Embase (Elsevier)

ORIGINAL ARTICLE

# Serum HSP27 Antibody Titers in Patients with Alzheimer's Disease and Vascular Dementia

**Mohammad Kiani[1], Naghmeh Mokhber[2], Majid Ghayour Mobarhan[3], Lida Jarrahi[4], Mohammad Reza Najarzadegan[5], Elham Ataei[6], Mehdi Rahimpour[7], Farzad Akbarzadeh[8]**

[1]Resident of Psychiatry, Psychiatry and Behavioral Sciences Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [2]Professor of Psychiatry, Psychiatry and Behavioral Sciences Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [3]Associate Professor of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [4]Assistant Professor of Social Medicine, Faculty of Community Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, [5]Resident of Psychiatry, Tehran Institute of Psychiatry, Faculty of Behavioral Sciences and Mental Health, Iran University of Medical Sciences, Tehran, Iran, [6]Neurologist, Khatamolanbia Hospital, Iranshahr University of Medical Sciences, Iranshahr, Iran, [7]Biological Chemistry Department, Medical School, University of Michigan, Ann Arbor, USA, [8]Assistant Professor of Psychiatry, Psychiatry and Behavioral Sciences Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

**Article Information**

**Received:** 06 Feb 16
**Accepted:** 18 Mar 2016
**Plagiarism software:** Turnitin

**Keywords:**
Alzheimer's disease,
Vascular dementia,
Heat Shock Protein 27

Mohammad Reza Najarzadegan

**ABSTRACT**

**Introduction:** It is possible that immunological factors, including the heat shock protein family, are involved in the development of the different forms of dementia. We aimed to compare the levels of serum HSP27 in patients with Alzheimer's disease and vascular dementia.

**Materials and Methods:** Thirty patients with grade 2 Alzheimer's disease, 30 patients with grade 2 vascular dementia and 30 normal subjects as a control group were recruited during November 2011 to November 2013. Diagnoses were based on DSM-IV-TR criteria. MMSE was conducted on all patients for assessing the severity of disease. Biochemical parameters, including HSP27 were measured in all subjects. Demographic characteristics were collected for all subjects.

**Results:** There were no statistically significant differences in age, gender and the levels of HSP27 between males and females in all groups. However there was a significant association between HSP27 with the severity and duration of dementia in patients groups. HSP27 levels were lower in the vascular dementia compared to those with Alzheimer's dementia, but were higher than for controls.

**Conclusion:** HSP27 antibody titers were positively related to the severity and duration of both Alzheimer's and vascular dementia and may be indicative of the role of this protein in the pathology of dementia.

## INTRODUCTION

Dementia is a progressive impairment of cognitive functions with full awareness.[1] The most common type of dementia is Alzheimer's disease that comprises 50-60% of all cases. Apart from Alzheimer's-type dementia, vascular dementia and mixed dementia (Alzheimer's-type and vascular dementia) are the most highly prevalent types, respectively.[2]

| Access this article online | |
|---|---|
| Website: www.actamedicainternational.com | Quick Response code |
| DOI: 10.5530/ami.2016.2.13 | |

Although the etiology of this neurodegenerative disease has not been indentified,[3] research continues to identify the molecular mechanisms of this disease, although complex genetic, bioneurohemical, neuroanatomic, immunologic, metabolic, and psychoneuroendocrinological factors are probably involved.[1]

Abnormal cellular and serum reactions against neurons and the presence of anti-brain-tissue antibodies can be the result of an external factor or an endogenous autoimmune disorder. One of the factors considered in this area are Heat Shok Protein (HSP) antibodies.[1]

The HSPs were originally identified in the salivary glands of drosophila following exposure to an increased temperature.[4]

**Corresponding Author:**
Farzad Akbarzadeh, Psychiatry and Behavioral Sciences Research Center, Ibn-e-Sina Hospital, Mashhad, Iran. Tel: +985117112540, Fax: +985117124184. E-mail: mn1272012@gmail.com

These proteins are also produced in other stressful conditions, including hypoxia, ischemia and the presence of stressor factors such as endotoxins, heavy metals, organic solvents and oxidants.[5] Different opinions have been expressed about the role of HSPs in several conditions.[6-11]

The HSPs consist of several of proteins based on their molecular weight.[12] One of the HSP is HSP27, a member of the small HSP family. It has also been called HSPB1, and is encoded by a gene on chromosome 7q11.23. HSP27 is involved in controlling protein folding and plays an important role in the genesis of neurodegenerative disorders in the case of mutation. This protein has also anti-oxidative and anti-apoptotic effects.[1,13]

Several neurodegenerative disorders are associated with the accumulation of insoluble proteins or amyloid fibril in neurons or glial cells. As a result, HSP27 expression increases in cells. This is also observed in Alzheimer's disease.[14] This process probably also has a role in other disorders such as multiple sclerosis, CVA, ALS, Parkinson's disease, etc.[15-20]

The potential involvement of HSP27 in the pathogenesis of Alzheimer's disease[9] and the varieties of dementia has not been investigated. Therefore, this study aims at comparing the level of serum HSP27 antibody titers in patients with Alzheimer's disease and vascular dementia with those of a control group.

## MATERIALS AND METHODS

The research protocol was approved by the Ethics Committee of Mashhad University Medical School. Written informed consent was obtained by all groups and their legal guardian. The subject groups included patients with Alzheimer's-type and vascular dementia referred to the clinic based at Ibn-e-Sina Psychiatric Hospital in Mashhad. The control group recruited from nursing home was consisting of no dementia volunteers had been matched for age and sex.

According to the sample size determination formula and similar studies,[21] Thirty patients with grade 2 Alzheimer's disease and 30 other with grade 2 vascular dementia were recruited during November 2011 to November 2013, and were diagnosed based on the Diagnostic and Statistical Manual of Mental Disorders 4$^{th}$ edition, Text Revision (DSM IV-TR) criteria and the clinical evaluation of two psychiatrists. In order to minimize the effect of confounding factors, all participants who had any of cerebrovascular diseases, cardiovascular diseases, seizure, head trauma, metabolic disorders, history of smoking, drug and substance abuse, consumption of drugs affecting the inflammatory system and concurrent axis I psychiatric disorders were excluded. Participants with vascular dementia who had a history or evidence of cerebrovascular disease were included. Thirty normal gender and age matched subjects were recruited as a control group.

After recruitment, the Mini Mental State Examination (MMSE) was used for the assessment of the severity of disease on all patients. Patients with the score ranging from 0-10, 11-19 and 20-24 were classified as severe, moderate and mild dementia, respectively.

In order to determine the HSP27 antibody titers and assess metabolic status (fasting blood glucose profile, hemoglobin and hematocrit, cholesterol, triglycerides, urea and creatinine), 15 ml of blood was taken from the patients of all groups in morning. The blood samples of all subjects were taken via a peripheral vein at baseline and divided in two separate test tubes with and without anti-coagulant. Afterward, the serum of one of the samples was separated and kept at –80°C. Metabolic and hs-CRP profiles were measured with routine enzymatic methods using autoanalyzer and serum HSP27 was measured using in-house ELISA technique. All biochemical tests were conducted using a Biotechnica BT-3000 plus Chemistry Analysis device manufactured in the U.S.A and the kit used for examining hs-CRP was hs-CRP biosystem manufactured in the U.S.A.

Demographic characteristics including their age, gender, severity of the disease, its duration and laboratory results were recorded. Data was analyzed by applied SPSS 15 and T-test, ANOVA, Kruskal-Walis test, Post-hoc tests (Tukey & Dunnett's T3), Pearson and Spearman correlation tests. p-value less than 0.05 were significant.

## RESULTS

Sixty patients with either vascular dementia or Alzheimer's disease and 30 normal subjects as control group were recruited. The mean age of patients groups was 78.1±6.3 and the mean age of control group was 77.8±6.2. Of the patients 38 individuals (63.3%) were male and 22 (36.7%) were female and of controls 17 individuals (56.6%) were male and 13 (43.4%) were female. There was no significant difference between groups regarding their age and gender (p=0.11 and 0.53, respectively). Demographic characteristics of all groups were shown in Table 1.

There was no significant correlation between age and the level of HSP27 antibody titers in the total patient cohort no in each of patient groups individually. (p=0.4, 0.14 and 0.76, respectively). Moreover, there was no significant differences between the mean HSP27 antibody titers in vascular dementia and Alzheimer's disease groups between males and females (p= 0.09 and 0.76, respectively).

In the comparison of the means of different profile variables, there were not significant differences between groups using ANOVA test for normally distributed variables and the Kruskal-Walis test for abnormally distributed variables. There was however a significant differences in HSP27 antibody titers (p<0.0001) (Table 2).

**Table 1: Demographic characteristics in patients with Alzheimer and vascular dementia and control subjects**

| Group / Variable | Vascular dementia | Alzheimer dementia | Controls | p-value |
|---|---|---|---|---|
| Age (mean±SD) | 76.8±5.5 | 79.4±6.4 | 77.8±6.2 | 0.11 |
| Sex (%) | | | | |
| Female | 12 (40.0) | 11 (36.6) | 13 (43.4) | 0.53 |
| Male | 18 (60.0) | 19 (64.4) | 17 (56.6) | |
| Severity of disease (%) | | | | |
| Mild | 7 (23.3) | 9 (30.0) | | 0.77 |
| Moderate | 11 (36.7) | 9 (30.0) | | |
| Severe | 12 (40.0) | 12 (60.0) | | |
| Duration of dementia (month) (mean±SD) | 90.40±36.46 | 101.73±37.01 | | 0.24 |

**Table 2: Comparison of the means of different profile variables in patients with Alzheimer and vascular dementia and controls**

| Variable | Vascular dementia | Alzheimer dementia | Controls | p-value |
|---|---|---|---|---|
| HSP27 antibody (absorption unit) | 0.227±0.08 | 0.279±0.08 | 0.067±0.03 | 0.00 |
| Hs-CRP (μg/l) | 3.39±0.42 | 3.41±0.48 | 3.40±0.41 | 0.84 |
| FBS (mg/dl) | 90.7±8.5 | 91.8±7.7 | 90.3±6.5 | 0.58 |
| BUN (mg/dl) | 18.8±4.1 | 19.3±4.1 | 18.9±4.0 | 0.66 |
| CHOL (mg/dl) | 182.5±12.1 | 180.3±13.5 | 181.4±12.7 | 0.51 |
| LDL (mg/dl) | 89.7±16.3 | 82.5±13.2 | 84.6±15.1 | 0.06 |
| HDL (mg/dl) | 49.8±5.3 | 48.3±8.6 | 48.9±6.6 | 0.44 |
| TG (mg/dl) | 190.4±19.4 | 184.1±33.6 | 187.8±21.4 | 0.37 |
| HCT (mg/dl) | 39.1±2.1 | 39.1±2.4 | 39.2±2.3 | 0.95 |
| HB (mg/dl) | 13.23±1.3 | 13.61±1.4 | 13.45±1.5 | 0.63 |
| Cr (mg/dl) | 0.963±0.119 | 0.964±0.116 | 0.965±0.117 | 0.70 |

Hsp27antibody: Heat shok protein antibody, Hs-CRP: High sensitive-C reactive protein, FBS: Fast blood suger, BUN: Blood urea nitrogen, CHOL: Cholestrol, LDL: Low density lipoprotein, HDL: High density lipoprotein, TG: Triglycerid, HCT: Hematocrit, HB: Hemoglobin, Cr: Creatinin

**Table 3: HSP27 in different severities of Alzheimer and vascular dementia**

| Variable / Severity of disease | Type of dementia | HSP27 (absorption unit) | p-value (T-test) |
|---|---|---|---|
| Mild | Vascular dementia | 0.131±0.04 | 0.02 |
| | Alzheimer dementia | 0.184±0.03 | |
| Moderate | Vascular dementia | 0.210±0.05 | 0.04 |
| | Alzheimer dementia | 0.250±0.02 | |
| Severe | Vascular dementia | 0.296±0.07 | 0.01 |
| | Alzheimer dementia | 0.361±0.05 | |

HSP27: Heat Shok Protein 27

T-test showed a significant difference between the levels of HSP27 antibody titers between the two dementia groups (p=0.03); HSP27 antibody titers were lower in patients with vascular dementia compared to those with Alzheimer's-type dementia. (Table 3)

ANOVA test showed a significant difference between the level of HSP27 antibody titers according to the severity of the disease (mild, moderate and severe) in both patients groups; antibody titers increased with increasing severity (p<0.000). Post-hoc tests (Tukey) also indicated that the levels of antibody titers in the vascular dementia differed with severity of disease (mild, moderate and severe) (p=0.02, <0.000 and 0.01, respectively). For the patients with Alzheimer's-type dementia, Post-hoc test (Dunnett's T 3) indicated that the antibody levels of all different dementia severities are different from each other (p= 0.003, <0.000 and <0.000, respectively) (Diagram 1).

According to t-test, the level of HSP27 antibody titers was different in similar Alzheimer's-type and vascular dementia severities in a way that the antibody levels in the vascular dementia group was significantly less than the Alzheimer's disease group for all levels of severity (p=0.02, 0.04 and 0.01, respectively).

Moreover, Spearman test between the duration of dementia and the levels of HSP27 antibody indicated high correlation in vascular and Alzheimer's-type dementia groups (r=0.84, p<0.000, r=0.93, p<0.000 respectively).

According to ANOVA test, there was a significant difference between the level of HSP27 antibody titers in all groups (p=0.000). Moreover, Post-hoc test (Tukey) indicated that level of HSP 27 antibody titers were different significantly between Alzheimer disease and vascular dementia compared to controls separately (p=0.000, p=0.000 respectively).

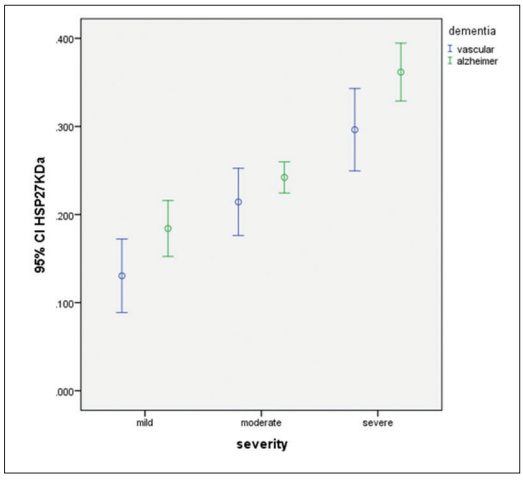


**Diagram 1:** Error bar diagram of the mean and CI 95% of HSP27 in different severities of Alzheimer and vascular dementia

## DISCUSSION

Our study indicated that the level of HSP27 antibody titers were high in individuals with vascular and Alzheimer's-type dementia than for controls. HSP27 antibody titers were lower in the vascular dementia patients compared to those with Alzheimer's dementia. There was also an association between antibody titers and the severity and duration of the disease. Shimura showed an association between HSP27 and hyperphosphorylated tau proteins in the brain of patients with Alzheimer's disease.[22]

In their investigation of the immunohistology of HSP27 expression in reactive gliosis in the brain of patients with Alzheimer's disease and other types of dementia, Renkawek *et al.* found out that the inductive incidence of this protein is high in the brain of patients with Alzheimer's disease. HSP27 expression was evident in a great number of proliferating astrocytes and the highest expression in degenerated astrocytes was in senile plaques. Neurofibrillary and Hirano bodies and a number of hippocampal neurons were also positive in this regard. HSP27 expression increased with increased intensity of morphological changes and duration of Alzheimer's disease. In brains from a control group, immune reactions were limited to vessels and specific astrocytes in brain white matter. Similar patterns of immune reactions were observed in individuals without dementia (Parkinson's disease, lacunar state or necrosis and regional ischemia). Patients with other dementia types (Parkinson's disease/complex dementia, multi-infarct dementia and normal pressure hydrocephalus) indicated lesser amounts of HSP27 expression in reactive astrocytes compared to the Alzheimer's disease group which was higher than the control group. Based on the results, especially in astrocytes which showed klazmatodendrosis, there is an association between increased HSP27 expression and increased pathology of Alzheimer's disease.[21] The results of this study regarding increase in the expression of HSP27 in patients with Alzheimer's disease and its association with the severity and duration of the disease is potentially consistent with our study.

Toth *et al.* investigated the effect of increased HSP27 expression on the improvement of the symptoms of Alzheimer's disease in a type of transgenic laboratory mouse, behavioral and electrophysiological tests indicated that learning and neuronal function improves in mice with Alzheimer's disease and increased HSP27 expression. Moreover, fewer amyloid plaques are formed in the brain of mice with increased HSP27 expression.[13]

Renkawek *et al.* studied patients with Alzheimer's dementia and age-matched control group patients without dementia indicated that under normal conditions, the human brain has a low incidence of HSP27 which is limited to vessels and single astrocytes in the white matter. However, the incidence of HSP27 significantly increases in brain cortex in patients with Alzheimer's dementia.[23]

It was also indicated that there is a strong association between Immune reactions of HSP27 incidence and the severity of specific Alzheimer's disease changes; especially in the number of tangles in the hippocampus.[17] These results are also consistent with the present study regarding the incidence of HSP27 and its association with the severity of Alzheimer's disease. Moreover, other studies indicated the role of HSP27 in Alzheimer's disease and the importance of the therapeutic role of this factor in the assessment and the application of protein-based therapies for the treatment of many neurodegenerative diseases, particularly Alzheimer's disease.[24-26]

## CONCLUSION

We have found that HSP27antibody titers are increased in patients with Alzheimer's disease and vascular dementia compared with healthy subjects of similar age. Antibody titers were also related to the severity of disease and its duration. For any severity of dementia, antibody levels were significantly lower in patients with vascular dementia compared to those with Alzheimer's disease. The results of our study constitute a basis to target HSP as the promising therapeutics approach for both dementia groups particularly Alzheimer's disease and many neurodegenerative diseases, but to make more comprehensive conclusions in this regard, further investigations are warranted in future studies.

## REFERENCES


1. Richards SS, Sweet RA. Dementia. In: Sadock BJ, Sadock VA, Ruiz P. (editors). Kaplan and Sadock's comprehensive textbook of psychiatry. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2009.
2. Grabowski TJ, DeKosky ST, Eichler AF., Clinical manifestations and diagnosis of Alzheimer disease, 2013.
3. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E. Alzheimer disease. Lancet 2011; 377(9770): 1019-31.
4. Tissieres A, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. J Mol Biol, 1974; 85: 389-98.
5. Mymrikov EV, Seit-Nebi AS, Gusev NB. Large potentials of small heat shock proteins. Physiol Rev 2011; 91(4): 1123-59.
6. Almeida-Souza L, Goethals S, de Winter V, Dierick I, Gallardo R, Van Durme J, et al. Increased monomerization of mutant HSPB1 leads to protein hyperactivity in Charcot-Marie-Tooth neuropathy. J Biol Chem 2010; 285: 12778-86.
7. Arya R, Mallik M, Lakhotia SC. Heat shock genes: integrating cell survival and death. J Biosci 2007; 32: 595-610.
8. Brundel BJ, Ke L, Dijkhuis AJ, Qi X, Shiroshita-Takeshita A, Nattel S, et al. Heat shock proteins as molecular targets for intervention in atrial fibrillation. Cardiovasc Res 2008; 78: 422-8.
9. Gooch C, Shy M. Hereditary motor neuropathy and heat shock

proteins: a shocking transformation. Neurology 2008; 71: 1656-7.
10. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 2002; 20: 395-425.
11. Zourlidou A, Payne Smith MD, Latchman DS. HSP27 but not HSP70 has a potent protective effect against alpha-synuclein-induced cell death in mammalian neuronal cells. J Neurochem 2004; 88: 1439-48.
12. Stock AD, Spallone PA, Dennis TR, Netski D, Morris CA, Mervis CB, et al. Heat shock protein 27 gene: chromosomal and molecular location and relationship to Williams's syndrome. Am J Med Genet A 2003; 120A: 320-5.
13. Toth ME, Szegedi V, Varga E, Juhasz G, Horvath J, Borbely E, et al. Overexpression of Hsp27 ameliorates symptoms of Alzheimer's disease in APP/PS1 mice. Cell Stress Chaperones 2013; 18(6): 759-71.
14. Outeiro TF, Klucken J, Strathearn KE, Liu F, Nguyen P, Rochet JC, et al. Small heat shock proteins protect against alpha-synuclein-induced toxicity and aggregation. Biochem Biophys Res Commun 2006; 351: 631-8.
15. Romi F, Helgeland G, Gilhus NE. Heat-shock proteins in clinical neurology. Eur Neurol 2011; 66(2): 65-9.
16. Vleminckx V, Van Damme P, Goffin K, Delye H, Van den Bosch L, Robberecht W. Upregulation of HSP27 in a transgenic model of ALS. J Neuropathol Exper Neurol 2002; 61(11): 968-74.
17. Renkawek K, Stege GJJ, Bosman GJCGM. Dementia, gliosis and expression of the small heat shock proteins HSP27 and alpha B-crystallin in Parkinson's disease. Neuroreport 1999; 10:2273-6.
18. Ecroyd H, Carver JA., Crystallin proteins and amyloid fibrils. Cell Mol Life Sci 2009; 66: 62-81.
19. Laskowska E, Matuszewska E, Kuczynska-Wisnik D. Small heat shock proteins and protein-misfolding diseases. Curr Pharm Biotechnol 2010; 11: 146-57.
20. Read DE, Gorman AM., Heat shock protein 27 in neuronal survival and neurite outgrowth. Biochem Biophys Res Commun 2009; 382: 6-8.
21. Renkawek K, Bosman GJ, de Jong WW., Expression of small heat-shock protein hsp 27 in reactive gliosis in Alzheimer disease and other types of dementia. Acta Neuropathol 1994; 87(5): 511-9.
22. Shimura H, Miura-Shimura Y, Kosik KS. Binding of tau to heat shock protein 27 leads to decreased concentration of hyperphosphorylated tau and enhanced cell survival. Biol Chem 2004; 279: 17957-62.
23. Renkawek K, Bosman GJ, Gaestel M. Increased expression of heat-shock protein 27 kDa in Alzheimer disease: a preliminary study. Neuroreport 1993; 5(1): 14-6.
24. Arrigo AP, Simon S, Gibert B, Kretz-Remy C, Nivon M, Czekalla A, et al. Hsp27 (HspB1) and alphaB-crystallin (HspB5) as therapeutic targets. FEBS Lett 2007; 581: 3665-74.
25. Nemes Z, Devreese B, Steinert PM, Van Beeumen J, Fesus L. Cross-linking of ubiquitin, HSP27, parkin, and alpha-synuclein by gamma-glutamyl-epsilon-lysine bonds in Alzheimer's neurofibrillary tangles. FASEB J 2004; 18: 1135-7.
26. Sharp PS, Akbar MT, Bouri S, Senda A, Joshi K, Chen HJ, et al. Protective effects of heat shock protein 27 in a model of ALS occur in the early stages of disease progression. Neurobiol Dis 2008; 30: 42-55.

**How to cite this article:** Kiani M, Mokhber N, Mobarhan MG, Jarrahi L, Najarzadegan MR, Ataei E, Rahimpour M, Akbarzadeh F. Serum HSP27 antibody titers in patients with Alzheimer's disease and vascular dementia. Acta Medica International. 2016;3(2):56-60.

**Source of Support:** Nil, **Conflict of Interest:** None declared.