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**Diagnostic Role for Antibodies against Heat Sock Protein 70 In Perilymphatic Fluid of Children with Cochlear Implant Surgery: A Case Control Study in Tehran, Iran**

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**KEYWORDS**

Cochlear implant,  
SNHL,  
HSP70,  
Perilymphatic  
fluid

**A B S T R A C T**

Serum antibodies against HSP 60 and HSP 70 are diagnostic for infectious diseases. Goal of study: quantification the antibodies against Heat Sock Protein 70Ab (HSP 70) in serum and perilymphatic fluid of Children with cochlear implant surgery. A cross sectional study had done upon 117 children undergoing cochlear implant surgery in the cochlear implant ward. Age of cases was between 6 months -14years ;mean :3. 4±3. 1years;. 58% male,42% female. Idiopathic SNHL diagnosed in 39. 4 % of cases. Blood samples (2 ml) were taken, then centrifuged and transferred to our research laboratory. During surgery period 0. 5-3 ml of Perilymphatic fluids were taken by ENT specialist in operation room. All samples were kept frozen and stored at -70°C. Hsp70 level determined by using the ELISA kits (STRESS XPRESS, stressgen biotechnologies Canada). The Heat Sock Protein70Ab (HSP 70) level determined in serum and perilymphatic fluid SNHL in cochlear implanted children, and compared between idiopathic type of SNHL and hereditary type. P-value <0. 05 was considered significant Findings: One hundred twenty four cases enrolled in this study. ; 58% male,42% female. Age of cases was between 6 months -14years ;mean :3. 4±3. 1years;. Idiopathic SNHL diagnosed in 39. 4 % of cases ( Fig-1) The Heat Sock Protein70 Ab detected in sera of 46. 8% of studied cases. Heat Sock Protein70 Ab level in perilymphatic fluid was higher in idiopathic SNHL cases (P = 0. 000). A ROC curve analysis showed an AUC(area under ROC curve) 0. 731 (95% CI; 0. 629-0. 834, P = 0. 000). HSP70 Ab (Cutoff level 0. 39 pg/dl ) in perilymphatic fluid had 76% sensitivity and 60% specificity for differentiating the idiopathic type of SNHL from hereditary ones. . Assessment the HSP70 level in perilymphatic fluid (76% sensitivity and 60 specificity ) might be useful for differentiating the idiopathic type of SNHL from hereditary ones. This test can potentially assist clinicians in faster diagnosis and specific treatment in cases with progressive idiopathic SNHL. Further studies are necessary in order to identify the immunologic role of the these antibodies and their impact on the prognosis of SNHL.

## **Introduction**

Sensorineural hearing loss can be mild, moderate, or severe, including total deafness. Neural hearing loss is the result of a problem with the auditory nerve or the brain itself. Most sensory hearing loss is due to poor hair cell function. The hair cells may be abnormal at birth, or damaged during the lifetime of an individual. There are both external causes of damage, like noise trauma and infection, and intrinsic abnormalities, like deafness genes. An acquired hearing loss refers to any type of hearing loss that appears after birth.

Acquired hearing loss can occur at any time in a person's life. This type of hearing loss may be caused by many things such as the result of a disease, a medical condition, or an injury. Some conditions that can result in an acquired hearing loss, especially in children are - ear infections (otitis media) ototoxic drugs, meningitis, measles, encephalitis, chicken pox, influenza, mumps, head injury and excessive noise exposure. Some possible causes of neural hearing loss include - heredity, exposure to drugs, excessive noise, prenatal exposure to rubella, cytomegalovirus, Rh incompatibility at birth, low birth weight caused by premature birth, elevated bilirubin levels (or jaundice), meningitis and some types of infectious diseases such as the mumps. Idiopathic hearing loss basically means unexplained sudden hearing loss. This type of hearing loss usually affects people who are between the ages of 30 to 60 years old. The condition affects both males and females equally. Although sudden idiopathic hearing loss (SIHL) is called sudden, it's not very likely that a person's loss of hearing happens so abruptly. Sudden hearing loss (SHL) is described as losing greater than 30 decibels of hearing over at least three contiguous frequencies within three days (72 hours) or

less. A more likely scenario would be that the person's hearing loss actually takes place over a few hours. Sudden hearing loss: Idiopathic (ISSHL: idiopathic sudden sensorineural hearing loss), H91. 2 Vascular ischemia of the inner ear or Cranial nerve, N8 Perilymph fistula, usually due to a rupture of the round or oval windows and the leakage of perilymph. The patient will most likely also experience vertigo or imbalance. A history of an event that increased intracranial pressure or caused trauma is usually present (20).

Autoimmune hearing loss is a plausible explanation for a certain percentage of the group categorized as the idiopathic type. SNHL in children can be caused by autoimmune disorders localized to the inner ear or secondary to systemic immune diseases (Merchant et al., 2005; Cadoni et al., 2003). Biomarkers are becoming increasingly important tools within all areas of medicine. Various biological markers like CRP, procalcitonin, and recently STREM-1 was useful for diagnosis and to differentiate between bacterial and aseptic meningitis<sup>5-7</sup>. Recently procalcitonin has been used for diagnosis of serious bacterial infections like bacterial meningitis. A soluble form of TREM-1 is released from the activated phagocytes and can be found in body fluids. STREM-1 is also detected in the blood of patients with endotoxemia and sepsis<sup>8</sup>. It has demonstrated that the presence of STREM-1 in CSF of patients has high specificity in the diagnosis of bacterial meningitis.<sup>9-12</sup> AECAs are of prognostic importance in these diseases and can be considered as a useful clinical tool to differentiate patients with idiopathic hearing loss (Vasama & Linthicum).

Bleotu et al<sup>12</sup> studied the significance of serum antibodies against HSP 60 and HSP 70 for the diagnostic of infectious diseases. High levels of anti-HSP60 were found in

patients with localized infections, anti-HSP70 were higher in generalized infections. ;anti-HSP 60 and anti-HSP70 were significantly increased in patients with Gram-negative bacterial infections, they recommend as additional diagnosis and prognosis markers in Gram-negative bacterial infections<sup>12</sup>. Kilic et al<sup>13</sup> assessed the levels of serum heat shock protein 70 in children with simple febrile convulsions<sup>13</sup>. They reported that Hsp70 were higher in the febrile convulsion) and epileptic convulsion and infection groups than in the controls (p<0. 0001) 12.

Karger AG reported the association between anti-HSP70 and anti-PLs antibodies with SSNHL, and a positive outcome of SSNHL. Further studies are necessary in order to identify and further clarify the immunologic role of the presence of autoantibodies and their impact on the prognosis of SSNHL

Most of unilateral deafness is diagnosed accidentally, usually between the age of 7 and 10 years. The children nor their parents could precisely determine the time of its onset, SNHL due to various etiologies is common in Iranian children, Cochlear implant surgery is needed for some cases

Many risk factors for hearing loss in infants and neonates have been explained by some iranian authors but he etiology of most cases remains unknown. Idiopathic sudden sensorineural hearing loss and its prognostic factors have also been discussed in many references. (Noorbakhsh et al., 2008; Noorbakhsh, Memari, Farhadi & Tabatabaei, 2008 ; Noorbakhsh et al., 2006; Noorbakhsh, Farhadi & Tabatabae, 2008; Noorbakhsh, Farhadi & Tabatabaei, 2005; Noorbakhsh, Siadati & Farhadi, 2006).

The main goal of study : to evaluate the antibodies against Heat Sock Potein 70Ab

(HSP 70) in serum and perilymphatic fluid OF Children with cochlear implant surgery

## **Materials and Methods**

A cross sectional study had done upon 124 children undergoing cochlear implant surgery in the cochlear implant ward.

This study was approved by the Ethical Committee in the ENT and head & Neck surgery Research Center affiliated by Iran University of Medical Sciences. Consent letter obtained from patients ( parents).

Data collection: Initially, a questionnaire was completed by an authorized physician for each cases (e. g. age, gender, Audio logic screenings (Auditory Brainstem Response, Evoked Otto-acoustic Emissions and Pure Tone Audiometry) appropriate for patients` age were performed in all cases. Type of SNHL were diagnosed by specialists based on history and complementary tests.

Blood samples (2 ml) were taken, then centrifuged and transferred to our research laboratory. During surgery period 0. 5-3 ml of Perilymphatic fluids were taken by ENT specialist in operation room. All samples were kept frozen and stored at -70° /c. Hsp70 level determined by using the ELISA kits (STRESS XPRESS, stressgen biotechnologies, Canada). Results were interpreted as suggested by the manufacturer.

## **Statistical Analysis**

Quantitative variables were summarized as mean ±standard deviation (SD) and qualitative variables as count with percentage. Comparison between qualitative variable and test results was assessed by chi-square (or Fisher exact test if proper). Chi square values (CI 95 %, p<0. 05) were

considered statistically significant. A ROC curve was constructed to illustrate various cut-offs of HSP 70 levels in differentiating the type of SNHL in case. Statistical calculation was performed with SPSS statistical software (version 13; SPSS Inc)

## **Results and Discussion**

One hundred seventy cases enrolled in this study. ; 58% male, 42% female. Age of cases was between 6 months -14 years; mean:3.4±3.1years;. Idiopathic SNHL diagnosed in 39.4 % of cases ( Fig-1)

Heat Shock Protein70 Ab level in perilymphatic fluid was higher in idiopathic SNHL cases (P = 0.000).

A ROC curve analysis showed an AUC(area under ROC curve) 0.731 (95% CI; 0.629-0.834, P = 0.000);

HSP70 Ab (Cutoff level 0.39 pg/dl) in perilymphatic fluid had 76% sensitivity and 60% specificity for differentiating the idiopathic type of SNHL from hereditary ones. Here, the present study found the higher level of Heat Shock Protein70 Ab in idiopathic type of SNHL (P = 0.000). HSP70 Ab (Cutoff level 0.39 pg/dl) in perilymphatic fluid had 76% sensitivity and 60% specificity for differentiating the idiopathic type of SNHL from hereditary ones.

Although many medical experts believe that sudden hearing loss is generally of an unknown cause, viral disease appears to be the cause for about 60-percent of all cases of SHL. These viruses include influenza type B, mumps, rubella, measles, herpes-1, and infectious mononucleosis. (20) Many of these viruses belong to the herpes family. It's possible that there could be a multitude of other causes for SHL including vascular disease, lyme disease, Meniere's disease, acoustic neuroma, otitis media, exposure to

ototoxic medications, tumors, stroke and Cerebrospinal fluid (CSF) leak. (20-22) Many people who have SIHL also recover suddenly by regaining half of their hearing within two weeks. Because people spontaneously recover their hearing at a high rate it's not always necessary to treat SIHL, especially if the hearing loss is minor.

Sudden sensorineural hearing loss (SSNHL) is frequently classified as 'idiopathic' since the causative factor responsible for its onset is not identified in most cases. (20)First Cadoni discussed the role of endothelial cell autoantibodies in the pathogenesis of sudden hearing loss (11) Naumann et al detected the humoral immune response to inner ear proteins in patients with sensorineural hearing loss. (8) Interferon-gamma production to inner ear antigens by T cells from patients with autoimmune sensorineural hearing loss.

Studied by Lorenz et al (9) Idiopathic sudden sensorineural hearing loss in children reported by Chen et al (10) Solares et al determined the interferon-gamma T-cell frequencies in patients with autoimmune sensorineural hearing loss. (13) Detection of autoantibodies to HSP70 and PLs offers a plausible explanation for the immune-mediated mechanism of SSNHL. Grosset al reported Prevalence and Clinical Significance of Anticardiolipin, Anti-β<sub>2</sub>-Glycoprotein-1, and Anti-Heat Shock Protein-70 Autoantibodies in Sudden Sensorineural Hearing Loss. (19) SSNHL was clinically associated with serum anti-heat shock protein-70 (anti-HSP70) and antiphospholipids (anti-PLs) autoantibodies and these autoantibodies had an impact on the prognosis of SSNHL. They concluded that the association between anti-HSP70 and anti-PLs antibodies with SSNHL, and is the first to identify a positive association between anti-HSP70 antibodies and a positive outcome of SSNHL (19).

*Area Under the Curve*

*Test Result Variable(s):hsp70*

<i>Area</i>	<i>Std. Error<sup>a</sup></i>	<i>Asymptotic Sig.<sup>b</sup></i>	<i>Asymptotic 95% Confidence Interval</i>	
			<i>Lower Bound</i>	<i>Upper Bound</i>
.731	.052	.000	.629	.834

*The test result variable(s): hsp70 has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.*

*a. Under the nonparametric assumption*

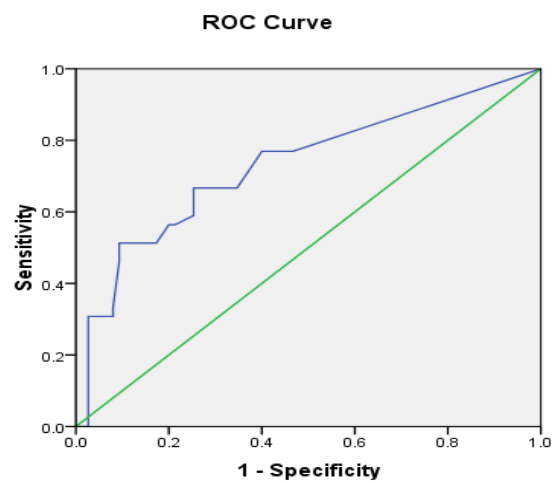
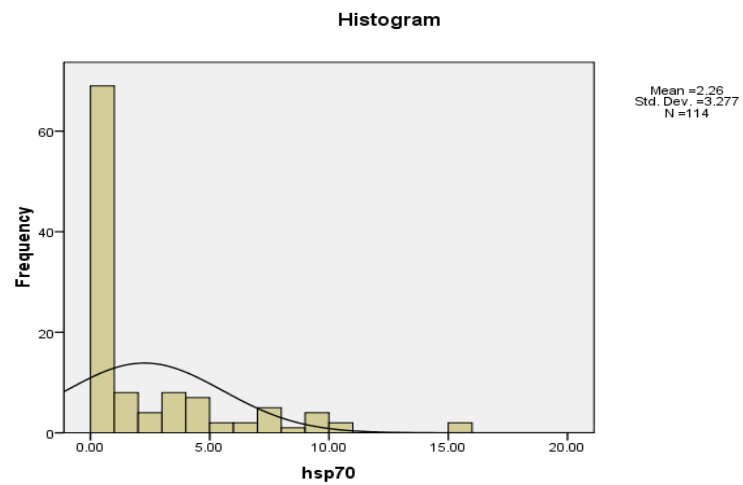
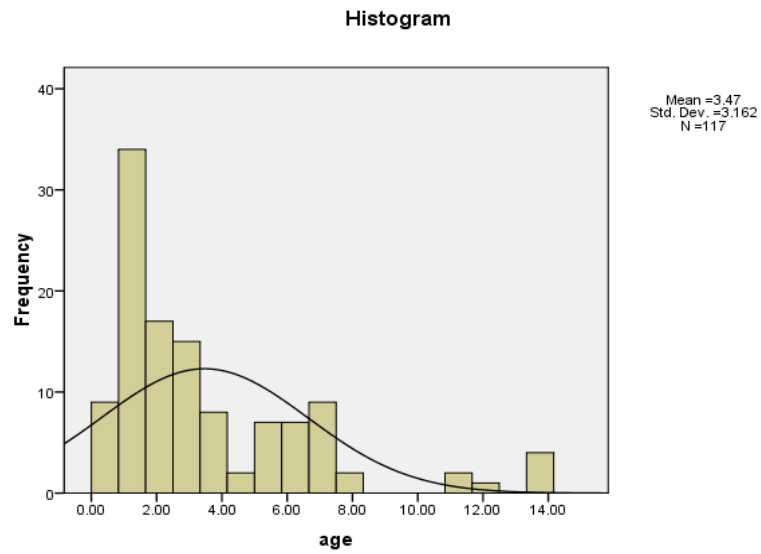
*b. Null hypothesis: true area = 0.5*

<i>Positive if Greater Than or Equal To<sup>a</sup></i>	<i>Sensitivity</i>	<i>1 - Specificity</i>
-.8000	1.000	1.000
.2650	.769	.467
.3500	.769	.440
.3750	.769	.427
.3850	.769	.413
<b>.3950</b>	<b>.769</b>	<b>.400</b>
.4700	.667	.347
.6350	.667	.333

*The test result variable(s): hsp70 has at least one tie between the positive actual state group and the negative actual state group.*

*a. The smallest cutoff value is the minimum observed test value minus 1, and the largest cutoff value is the maximum observed test value plus 1. All the other cutoff values are the averages of two consecutive ordered observed test values.*

Serum Hsp 70 –Ab Detected in 46. 8% of Cases



Diagonal segments are produced by ties.

Karger AG reported the association between anti-HSP70 and anti-PLs antibodies with SSNHL, and a positive outcome of SSNHL. Further studies are necessary in order to identify and further clarify the immunologic role of the presence of autoantibodies and their impact on the prognosis of SSNHL.

There are various types of treatment that have been used for SIHL including oxygen therapy, vitamin E, anti virals, diuretics, anticoagulants, plasma expanders, and carbogen. However many medical professionals believe that steroids are the best treatment for the condition. (14-17) Tucci et al treated the SSNHL cases with systemic steroids and valacyclovir (18)

Although SNHL due to various etiologies is common in Iranian children, The etiology of most cases remains unknown. Cochlear implant surgery is needed for some cases (21-25)

### **Conclusion**

Assesment the HSP70 level in perilymphatic fluid (76% sensitivity and 60 specificity ) might be useful for differentiating the idiopathic type of SNHL from hereditary ones. This test can potentially assist clinicians in faster diagnosis and specific treatment in cases with progressive idiopathic SNHL. Further studies are necessary in order to identify the immunologic role of the these antibodies and their impact on the prognosis of SNHL.

### **Acknowledgement**

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