphocytes and pro-inflammatory cytokines such as interleukin 1, Interleukin 6, Interleukin 8, etc. play an active role in alopecia areata, there is inflammatory secretion with predominance of Th1 lymphocyte around hair follicles, with simultaneous reduction of their serum levels.²⁷ Interleukin 6 facilitates interactions between leukocytes and interleukin 8 makes chemotaxis of neutrophils and polymorphonuclears to inflammatory position.²⁸ Due to the inflammatory process of the alopecia areata, which is associated with the accumulation of self-reactive lymphocytes and increased HSP70 in other inflammatory diseases, such as rheumatoid arthritis, as an inflammatory disease Vitiligo, which is similar to alopecia areata in its pathogenesis, and inflammatory mediators such as GM-CSF IL1, IL8, IL6, and TNF, are involved in this phenomenon,²⁹ HSP70 can be used to diagnose and treat alopecia areata disease and as a marker in the active form of the disease. Data of another study showed that interaction between Heat shock protein70 and Plasmacytoid dendritic cells in vitiligo is a essential for the increase of IFN- α production, and might be an interesting target.³⁰ A recent study showed concurrent activation of IL-23 and IL-32 cytokine pathways as well as Th1 and Th2 immune axes in lesions of scalp alopecia areata.³¹

Another study showed potentially, elevated pre-treatment serum levels of IL-12 and IL-4 can be utilized as favorable and unfavorable prognosticators of topical diphenylcyclopropenone therapeutical effect, respectively. Whether serum cytokine expression levels may be utilized as prognosticators of response to other forms of treatment is unknown, but it might warrant investigation in the development of personalized treatments for alopecia areata.³²

Alopecia areata is occurred by Th2/Tc2 activation in skin-homing and systemic subsets, accord with disorder severity, while IFN- γ is linked to disorder chronicity. These data suggestion for a possible role of diverse T-cells subsets in disorder pathogenesis and accentuate the systemic nature of Alopecia areata supporting the demand for systemic therapeutic strategies in severe patients.^{33,34}

Result

Due to the high presence of HSP70 in patients with alopecia areata compared to the control group in the current study and the role of HSP70 in inflammation, the effect of this protein can be useful in the diagnosis and treatment of alopecia areata disease. HSP70 can also be a marker in the active form of the disease. Perhaps in the future, it will be used as a target for treatment, which is suggested in further studies.

Conclusion

According to the results of this study, there is a relationship between the increase in serum HSP70 and alopecia areata disease. This association can be a causative factor or aggravating factor in alopecia areata.

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None.

Conflicts of interest

The author declares that there are no conflicts of interest.

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