Original Article* / *Çalışma - Araştırma

Prognostic value of serum anti-heat-shock protein 70 and paraoxonase levels in idiopathic sudden sensorineural hearing loss

İdiyopatik ani sensörinöral işitme kaybında anti-ısı-şok protein 70 ve paraoksonaz serum düzeylerinin prognostik değeri

Sertaç Düzer, M.D.,[1] Hayrettin Cengiz Alpay, M.D.,[2] Dilara Kaman, M.D.,[3] Öner Sakallıoğlu, M.D.,[1] Şinasi Yalçın, M.D.,[2] İrfan Kaygusuz, M.D.,[2] Turgut Karlıdağ, M.D.,[2] Erol Keleş, M.D.[2]

[1]*Department of Otolaryngology, Elazığ Training and Research Hospital, Elazığ, Turkey*
[2]*Department of Otolaryngology, Medical Faculty of Fırat University, Elazığ, Turkey*
[3]*Department of Biochemistry, Medical Faculty of Fırat University, Elazığ, Turkey*

Objectives: This study aims to investigate the correlation between serum anti-heat-shock protein 70 (anti-HSP 70) levels, serum paraoxonase (PON) levels and prognosis of idiopathic sudden sensorineural hearing loss (ISSHL).

Patients and Methods: Twenty-five patients with ISSHL as the study group and 25 healthy volunteers as the control group were enrolled in this study. Blood samples were obtained from all patients before the treatment initiation and on the 10th day of the treatment from only patients of the study group. Idiopathic sudden sensorineural hearing loss was defined as the hearing loss between 250-6.000 Hz frequencies. The recoveries in the hearing thresholds were evaluated at 10 days of the treatment.

Results: When the pre-treatment serum PON, anti-HSP 70 levels and the post-treatment serum PON, anti-HSP 70 levels of the patients with ISSHL were compared, we observed that the post-treatment serum PON levels of the recovered patients increased, while the post-treatment serum anti-HSP 70 levels of recovered patients decreased.

Conclusion: We believe that serum levels of anti-HSP 70 and PON can be used as markers for estimating and evaluating the prognosis of ISSHL patients.

Key Words: Heat-shock protein 70; idiopathic sudden sensorineural hearing loss; paraoxonase; prognosis.

Amaç: Bu çalışmada serum anti-ısı-şok protein 70 (anti-IŞP 70) düzeyleri ve serum paraoksonaz (PON) düzeyleri ve idiyopatik ani sensörinöral işitme kaybı (İASNİK) prognozu arasındaki ilişki araştırıldı.

Hastalar ve Yöntemler: Çalışmaya 25 İASNİK'li hasta çalışma grubu olarak ve 25 sağlıklı gönüllü kontrol grubu olarak dahil edildi. Tüm hastalardan tedaviye başlamadan önce ve yalnızca çalışma grubundaki hastalardan tedavinin 10. gününde kan örneği alındı. İdiyopatik ani sensörinöral işitme kaybı, 250-6.000 Hz arasındaki işitme kaybı olarak tanımlandı. Tedavinin 10. gününde işitmede düzelme olup olmadığı değerlendirildi.

Bulgular: Tedavi öncesi serum PON ve anti-IŞP 70 düzeyleri tedavi sonrası serum PON ve anti-IŞP 70 düzeyleri ile karşılaştırıldığında, iyileşen hastalarda tedavi sonrası serum PON düzeylerinin arttığı, yine iyileşen hastalarda tedavi sonrası anti-IŞP 70 serum düzeylerinin ise azaldığı belirlendi.

Sonuç: Anti-IŞP 70 ve PON serum düzeylerinin, İASNİK'li hastalarda prognozu tahmin etmede ve değerlendirmede kullanılabileceğini düşünmekteyiz.

Anahtar Sözcükler: Isı şok protein 70; idiyopatik sensörinöral ani işitme kaybı; paraoksonaz; prognoz.

Available online at
www.kbbihtisas.org
doi: 10.5606/kbbihtisas.2014.02603
QR (Quick Response) Code

Received / *Geliş tarihi:* May 06, 2013 Accepted / *Kabul tarihi:* December 20, 2013

Correspondence / İletişim adresi: Öner Sakallıoğlu, M.D. Elazığ Eğitim ve Araştırma Hastanesi Kulak Burun Boğaz Hastalıkları Kliniği, 23200 Elazığ, Turkey.
Tel: +90 532 - 690 55 20 e-mail *(e-posta):* onersakallioglu@yahoo.com

Sudden hearing loss is a sensorineural hearing loss (SHL) exceeding 30 dB over three contiguous pure-tone frequencies occurring within three days. The incidence of SHL in the population is 5-20/100,000. It generally occurs between the ages of 30 and 60 years, 90% is unilateral, and it has various causes. Nevertheless, no identifiable cause is detected in 80-90% of patients; this is known as idiopathic sudden sensorineural hearing loss (ISSHL).[1,2] Generally, the prognosis is bad in patients with advanced age, bilateral and total hearing loss, objective vestibular symptoms, vascular disorders, and delayed treatment. If the hearing loss curve decreases from low- to high-pitched sound in the audiogram in SHL, it is called a rising curve and has a good prognosis.[1] However, no serum prognostic marker has been identified for ISSHL.

In 1990, Harris and Sharp[3] discovered antibodies against a 68-kDa protein derived from inner ear extracts of cattle in the serum of SHL patients. Subsequent studies examined the role of these anti-68-kDa antibodies in SHL and Ménière's disease. The antibody, which was produced in response to an infectious agent, can cross-react with human heat-shock protein 70 (HSP70), and can trigger an autoimmune reaction in human target organs, as in the excessive secretions caused by chronic inflammation in patients with Ménière's disease and hearing loss.[4] Although many studies have examined serum anti-HSP antibodies in the majority of inner ear diseases, few have investigated the relationship between the anti-HSP70 level in ISSHL patients and prognosis.

It has been postulated that human serum paraoxonase (PON) is related to high-density lipoprotein (HDL), and that it has an antioxidant function. Experimental studies have shown relationships between PON and the HDLs apolipoprotein-A1 (Apo-A1) and apolipoprotein J (Apo-J)/clusterin.[5-11] Paraoxonase is thought to exert its antioxidant effect by protecting low density lipoprotein-K (LDL-K) from the oxidation induced by copper ions and free radicals.[6,7-12] One study found an association between a PON2 gene polymorphism and SHL and postulated that it could represent a marker of susceptibility to noise-induced hearing loss.[13]

As mentioned, few studies have evaluated the relationship between anti-HSP70 levels and the prognosis of ISSHL patients. In addition, no study has directly investigated the correlation between serum PON activity and hearing loss. Therefore, we investigated the correlations between serum anti-HSP70 and PON levels and the prognosis of ISSHL.

PATIENTS AND METHODS

Fifty participants were enrolled in this study: 25 patients (8 females, 17 males; mean age 39.48 years; range 16 to 65 years;) diagnosed with ISSHL and treated at the Otorhinolaryngology Clinic of Firat University, Faculty of Medicine and 25 healthy volunteers (11 females, 14 males; mean age 34.16 years; range 21 to 59 years) with no history of hearing loss, and no systemic or ear diseases. In the study group, 14 patients (56%) had lost hearing in the right ear, and 11 (44%) in the left ear. No patient had bilateral hearing loss. Informed consent was obtained from all participants, and the study followed the guidelines of the Declaration of Helsinki. This study was approved by the local Ethics Committee.

Detailed histories were obtained from all patients with a diagnosis of ISSHL and the control volunteers. Detailed ear, nose, throat, and systemic examinations were performed. The routine biochemical parameters, hemogram, prothrombin time (PT), and active partial thromboplastin time (aPTT) were measured. Patients seen within seven days of the onset of hearing loss with normal preprandial blood biochemistry were included in the study. Patients who had previously suffered from hearing loss, had any additional systemic diseases, any ear disease, retrocochlear pathology detected by temporal bone magnetic resonance imaging (MRI), coagulation disorders detected when the serum PT and aPTT were examined, and immunoglobulin M (IgM) for viruses (including CMV, roseola, rubella, chicken pox, HSV-1, HSV-2, parainfluenza, influenza A and B, EBV, or mumps) were excluded.

The diagnosis of ISSHL was established as a hearing loss ≥30 dB detected at 250, 500, 1000, 2000, 4000, and 6000 Hz on pure-tone audiometry. Recovery of the hearing thresholds was evaluated 10 days after initiating treatment and was classified in accordance with Siegel's classification for the standardization of the recovery of patients with hearing loss, as follows:[14]

Category I: Complete recovery;

Category II: Despite a recovery exceeding 15 dB, the patient does not have a normal hearing threshold, but has a pure-tone audiometry average better than 45 dB (partial recovery);

Category III: Despite a recovery exceeding 15 dB, the patient's pure-tone audiometry average is worse than 45 dB (partial recovery);

Category IV: The recovery is less than 15 dB or there is no recovery of the hearing thresholds (no recovery).

Corticosteroid treatment was initiated after the diagnosis of ISSHL, and serum samples were obtained from the patients. The patients were administered 1 mg/kg prednisone (Prednol tablets, Mustafa Nevzat Drug Industry, Turkey) orally, and the dose was reduced by 8 mg per day. While on the corticosteroid, the patients were administered the proton pump inhibitor lansoprazole (Lanzedin, Biofarma Drug Industry, Turkey) 2x30 mg orally. Parenteral 76% sodium amidotrizoate 2x1/2 ampoules (Urografin, Bayer Turkish Chemistry Drug Industry, Turkey) was infused intravenously for five days. Oral vitamin B1 (2x250 mg thiamine hydrochloride) and B6 (250 mg pyridoxine hydrochloride; Nerox B tablet, Abdi İbrahim Pharmaceutical Company) were given for three months. Carbogen therapy (5% CO_2 + 95% O_2) was applied for 10 days, four times per day, for 30 min each via inhalation. The antiviral brivudine 125 mg orally (Zostex, Ufsa Drug Industry, Turkey) was administered for seven days and the microcirculation regulating agent trimetazidine 3x20 mg (Vastarel, Servier Drug Industry, Turkey) was added for three months.

Blood samples (4 ml) were collected in anticoagulant-free tubes from the patients before the treatment started and on the tenth day of treatment, and once from the healthy controls. The samples were centrifuged for 10 min at 4000 rpm, +4 °C and the serum was stored at -80 °C until the PON and anti-HSP70 levels were determined. The serum PON activity was measured using the method of Eckerson et al.,[15] which is based on the spectrophotometric measurement of the activity of 4-nitrophenol produced via enzymatic hydrolysis with the addition of 1 mol/L NaCl at a wavelength of 412 nm (Techcomp 8500 II UV/vis, Techcomp, China). An ELISA kit (Stressgen, catalogue no: EKS-750, Ann Arbor, USA) was used to assay anti-HSP70. The anti-HSP70 IgG, IgA, and IgM (total) levels were determined spectrophotometrically.

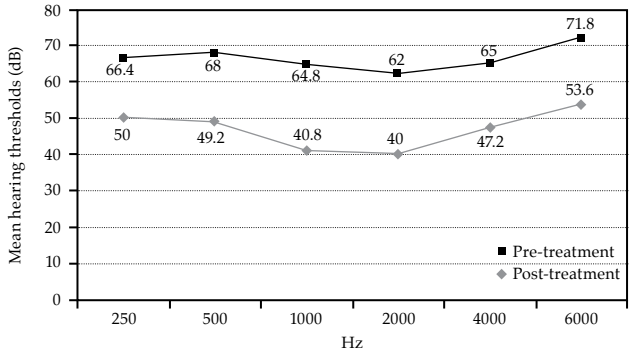

Figure 1. *Mean hearing thresholds (dB) at various frequencies pre- and post-treatment.*

For statistical analysis, Student's t-test was performed using the SPSS version 12.0 for Windows software program (SPSS Inc., Chicago, IL, USA) A value of $p<0.05$ was taken to indicate statistical significance.

RESULTS

In the study group, 13 patients first visited our clinic within three days of their hearing loss, and the other 12 patients visited 4-7 days after their hearing losses. Their treatments were started on the first visit. When the frequency-specific hearing thresholds before and after treatment were compared, significant recovery was observed

Table 1. Pre-treatment paraoxonase and anti-heat-shock protein 70 levels in the study group and controls

	Study group	Controls	*p**
	Mean±SD	Mean±SD	
Paraoxonase (U/mL)	265.51±11.69	205.17±18.04	<0.05
Anti-heat-shock protein 70 (µg/ml)	97.90±7.21	84.25±5.15	>0.05

* Student's t-test; SD: Standard deviation; $p<0.05$ indicates a statistically significant difference.

Table 2. Post-treatment paraoxonase and anti-heat-shock protein 70 levels of the study group and controls

	Study group	Controls	*p**
	Mean±SD	Mean±SD	
Paraoxonase (U/mL)	284.44±17.76	205.17±18.04	<0.05
Anti-heat-shock protein 70 (mg/ml)	77.48±5.73	84.25±5.15	>0.05

* Student's t-test; SD: Standard deviation; p<0.05 indicates a statistically significant difference.

Table 3. Pre- and post-treatment mean paraoxonase and anti-heat-shock protein 70 levels of the patients

	Study group	Controls	*p**
	Mean±SD	Mean±SD	
Paraoxonase (U/mL)	265.51±11.69	284.44±17.76	>0.05
Anti-heat-shock protein 70 (mg/ml)	97.90±7.21	77.48±5.73	<0.05

* Student's t-test; SD: Standard deviation; p<0.05 indicates a statistically significant difference.

(Figure 1), with complete recovery (category I) in six (24%), partial recovery (categories II and III) in nine (36%), and no recovery (category IV) in 10 (40%) of the 25 ISSHL patients.

Pre-treatment serum PON levels were significantly (p<0.05) higher in the study group than in controls. Although the pre-treatment serum anti-HSP70 levels were higher in the study group, the difference was not significant (p>0.05) (Table 1). The post-treatment serum PON levels were significantly (p<0.05) higher in the study group than in the controls. Although the post-treatment serum anti-HSP70 levels were lower in the study group than the controls, the difference was not significant (p>0.05) (Table 2).

In the ISSHL patients, there was no significant (p>0.05) difference between the pre- and post-treatment serum PON levels, but a significant (p<0.05) difference was identified between the pre- and post-treatment serum anti-HSP70 levels (Table 3). In the ISSHL patients with either complete or partial recovery, the post-treatment serum anti-HSP70 levels were significantly (p<0.05) lower than the pre-treatment levels. The decrease was not significant in the patients who did not recover (Table 4). Conversely, the pre-treatment serum PON levels were lower than the post-treatment serum PON levels in the patients who recovered, but were higher in those who did not recover; however, these differences were not significant (p>0.05) (Table 5).

DISCUSSION

Idiopathic sudden sensorineural hearing loss is an otological emergency. Despite intensive investigation of the physiopathology and histopathology of SHL, the etiology is not understood completely. The treatment of ISSHL is based on its etiology. In ISSHL, treatment has no superiority to spontaneous recovery which is observed in 60-65% of patients, even without

Table 4. Mean anti-heat-shock protein 70 levels of patients who did and did not recover before and after treatment

	Complete or partial recovery	*p**	No recovery	*p**
	Mean±SD		Mean±SD	
Pre-treatment anti-HSP70 (μg/ml)	113.39±8.48	<0.05	74.67±8.89	>0.05
Post-treatment anti-HSP70 (μg/ml)	82.57±6.65	<0.05	69.84±10.26	>0.05

* Student's t-test; SD: Standard deviation; anti-HSP70: Anti-heat-shock protein 70; p<0.05 indicates a statistically significant difference.

Table 5. Mean paraoxonase levels of patients who did and did not recover before and after treatment

	Complete or partial recovery	*p**	No recovery	*p**
	Mean±SD		Mean±SD	
Pre-treatment paraoxonase (U/mL)	288.19±15.71	>0.05	231.47±11.02	>0.05
Post-treatment paraoxonase (U/mL)	325.22±20.80	>0.05	223.26±19.99	>0.05

* Student's t-test; SD: Standard deviation; p<0.05 indicates a statistically significant difference.

treatment, in the first two weeks after disease onset.[16]

For the last 50 years, ISSHL has been thought to have an autoimmune origin. In animal studies, the cochlea secretes HSP70 upon ototoxic stimulation.[17] Mathews et al.[18] found that ISSHL patients possessed antibody against HSP70. Loveman et al.[19] detected anti-HSP70 in the serum of 27 of 30 patients (90%) with ISSHL. Although the existence of serum anti-HSP70 antibody has been investigated in most inner ear diseases, few studies have evaluated the relationship between the anti-HSP70 level and the prognosis of patients with ISSHL.

Similarly, a PON2 gene polymorphism may be associated with SHL and could represent a marker of susceptibility to noise-induced hearing loss.[13] However, no study has examined the serum PON levels of patients with ISSHL. We found no significant difference between the pre-treatment serum anti-HSP70 levels of the study and control groups. However, in the study group, the pre- and post-treatment serum anti-HSP70 levels differed significantly. In addition, serum anti-HSP70 levels were significantly lower in patients who recovered (either complete or partial recovery) than those who did not.

Süslü et al.[20] investigated anti-HSP70, tumor necrosis factor-alpha (TNF-α), and antinuclear antibody (ANA) levels, and erythrocyte sedimentation rate (ESR), in ISSHL and found no increase in anti-HSP70 levels in the study group compared to controls, but significant increases in ANA and ESR and a decrease in TNF-α levels. Park et al.[21] reported a significant difference in the anti-HSP70 levels of patients compared to a control group before treatment for sudden hearing loss. Similarly, Gross et al.[22] detected anti-HSP70 antibodies in 60 of 63 SHL patients. In our study, there was no significant difference between the pre-treatment serum anti-HSP70 levels of the study group and those of the controls, similar to the findings of Süslü et al.[20]

Paraoxonase exists in many tissues, including the liver, kidneys, small intestine, and in serum.[6,23] Non-genetic factors, such as diet, acute-phase reactants, pregnancy, hormones, smoking, and simvastatin treatment, can modulate serum PON levels.[24] Moreover, PON activity decreases with increased age.[25] In our study, the pre-treatment serum PON levels of the ISSHL patients were significantly (p<0.05) higher than those of controls. The pre-treatment serum PON levels were high in recovered (either complete or partial recovery) patients after treatment. In the patients who did not recover, the post-treatment serum PON levels were lower than the pre-treatment levels. However, these differences were not significant (p>0.05).

Conclusion

When the ISSHL patients were subdivided into groups that did (either complete or partial recovery) or did not (no recovery) recover, the pre-treatment serum anti-HSP70 levels of the recovered patients were higher than the post-treatment levels. Conversely, the pre-treatment serum PON levels of the recovered patients were lower than the post-treatment levels. An increase in the post-treatment serum PON levels suggests a good prognosis; moreover, the prognosis of ISSHL might be better in those with higher pre-treatment serum anti-HSP70 levels compared to those post-treatment. Therefore, serum anti-HSP70 and PON levels might represent novel prognostic markers for ISSHL patients; however more comprehensive studies of this topic are needed.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors thank the Fırat University, Scientific Research and Projects Unit for financial support.

REFERENCES

1. Byl FM Jr. Sudden hearing loss: eight years' experience and suggested prognostic table. Laryngoscope 1984;94:647-61.
2. Mattox DE, Simmons FB. Natural history of sudden sensorineural hearing loss. Ann Otol Rhinol Laryngol 1977;86:463-80.
3. Harris JP, Sharp PA. Inner ear autoantibodies in patients with rapidly progressive sensorineural hearing loss. Laryngoscope 1990;100:516-24.
4. Bloch DB, Gutierrez JA, Guerriero V Jr, Rauch SD, Bloch KJ. Recognition of a dominant epitope in bovine heat-shock protein 70 in inner ear disease. Laryngoscope 1999;109:621-5.
5. Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN. Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. Arterioscler Thromb Vasc Biol 1995;15:1812-8.
6. Heijmans BT, Westendorp RG, Lagaay AM, Knook DL, Kluft C, Slagboom PE. Common paraoxonase gene variants, mortality risk and fatal cardiovascular events in elderly subjects. Atherosclerosis 2000;149:91-7.
7. Kelso GJ, Stuart WD, Richter RJ, Furlong CE, Jordan-Starck TC, Harmony JA. Apolipoprotein J is associated with paraoxonase in human plasma. Biochemistry 1994;33:832-9.
8. Hasselwander O, McMaster D, Fogarty DG, Maxwell AP, Nicholls DP, Young IS. Serum paraoxonase and platelet-activating factor acetylhydrolase in chronic renal failure. Clin Chem 1998;44:179-81.
9. Mackness B, Hunt R, Durrington PN, Mackness MI. Increased immunolocalization of paraoxonase, clusterin, and apolipoprotein A-I in the human artery wall with the progression of atherosclerosis. Arterioscler Thromb Vasc Biol 1997;17:1233-8.
10. McElveen J, Mackness MI, Colley CM, Peard T, Warner S, Walker CH. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 1986;32:671-3.
11. Mackness B, Durrington PN, Mackness MI. Human serum paraoxonase. Gen Pharmacol 1998;31:329-36.
12. Aviram M, Hardak E, Vaya J, Mahmood S, Milo S, Hoffman A, et al. Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. Circulation 2000;101:2510-7.
13. Fortunato G, Marciano E, Zarrilli F, Mazzaccara C, Intrieri M, Calcagno G, et al. Paraoxonase and superoxide dismutase gene polymorphisms and noise-induced hearing loss. Clin Chem 2004;50:2012-8.
14. Siegel LG. The treatment of idiopathic sudden sensorineural hearing loss. Otolaryngol Clin North Am 1975;8:467-73.
15. Eckerson HW, Romson J, Wyte C, La Du BN. The human serum paraoxonase polymorphism: identification of phenotypes by their response to salts. Am J Hum Genet 1983;35:214-27.
16. Booth JB. Sudden and fluctuant sensorineural hearing loss. In: Kerr AG, editor. Scott Brown's Otolaryngology. 6th ed. London: Butterworths-Heinemann 1997. p. 1-82.
17. Gong SS, Yan Z. Expression of heat shock protein 70 in the cochlea in experimental autoimmune inner ear disease. Ann Otol Rhinol Laryngol 2002;111:275-80.
18. Mathews J, Rao S, Kumar BN. Autoimmune sensorineural hearing loss: is it still a clinical diagnosis? J Laryngol Otol 2003;117:212-4.
19. Loveman DM, de Comarmond C, Cepero R, Baldwin DM. Autoimmune sensorineural hearing loss: clinical course and treatment outcome. Semin Arthritis Rheum 2004;34:538-43.
20. Süslü N, Yilmaz T, Gürsel B. Utility of anti-HSP 70, TNF-alpha, ESR, antinuclear antibody, and antiphospholipid antibodies in the diagnosis and treatment of sudden sensorineural hearing loss. Laryngoscope 2009;119:341-6.
21. Park SN, Yeo SW, Park KH. Serum heat shock protein 70 and its correlation with clinical characteristics in patients with sudden sensorineural hearing loss. Laryngoscope 2006;116:121-5.
22. Gross M, Eliashar R, Ben-Yaakov A, Ulmansky R, Elidan J. Prevalence and clinical significance of anticardiolipin, anti-beta2-glycoprotein-1, and anti-heat shock protein-70 autoantibodies in sudden sensorineural hearing loss. Audiol Neurootol 2008;13:231-8.
23. bin Ali A, Zhang Q, Lim YK, Fang D, Retnam L, Lim SK. Expression of major HDL-associated antioxidant PON-1 is gender dependent and regulated during inflammation. Free Radic Biol Med 2003;34:824-9.
24. Biasioli S, Schiavon R, Petrosino L, De Fanti E, Cavalcanti G, Battaglia P, Fasolin A. Paraoxonase activity and paraoxonase 1 gene polymorphism in patients with uremia. ASAIO J 2003;49:295-9.
25. Seres I, Paragh G, Deschene E, Fulop T Jr, Khalil A. Study of factors influencing the decreased HDL associated PON1 activity with aging. Exp Gerontol 2004;39:59-66.