New insight into potential role of inflammatory factors in the pathogenesis of vitiligo

Abstract

Vitiligo is a chronic autoimmune disease that leads to skin depigmentation (as a result of melanocytes destruction). Since inflammatory factors have roles in pathogenesis of vitiligo disease, this study aimed to compare serum level of inflammatory marker HSP70 (Heat shock protein 70) in patients with vitiligo in comparison with control group.

Methods: In this case control study, 50 patients with vitiligo and 50 healthy volunteer individuals were recruited. The two groups were matched for sex and age. 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 in patients with vitiligo (67.10 ± 62.05) is higher in comparison with control group (51.19 ± 35.68), but this difference is not statistically significant (p=0.95). The mean serum level of HSP70 in patients with vitiligo was 72.23 in acute phase and 66.66 in chronic phase that was not statistically significant. The mean HSP70 in patients with vitiligo in diffused form (77.77 ± 68.52) is higher in comparison with patients with localized form (54.58 ± 52.17), but this difference is not statistically significant (p=0.21). Also the mean HSP70 in the mild, moderate and severe forms was respectively 91.58, 82.54 & 93.31 which was not statistically significant.

Conclusion: The results of this study showed that there is no relationship between serum level of HSP70 and vitiligo. But the potential role of inflammatory and immunological agents in the pathogenesis of vitiligo remains as an essential factor to the development of this autoimmune disease. Further studies with more sample size and also investigating other biomarkers and inflammatory and autoimmune factors are suggested.

Keywords: vitiligo, HSP70, serum level, biomarker

Introduction

Vitiligo is an autoimmune disorder which causes skin depigmentation along with disorder in pro-inflammatory cytokines like INF-γ & TNF and so leads to dysfunction of melanocytes. Depigmentation in these areas occurs with progressive disappearance of melanocytes from basal layer of skin. The most common form of vitiligo disease is uniform amelanocytic macules or patches surrounded by normal skin. The color of lesions is usually milky or white. The macules are seen round, oval or linear and usually have convex and hyperpigmented margins.

The pathogenesis of vitiligo is unknown but there are various theories include autoimmune origin, existence of toxic metabolites or oxidative stimulants, neurogenic inflammatory factors and lack of melanocytes growth factors. Various studies show that vitiligo is a multifactorial and polygenic disease. Cytokines produced by Th17 cells play a major role in maintenance and extension of vitiligo. The most common form of vitiligo is the area of trigeminal nerve, and in generalized form is developed with autoimmune mechanism against melanocytes. Vitiligo may be associated with other autoimmune disorders, including alopecia areata, sutton’s naevus, addison’s disease, thyroid diseases, diabetes mellitus, pernicious anemia and rheumatoid arthritis.

Heat shock protein (HSP) was first introduced in 1962 by Ritossa. Drosophila busckii 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.

Heat shock protein exists in normal cells and prevents creation of incorrect spatial structure which is made by incorrect folding of protein, but it increases as a result of biologic stresses and toxic and inflammatory chemicals in order to protect cells against stresses. These proteins have antioxidant and anti-inflammatory effect and cause protection of cell nucleus and lipid layer against injury.
Oxidative stress is a marker of imbalance between existence of oxygen free radicals and ability of biologic system for detoxification or repair of their destructive effects. HSP70 is a biomarker which leads to cell survival in deadly condition through unknown mechanisms and it has anti-apoptosis effects and is produced in cells which are exposed to heat shock. Change in intracellular pH and exposure of the cell to glucose analogs, nitric oxide compounds and heavy metals and ischemia lead to HSP70 expression. It is reported that factors increasing Ca\(^{2+}\) and hormones and antibiotics also lead to HSP70 expression. The family of HSP70 are the most sensitive proteins toward temperature and have the most protected structure. The family of HSP70 are proteins which bind to ATP and are found in 60-80% of eukaryotic cells. Its serum level in young individuals is 60-3000ng/ml. Serum level of HSP70 decreases as the age increases, but its level reaches to 500-6021ng/ml in inflammatory conditions.

HSP70 is vital and important for both cell function and survival after stress. In addition to heat shock, various stimulants such as hypoxia, acidosis, reactive oxygen species, reactive nitrogen species, viral infections, malignancies, autoimmune diseases, etc. lead to its transcription induction.

HSP70 is found in serum of healthy individuals, in renal and peripheral vessels disease, atherosclerosis, diabetes mellitus and after surgery and it is released from tumoral cells, peripheral blood mononuclear cells, T lymphocytes, amniotic fluid cells and macrophages. HSP70 is found in serum of healthy individuals, in renal and peripheral vessels disease, atherosclerosis, diabetes mellitus and after surgery and it is released from tumoral cells, peripheral blood mononuclear cells, T lymphocytes, amniotic fluid cells and macrophages.

Since inflammatory factors have roles in pathogenesis of vitiligo disease, we decided to investigate the relationship between serum level of HSP70 and vitiligo disease in this study.

**Methods**

In this case control study, 50 patients with vitiligo and 50 healthy volunteer individuals were recruited. Patients who were clinically diagnosed with vitiligo by a dermatologist and had following conditions were entered:

a. Non-existence of non-epithelial ovarian cancer, endometrial cancer, breast, lung, pancreas cancer
b. Lack of diseases such as endometriosis, lymphoma, pelvic inflammatory disease, infections, cirrhosis.

c. Non-use of antibiotics, hormonal therapy, nitric oxide compounds and heavy metal
d. Non-existence of Cardiovascular or brain ischemic heart disease

50 people as control group among those who had the above inclusion criteria but were not affected by vitiligo.

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. He also received a blood sample to examine the serum level of HSP70. Sampling 5ccs in clot tube containing Separator Clot Activator Gel was done from the patients after at least 4 hours fasting and it was centrifuged within 1 hour and serum of patients and control group was transferred to the -20\(^{\circ}\)C freezer. After collecting all the control samples and sample, on a specified day all the samples were extracted from the freezer and were de-freeze and homogenized and prepared for measurement. Measurement of HSP70 was done by using HSP70 kit from EASTBIOPHARM Company made in Germany which was done by ELISA method with devices of American Awareness Company containing Washer, Stat Fax and Stat Fax incubator shaker device. 30% of tests were done again for control and ultimately the result was recorded in a form specific to each person after final control. After determining the mean serum level of HSP70, the data of two groups were compared together and the results were analyzed statistically using SPSS software. 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 were used for comparison.

**Results**

In this study, 50 individuals with vitiligo and 50 individuals as the control group were selected. 46% of the cases were males, and 54% were females. In control group, 44% of the case was males and 56% were females. The mean age of control group was 27.54±13.19 and in case group was 26.52±13.75.

Our findings showed that the mean HSP70 among individual of case group (67.10±62.05) in comparison with control group (51.19±35.68) is higher, but this difference is not statistically significant (p=0.95) (Table 1).

**Table 1** Comparison of the mean HSP70 among individuals of case and control group

| Group     | Mean  | Standard deviation | Mann-Whitney U test |
|-----------|-------|--------------------|---------------------|
| Case      | 67.1  | 62.05              | U=1241.00           |
| Control   | 51.19 | 35.78              | p=0.95              |

Also plasma level of HSP70 in acute phase of the disease was higher than chronic phase, but there was no significant difference (p=0.90) (Table 2).

**Table 2** Comparison of plasma level of HSP70 in acute and chronic phase of vitiligo

| Duration of the disease | Mean    | Standard deviation | Mann-Whitney U test |
|-------------------------|---------|--------------------|---------------------|
| Acute phase             | 72.23   | 88.06              | U=88.00             |
| Chronic phase           | 66.66   | 60.6               |                     |

In the present study, serum level of HSP70 was measured in various forms of the disease. Our findings herein showed that the mean HSP70 in patients with vitiligo with diffused form of disease (77.77±68.52) in comparison with patients with Localized form (54.58±52.17) is higher, but this difference is not statistically significant (p=0.21) (Table 3).

**Table 3** Comparison of the mean HSP70 in patients with vitiligo with diffused and limited form

| Area of involvement | Mean   | Standard deviation | Mann-Whitney U test |
|---------------------|--------|--------------------|---------------------|
| Localized form      | 54.58  | 52.17              | U=246.50            |
| Diffused form       | 77.77  | 68.52              |                     |
Also the mean HSP70 in mild, moderate and severe forms was respectively 91.58, 82.54 and 93.31 that there was not a significant difference ($p=0.19$) (Table 4).

### Table 4 Comparison of the mean HSP70 in mild, moderate and severe form of vitiligo

| Severity of the disease | Mean  | Standard deviation | Statistical test |
|-------------------------|-------|--------------------|------------------|
| Mild                    | 54.58 | 52.17              |                  |
| Moderate                | 31.91 | 4.11               | $\chi^2=246.50$  |
| Severe                  | 93.82 | 73.31              | $df=2$           |
| Sum total               | 67.1  | 62.05              | $p=0.19$         |

### Discussion

Our findings showed that the mean HSP70 in individuals of case group (67.1±62.05) in comparison with control group (51.19±36.68) was higher, but this difference was not statistically significant ($p=0.95$). Also, plasma level of HSP70 in acute phase of the disease was higher than chronic phase, but there was not a significant difference. In the present study, serum level of HSP70 in patients with vitiligo with diffused form of disease (77.7±68.52) in comparison with patients with Localized form (54.58±52.17) was higher, but this difference was not statistically significant ($p=0.21$). Also the mean HSP70 in mild, moderate and severe form was respectively 91.58, 82.54 and 93.31 that there was not a significant difference.

Since our efforts by keywords serum level, HSP70 and vitiligo on various databases such as UP To Date, JoVE, Pubmed, Elsevier, Ovid, Google Scholar and other scientific references did not lead to finding an article with the same title, so comparing the results with the same article was not possible; hence the results of investigations in some relatively similar articles are mentioned below:

Contradictory result is reported in a study done by Jeffrey et al in 2014 that HSP70 in vitiligo disease appears on perilesional and lesions skin in comparison with healthy areas.21

Also in another study done by Reham William Doss et al in 2016, the results of skin lesion biopsy showed that HSP70 expression was higher in comparison with healthy areas; maybe this contradiction between these studies and our study is in the method, which we have investigated serum level and the results of these studies are outcomes of investigating skin samples. The results show that HSP70 is found in serum of healthy people.24

HSP70 has antioxidant and anti-inflammatory effects and causes protection of cell nucleus and lipid layer against injury.25 In an investigation done by Briganti S et al in 2012 on 29 patients with active form of vitiligo, they analyzed oxidation-reduction reactions of glutathione, ubiquinone, catalase, superoxide dismutase and GSH peroxidase as enzymatic and chemical antioxidants in skin samples and peripheral blood mononuclear cells and the result is that increase in activity of antioxidants in affected skin and similarly increase of catalase and superoxide dismutase are seen in peripheral blood mononuclear cells.26 This study is done on skin biopsy samples, while we have investigated on serum level.

In addition to heat shock, various stimulants such as hypoxia, acidosis, reactive oxygen species, reactive nitrogen species, viral infections, malignancies, autoimmune diseases, etc. cause its transcription induction.18

HSP70 also increases by biologic stresses and toxic and inflammatory chemicals and malondialdehyde is a marker for measurement of oxidative stress level that in a study done by Dina A. Mehaney in January 2014 on 89 patients with vitiligo and 90 individuals as control group, high level of malondialdehyde (MDA) was seen in patients with vitiligo in comparison with control group, and in another study done by Agrawal S on 80 patients with vitiligo and 80 healthy individuals, he reported high level of MDA.27

Contradictory our results, data of another study showed that interaction between Heat shock protein70 and Plasmacytoid dendritic cells in vitiligo is essential for the increase of IFN-α production, and might be an interesting target.28

### Conclusion

The results of this study showed that the mean HSP70 among the individuals of case group in comparison with control group is higher, but this difference was not statistically significant. Also plasma level of HSP70 in acute phase of the disease was more than chronic phase, but there was not a significant difference. Also the mean HSP70 in patients with diffused form of vitiligo in comparison with limited form is higher, but this difference was not statistically significant. The mean HSP70 in patients with vitiligo according to the duration and severity of the disease did not have any significant difference. The results of this study showed that there is no relationship between serum level of HSP70 and vitiligo. But the potential role of inflammatory and immunological agents in the pathogenesis of vitiligo remains as an essential factor to the development of this autoimmune disease.

Further studies with more sample size and also investigating other biomarkers and inflammatory and autoimmune factors are suggested.

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### Conflict of interest

The author declares that there is no conflict of interest.

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