Research Article
Open Access
CrossMark

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

Volume 6 Issue 3 - 2018

Reza Ghaderi
Department of Dermatology, Faculty of Medicine, Birjand University of Medical Science, Iran

Correspondence: Reza Ghaderi, Professor of Dermatology, Department of Dermatology, faculty of Medicine, Birjand University of Medical Science, Birjand, Member of Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Tel +9832 4430 01, Email rezaghaderi@yahoo.com

Received: July 12, 2018 | **Published:** July 25, 2018

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.[1] Depigmentation in these areas occurs with progressive disappearance of melanocytes from basal layer of skin.[2] 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.[1–3]

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.[4–5] Vitiligo has 3 types: focal, segmental and generalized. In segmental type, unilateral spots are formed in the direction of neural segment (dermatome) which is a stable form of vitiligo. The most neural segment is in the area of trigeminal nerve, and in generalized form is developed with autoimmune mechanism against melanocytes.[6] 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.[6–7]Accomplishment with both humoral and cellular immunity is reported. In pathology, lack of melanocytes and replacement of Langerhans cells are seen.[8]

Vitiligo doesn't have any effect on physical activity but vitiligo can lead to serious cosmetic problems, and various studies show that many mental disorders have been observed in patients with vitiligo. Level of BDNF which is a neurotrophic factor derived from the brain in these patients is higher than healthy people in terms of vitiligo.[8–11]

Heat shock protein (HSP) was first introduced in 1962 by Ritossa. 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.[12]

Heat shock protein exists in normal cells and prevents creation of incorrect spatial structure which is made by incorrect folding of protein,[12] but it increases as a result of biologic stresses and toxic and inflammatory chemicals in order to protect cells against stresses.[13] These proteins have antioxidant and anti-inflammatory effect and cause protection of cell nucleus and lipid layer against injury.[14]

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.[15] 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.[16] 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.[17–18] Its serum level in young individuals is 60-3000ng/ml. Serum level of HSP70 decreases as the age increases,[19] but its level reaches to 500-6021ng/ml[20–21] 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.[18]

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.[22]

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˚C freezer. After collecting all the control samples and sample, on a specified day all the samples were extracted from the freezer and ware 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	Mann-Whitney U=1241.00 p=0.95
Control	51.19	35.78	

Also plasma level of HSP70 in acute phase of the disease was higher than chronic phase, but there was no significant difference (p=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	Mann-Whitney 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	Mann-Whitney U=246.50
Diffused form	77.77	68.52	

Citation: Ghaderi R. New insight into potential role of inflammatory factors in the pathogenesis of vitiligo. *MOJ Immunol.* 2018;6(3):100–103.
DOI: 10.15406/moji.2018.06.00202

Also the mean HSP70 in mild, moderate and severe forms was respectively 91.58, 82.54 & 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	x^2=246.50 df=2 p=0.19
Severe	93.82	73.31	
Sum total	67.1	62.05	

Discussion

Our findings showed that the mean HSP70 in individuals of case group (67.10±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 (77.77±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 lesional skin in comparison with healthy areas.[23]

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 induaction.[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.

Acknowledgements

I wish to thank Dr. Arefinezhad, Ms. Mina Ghaderi and Ms. Mandana Ghaderi (medical students of Tehran University of Medical Sciences) without whose help this study could not have been completed.

Conflict of interest

The author declares that there is no conflict of interest.

References

1. Banerjee P1, Venkatachalam S, Mamidi MK, Bhonde R, Shankar K, Pal R. Vitiligo patient derived keratinocytes exhibit characteristics of normal wound healing via epithelial to mesenchymal transition (EMT). *Exp Dermatol*. 2015;24(5):391–3.
2. Webb KC1, Eby JM, Hariharan V, et al. Enhanced bleaching treatment: opportunities for immune-assisted melanocyte suicide in vitiligo. *Exp Dermatol*. 2014;23(8):529–33.
3. Kotb El-Sayed MI, Abd El-Ghany AA, Mohamed RR. Neural and Endocrinal Pathobiochemistry of Vitiligo: Comparative Study for a Hypothesized Mechanism. *Front Endocrinol (Lausanne)*. 2018;25(9):197.
4. Sushama S, Dixit N, Gautam RK, et al. Cytokine profile (IL-2, IL-6, IL-17, IL-22, and TNF-α) in vitiligo-New insight into pathogenesis of disease. *J Cosmet Dermatol*. 2018.
5. Zhou L1, Shi YL, Li K, et al. Increased circulating Th17 cells and elevated serum levels of TGF-beta and IL-21 are correlated with human non-segmental vitiligo development. *Pigment Cell Melanoma Res*. 2015;28(3):324–9.

Citation: Ghaderi R. New insight into potential role of inflammatory factors in the pathogenesis of vitiligo. *MOJ Immunol.* 2018;6(3):100–103. DOI: 10.15406/moji.2018.06.00202

6. Kotb El-Sayed MI, Abd El-Ghany AA, Mohamed RR. Neural and Endocrinal Pathobiochemistry of Vitiligo: Comparative Study for a Hypothesized Mechanism. *Front Endocrinol (Lausanne)*. 2018;9:197.
7. Marti A, Taieb A, Boniface K, et al. Elevated total Serum IgE in vitiligo might be protective for other autoimmune diseases. Br *J Dermatol.* 2018.
8. Yanik ME, Erfan G, Albayrak Y, et al. Reduced serum brain-derived neurotrophic factor in patients with first onset vitiligo. *Neuropsychiatr Dis Treat*. 2014;10:2361–2367.
9. Nagarajan A, Masthan MK, Sankar LS, et al. Oral Manifestations of Vitiligo. *Indian J Dermatol*. 2015;60(1):103
10. Yong Hyun Jang, Soo-Eun Jung, Jaeyoung Shin, et al. Triple Combination of Systemic Corticosteroids, Excimer Laser, and Topical Tacrolimus in the Treatment of Recently Developed Localized Vitiligo. *Ann Dermatol*. 2015;27(1):104–107.
11. Rzepecki AK, McLellan BN, Elbuluk N. Beyond Traditional Treatment: The Importance of Psychosocial Therapy in Vitiligo. *J Drugs Dermatol*. 2018;17(6):688–691.
12. Bae JM, Lee JH, Kwon HS, et al. Motorized 0.8 mm micro-punch grafting for refractory vitiligo*: A retrospective study of 230 cases. J Am Acad Dermatol*. 2018.
13. Leonardi R,Caltabiano M,Cascone P, et al. Experission of heat shock protein 27 in human temporomandibular joint discs of patients with internal derangement. *J Cranifac Surg.* 2002;13:713–7.
14. Alexandrovv VY. Functional aspects of cell response to heat shock. *Int Rev Cytol.* 1994;148:171–227.
15. Arican O1, Kurutas EB, Sasmaz S. Oxidative stress in patients with acne vulgaris Mediators Inflamm. *Mediators Inflamm*. 2005;2005(6):380–4.
16. Kevin C.Kregel. Invented Review: Heat shock proteins: Modifying factors in physiological stress responses and acquired thermotolerance. *J Appl Phsiol*. 2002;92:2177–2186.
17. Juliann G, Kiang George C, Tsokos. Heat shock protein 70 KDa: Molecular Biology,Biochemistry, and physiology. *Pha rmacol Ther.* 1998;80(2):183–201.
18. Schmitt E, Gehrmann M, Brunet M, et al. Intracellular and extracellular functions of heat shock proteins: repercussions in cancer therapy. *J Leukoc Bio.l* 2007;81(1):15–27.
19. Njemini R, Lambert M, Demanet C. Mets T. Elevated serum shock ptotein 70 level in patients with acute infection: use of an optimized enzyme-linked immunosorbent assay. *Scandinavian journal of immunology*. 2003:58(6):664– 9.
20. Motor S, Ozturk S, Ozcan O, et al. Evaluation of total antioxidant status, total antioxidant status and oxidative stress index in patient with vitiligo. *Int J Clin Exp Med*.2014;7(4):1089–93.
21. Wikramanayake TC, Alvarez Connelly E, Simon J, et al. Heat treatment increase the incidence of alopecia areata in the C3H/HeJ mouse model. *cell stress Caperon*.2010;15(6):985–91.
22. Keren A, Shemer A, Ullmann Y, et al. The PDE4 inhibitor,apremilast, suppresses experimentally induced alopecia areata in human skin in vivo. *Journal of dermatological acience*. 2015;77(1):74–6.
23. Jeffrey A, Mosenson, Kelsey Flood, et al. Preferential secretion of inducible hsp70 by vitiligo melanocytes under stress. *Pigment Cell Melanoma Res*. 2014;27(2):209–220.
24. Reham William Doss, Abdel-Aziz A El-Rifaie, Amr M Abdel-Wahab, et al. Heat Shock Protein-70 Expression in Vitiligo and its Relation to the Disease Activity. *Indian J Dermatol*. 2016;61(4):408–412.
25. Abdou AG1, Maraee AH, Reyad W. Immunohistochemical expression of heat shock protein 70 in vitiligo. *Ann Diagn Pathol 2013*;17(3):245–9.
26. Briganti S1, Caron-Schreinemachers AL, Picardo M, et al. Anti-oxidant defence mechanism in vitiliginous skin increases with skin type. *J Eur Acad Dermatol Venereol*. 2012;26(10):1212–9.
27. Mehaney DA, Darwish HA, Hegazy RA et al. Analysis of oxidative stress status, catalase and catechol-O-methyltransferase polymorphisms in Egyptian vitiligo patients. PLoS One. 2014;9(6):e99286.
28. Jacquemin C, Rambert J, Guillet S, et al. Heat shock protein 70 potentiates interferon alpha production by plasmacytoid dendritic cells: relevance for cutaneous lupus and vitiligo pathogenesis. *Br J Dermatol*. 2017;177(5):1367–1375.

Citation: Ghaderi R. New insight into potential role of inflammatory factors in the pathogenesis of vitiligo. *MOJ Immunol.* 2018;6(3):100–103.
DOI: 10.15406/moji.2018.06.00202