Int. Adv. Otol. 2011; 7:(1) 55-61

ORIGINAL ARTICLE

Diagnostic Value of Serum Anti-Heat Shock Protein 70 (Anti-HSP70) in Cases of Autoimmune Inner Ear Disease

Rania Hamdy El-Kabarity, Rasha Hamdy El-Kabarity

Clinical Pathology and Immunology- Ain Shams University, (RHEK)
Audiology –ENT Department- Ain Shams University (RHEK)

Objective: The aim of the current study was to assess the diagnostic reliability of serum anti-heat shock protein 70 (Anti-HSP70) in cases of autoimmune inner ear disease (AIED).

Materials and Methods: The current study was carried out on 43 patients with idiopathic SNHL. Included women had SNHL greater than 30 decibels (dB) hearing loss, over at least three contiguous frequencies, occurring over 72 hours or less over a few hours at one ear, accompanied by tinnitus, vertigo, or both. The degree of hearing loss may vary from mild to severe, and may involve different configuration Patients were recruited from the Audiology Unit in Ain Shams University Hospitals. A control group consisting of 30 healthy persons was included. All were subjected to history, clinical examination, audiological evaluation and immunological tests. The immunological tests included both non-specific tests (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], antinuclear antibody levels [ANA] and rheumatoid factor [RF]) and specific test which is the serum anti-HSP70 (using WB immunoassay).

Results: A total of 73 subjects were included in the current study. They were divided into two groups: group I (cases group) included 43 cases with idiopathic SNHL and group II (control group) included 30 healthy persons without hearing loss. The mean age of group I was 30.79 ± 12.42 years (range: 14 – 60 years); 20 (46.51%) were males and 23 (53.49%) were females. The mean age of group II was 32.2 ± 12.26 years (range: 14 – 58 years).;12 (40%) were males and 18 (60%) were females. According to pure tone audiometry, of group I, 16 (37.21%) cases had mild to moderate sensorineural hearing loss (HL) [>25-55 db], 12 (27.91%) cases had moderately severe sensorineural HL [56-70 db] and 15 (34.88%) cases had severe to profound HL [> 70 db]. There was a significantly higher mean values of ESR [17.42 ± 10.92 mm/hour vs. 6.9 ± 2.43 mm/hour, respectively, p<0.001], CRP [8.53 ± 7.48 mg/L vs. 3.5 ± 1.42 mg/L, respectively, p=0.001] and RF [26.83 ± 11.96 IU/ml vs. 19.36 ± 6.68 IU/ml, respectively, p=0..03] among group I when compared to group II. There was, however, no significant difference between subjects of both group concerning ANA. There was a significantly higher proportion of subjects with positive ESR (≥ 10 mm/hour) [25/43 (58.1%) vs. 2/30 (6.7%), respectively, p<0.001] and positive CRP (≥ 5 mg/L) [17/43 (39.1%) vs. 3/30 (10%), respectively, p=0.007] among cases with AIED when compared to subjects of the control group. There was a slightly higher proportion of subjects with positive RF (≥ 40 IU/ml) [4/43 (9.3%) vs. 0/30 (0%), respectively, p>0.05] and a slightly higher proportion of subjects with positive ANA (≥ 1 IU/ml) [2/43 (4.7%) vs. 0/30 (0%), respectively, p>0.05] among cases with AIED when compared to subjects of the control group. Yet, these latter 2 differences did not reach a statistical significance. Of the 43 cases with AIED, 34 (79.1%) cases had a positive serum anti-HSP70, compared to none in subjects of the control group [p<0.001]. Anti-HSP70 had the best diagnostic accuracy in cases of AIED having a sensitivity of 79.07%, specificity of 100%, positive predictive value of 100%, negative predictive value of 76.92% and an overall accuracy of 87.67%.

Conclusion: In conclusion, cases with idiopathic SNHL may benefit from testing serum anti-HSP70 shortly after onset of symptoms, in order to identify cases of AIED, as, in such cases, it had a higher sensitivity when compared to non-specific immunological tests, namely ESR and CRP.

Submitted : 29 May 2010 *Accepted : 03 January 2011*

Introduction

Many cases of idiopathic sensori-neural hearing loss (SNHL) of adult onset are attributable to underlying autoimmune pathology, a phenomenon referred to as autoimmune inner cell disease (AIED) [1]. Although the mechanisms involved in the pathogenesis of AIED is not known, autoimmunity may be induced either within the inner ear (as a primary end organ response) or outside the inner ear and gain access to the inner ear (as a secondary response) [2]. The importance of diagnosis of AIED is highlighted in the context of its being one of few forms of treatable inner ear disorders with a good response to immunosuppressive drugs. Early diagnosis of AIED with prompt treatment may prevent irreversible damage to the inner ear

Corresponding address:
Rasha Hamdy El-Kabarity
Audiology Unit-ENT Department, Faculty of Medicine, Ain Shams University, Abbasyia - Cairo
E-mail: rasha_hamdi@hotmail.com

structures[1]. Heat shock proteins (HSPs) are believed to protect cells by dissolving and refolding misfolded or denatured proteins. They are induced by various forms of stress including heat, ischemia, toxic agents as well as free radicals [3]. The use of heat shock protein 70 (HSP70) is based on having a similar molecular weight (68 KDa) with a protein extracted from inner ear tissues. HSP70 reacts with antibodies in the sera of many patients with AIED [18]. Hughes (1996) proposed that the two most clinically helpful tests for diagnosing AIED are the lymphocyte transformation test and the Western Blot immunoassay [4]. The Western Blot (WB) technique is the most beneficial test used to establish the diagnosis of AIED. WB determines the reactivity of sera from patients with idiopathic progressive SNHL against bovine inner ear material (HSP70) [5]. Bonaguri et al. (2007) confirmed the value of the anti-HSP70 test in the serological diagnosis of autoimmune hearing loss. It is probably the only available diagnostic marker that identifies an autoimmune origin of hearing loss [6].

Patients and Methods

The current study was carried out on 43 patients with idiopathic SNHL (group I). Selected patient had SNHL greater than 25decibels (db) hearing loss, over at least three contiguous frequencies, occurring over 72 hours or less over a few hours at one ear, accompanied by tinnitus, vertigo, or both. The degree of hearing loss may vary from mild to severe, and may involve different parts of the hearing frequency range. Patients were selected from the Audiology Unit in Ain Shams University Hospitals. A control group consisting of 30 healthy persons was included. All included cases were subjected to history, clinical examination, basic audiological evaluation (pure tone audiometry, speech audiometry, discrimination scores and immittancemetry with acoustic reflex testing and immunological tests. The immunological tests included both non-specific tests (erythrocyte sedimentation rate [ESR], C-reactive protein [CRP], antinuclear antibody levels [ANA] and rheumatoid factor [RF]) and specific test which is the serum anti-HSP70 (using WB immunoassay).

Gel Electrophoresis Detection of Anti-68kDa (Anti-HSP70) Antibodies by Enzyme-linked Immunoelectrotransfer Technique (Western Blot)

Principle:

HSP70 antigen, which was purified from bovine kidney cell line and mixed with a marker of 61 KDa molecular weight, was subjected to polyacrylamide gel electrophoresis (PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. The membranes were cut into 3 mm x 5 cm strips. Using blunt forceps, the strips were labeled side up into individual wells of the assay tray. To perform the test, strips were soaked in blocking diluent to block non-specific binding sites and then incubated with diluted patients' sera. Antibodies specifically bind to HSP70 protein on the strip. After washing and incubation steps, HSP70 antibody positive reactions appeared as blue-violet bands at 70 KDa.

Procedure:

The strips were placed up into individual wells of the assay tray, soaked in blocking dilutent (1 ml / strip) to block non-specific binding sites, and incubated for at least 30 minutes. 10 µl of positive, negative controls and serum samples from patients and controls were added into appropriate wells to obtain a 1:100 dilution and incubated for 60 minutes at room temperature with shaking. All strips were washed 3 times, 5 minutes for each, with gentle agitation by washing buffer. 1ml / strip of the enzyme conjugate, diluted into 1:100 in blocking dilutent, was added and incubated for 30 minutes at room temperature with shaking. All strips were washed 3 times as above. 1 ml of substrate was added into each strip and incubated with gentle shaking for 20 minutes. Finally, all strips were removed from assay tray, placed gently to dry onto absorbent filter paper for 15 - 20 minutes. The strips were blotted with three proteins of MW 61, 70 and 72 KDa. The 61 KDa protein served as MW marker, the 70 KDa band composed of inducible HSP70 protein. and 72 KDa bands consisted of an unrelated protein co-purified with the HSP70 protein. As described by the manufacturer, in order to o read the final results, test strip was held between the positive and negative control reactions on the provided, laminated control card and aligned using the 61 KDa MW marker as the reference point. The control strips were mounted on gridlines to facilitate accurate alignment of the test strips with the control strips. Test strip was compared with those of the controls on either side. A magnifying glass was used to facilitate proper alignment of test strips along the MW marker and assist in observation of weak reactions. Crisp band was checked on the test strip that aligns with the 70 KDa band on the positive strip. Positive reactions occurred in varying intensities from weak to stronger. Weak reactions were compared

with baseline reaction intensities at the corresponding position on the negative control strip. Figure 1 represents the strips of negative and positive controls and some cases of patients group.

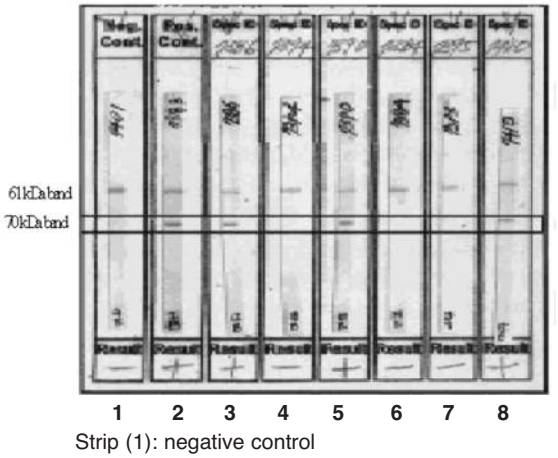

Figure 1. Strips of Anit-HSP70 by WB Technique in Some of Included Subjects and in Negative and Positive Controls

Statistical analysis:

All clinical and demographic data were recorded on an investigative report form. These data were analyzed with the statistical program: SPSS® for Windows®, version 15.0 (SPSS, Inc, USA). Description of quantitative (numerical) variables was performed in the form of mean, standard deviation (SD) and range. Description of qualitative (categorical) data was performed in the form of number of cases and percent. Analysis of numerical variables was performed by using student's unpaired t-test. Analysis of categorical data was performed by using Fischer's exact test and Chi-squared test. Diagnostic accuracy was assessed using the following terms: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy. Association between rank variables was assessed using Spearman's rank correlation coefficient. Significance level was set at 0.05.

Results

A total 73 subjects were included in the current study. They were divided into two groups: group I (cases group) included 43 cases with idiopathic SNHL and group II (control group) included 30 healthy persons without hearing loss. The mean age of included cases of group I was 30.79 ± 12.42 years (range: 14 – 60 years). Of the 43 cases, 20 (46.51%) were males and 23 (53.49%) were females. The mean age of included controls was 32.2 ± 12.26 years (range: 14 – 58 years). Of the included 30 controls, 12 (40%) were males and 18 (60%) were females. There were no statistically significant difference between both groups concerning age and gender distribution. According to pure tone audiometry, of the included 43 cases, 16 (37.21%) cases had mild to moderate SNHL (HL>25-55 db], 12 (27.91%) cases had moderate SNHL [56-70 db] and 15 (34.88%) cases had severe to profound HL [> 70 db].

Blood samples were taken from all included subjects for checking ESR, CRP, RF, ANA as well as anti-HSP70. There was a significantly higher mean values of ESR [17.42 ± 10.92 mm/hour vs. 6.9 ± 2.43 mm/hour, respectively, p<0.001], CRP [8.53 ± 7.48 mg/L vs. 3.5 ± 1.42 mg/L, respectively, p=0.001] and RF [26.83 ± 11.96 IU/ml vs. 19.36 ± 6.68 IU/ml, respectively, p=0..03] among cases with AIED when compared to control group. There was, however, no significant difference between subjects of both group concerning ANA (Table 1 and Figure 2).

Table 1. Difference between Study Groups concerning non-specific immunological tests (quantitative)

	Group I Cases with AIED (n=43)	Group II Control Group (n=30)	p*
ESR (mm/hr)	17.42 ± 10.92	6.9 ± 2.43	<0.001 HS
CRP (mg/L)	8.53 ± 7.48	3.5 ± 1.42	0.001 S
RF (IU/ml)	26.83 ± 11.96	19.36 ± 6.68	0.003 S
ANA (IU/ml)	0.31 ± 0.27	0.29 ± 0.12	>0.05 NS

Data expressed as mean ± SD
** Analysis using independent student's t-test*
NS non-significant – S significant – HS highly significant

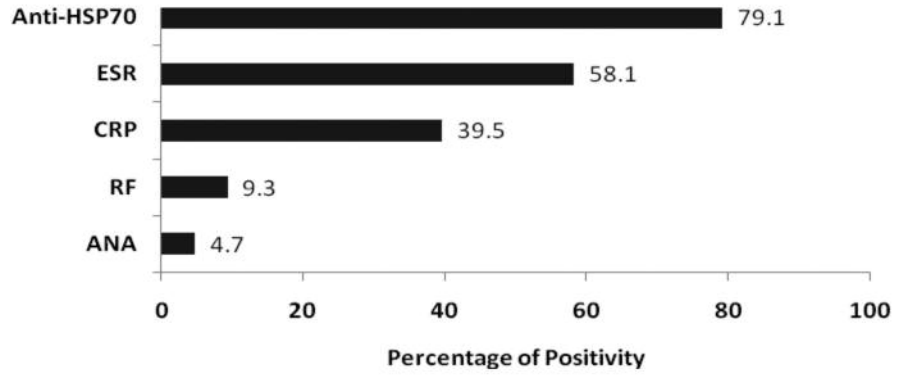

Figure 2. Bar-chart showing difference between study groups concerning positivity of Anti-HSP70 and non-specific immunologic tests in AIED cases

There was a significantly higher proportion of subjects with positive ESR (≥ 10 mm/hour) [25/43 (58.1%) vs. 2/30 (6.7%), respectively, p<0.001] and positive CRP (≥ 5 mg/L) [17/43 (39.1%) vs. 3/30 (10%), respectively, p=0.007] among cases with AIED when compared to subjects of the control group (Table 2).

Table 2. Difference between study groups concerning non-specific immunological tests (qualitative)

	Group I Cases with AIED (n=43)	Group II Control Group (n=30)	p*
Positive ESR (> 10 mm/hr)	25 (58.1%)	2 (6.7%)	<0.001 HS
Positive CRP (> 5 mg/L)	17 (39.5%)	3 (10%)	0.007 S
Positive RF (> 40 IU/ml)	4 (9.3%)	0 (0%)	>0.05 NS
Positive ANA (> 1 IU/ml)	2 (4.7%)	0 (0%)	>0.05 NS

Data expressed as number (percentage)
** Analysis using Fischer's exact test*
NS non-significant – S significant – HS highly significant

There was a slightly higher proportion of subjects with positive RF (≥ 40 IU/ml) [4/43 (9.3%) vs. 0/30 (0%), respectively, p>0.05] and a slightly higher proportion of subjects with positive ANA (≥ 1 IU/ml) [2/43 (4.7%) vs. 0/30 (0%), respectively, p>0.05] among cases with AIED when compared to subjects of the control group. Yet, these latter 2 differences did not reach a statistical significance (Table 3).

Table 3. Difference between study groups concerning Anti-HSP70

	Group I Cases with AIED (n=43)	Group II Control Group (n=30)	p*
Positive Anti-HSP70	34 (79.1%)	0 (0%)	<0.001 HS

Data expressed as number (percentage)
** Analysis using Fischer's exact test*
HS highly significant

Further analysis of the results revealed that among the 34 cases of AIED that had a positive anti-HSP70, 24 (70.6%) cases had a positive ESR, 17 (50%) cases had a positive CRP, 4 (11.8%) had a positive RF and none (0%) had a positive ANA. Anti-HSP70 had the best diagnostic accuracy in cases of AIED having a sensitivity of 79.07%, specificity of 100%, positive predictive value of 100%, negative predictive value of 76.92% and an overall accuracy of 87.67% (Table 4). There was no significant association between serum anti-HSP70 and degree of HL in cases of AIED (r=0.255, p>0.05). serum anti-HSP70 was positive in 10/16 (62.5%) cases of mild to moderate HL, 11/12 (91.7%) cases of moderate HL and 13/15 (86.7%) cases of severe to profound HL. The difference, however, did not reach a statistical significance

Discussion

Autoimmune inner ear disease (AIED) is a rare disease accounting for less than 1% of all cases of hearing impairment or dizziness, characterized by a rapidly

Table 4. Diagnostic accuracy of Anti-HSP70 and non-specific immunological tests in aied in included cases

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Overall Accuracy
Positive Anti-HSP70	79.07%	100%	100%	76.92%	87.67%
Positive ESR	58.14%	93.33%	92.59%	60.87%	72.6%
Positive CRP	39.53%	90%	85%	50.94%	60.27%
Positive RF	9.3%	100%	100%	43.38%	46.58%
Positive ANA	4.65%	100%	100%	42.25%	43.84%

Table 5. Relationship between Anti-HSP70 and degree of HL in AIED cases

	Moderate HL [41 – 55 db] (n=16)	Severe HL [56 – 70 db] (n=12)	Profound HL [> 70 db] (n=15)	p*
Positive Anti-HSP70	10 (62.5%)	11 (91.7%)	13 (86.7%)	>0.05 NS

Data expressed as number (percentage)
** Analysis using Chi-squared test*
NS non-significant

progressive, often fluctuating, bilateral sensori-neural hearing loss (SNHL) over a period of weeks to months [7]. The diagnosis is based on history, clinical findings, blood tests and the results of hearing and vestibular tests [8]. There is no specific test for AIED. A common approach is to look for other evidence for autoimmune involvement [9]. Non-specific immunologic tests, namely ESR, CRP and collagen disease markers (RF and ANA) are, by nature, non-specific. The utility of anti-HSP70 test has been recently questioned, as HSP70 has a similar molecular weight to a protein extracted from the inner ear [6]. The current study showed that serum anti-HSP70 had the highest sensitivity (79.1%) and NPV (76.92%) when compared to the non-specific immunological tests (ESR, CRP, RF and ANA), which all had relatively much lower sensitivities and NPVs. These findings suggest that these non-specific immunological tests are not suitable to be used as a screening panel for cases of idiopathic SNHL owing to the unacceptably high false negative rates (particularly with RF and ANA). Serum anti-HSP70, by having the highest sensitivity and NPV, should be used as a screening test in cases of idiopathic SNHL to define an autoimmune etiology.

Several trials investigated the value of serum anti-HSP70 in cases of AIED [6,10-14,16-17, 18-19]. The sensitivity of serum anti-HSP70 in such cases had a wide range (13% - 59.5%). These variable results may be explained by different timing of serum anti-HSP70 detection (the shorter the time between onset of symptoms and serum anti-HSP70 testing, the higher the sensitivity), different types of the antigen used (whether from bovine kidney cell line or not) and whether cases were under treatment or not (cases under treatment had higher negative results than those not receiving treatment yet). Previous relevant studies reported relatively higher sensitivities of ESR and CRP [20,21]. ESR and CRP are acute phase reactants, and are supposed to markedly elevated in the acute phase of AIED [22]. Not all the subjects included in the current study were during the acute phase of AIED. This may explain the relatively lower sensitivities of ESR and CRP shown by the current study. Similar to the findings of the current study, Toubi et al. reported a poor sensitivity of RF in cases of AIED. The authors' conclusion was to exclude RF as an initial screening test for the disease [23]. On the contrary, ANA was shown to have a higher sensitivity in cases of AIED

than that reported by the current study. Berrocal et al. (2002) studies 125 cases of AIED and found that ANA had a sensitivity of 34.4% [10]. Mafong et al. investigated 114 children with idiopathic SNHL and found that ANA in such cases had a sensitivity of 25% [25]. The discrepancy between these results and ours may be explained by the diverse clinical forms of the disease in the former study and the limited pediatric age group in the latter one.

Added to its benefit in diagnosing AIED among cases of idiopathic SNHL, serum anti-HSP70 has been shown to have value in prognosis and response to treatment [14].

In conclusion, cases with idiopathic SNHL may benefit from testing serum anti-HSP70 shortly after onset of symptoms, in order to identify cases of AIED, as, in such cases, it had a higher sensitivity when compared to non-specific immunological tests, namely ESR and CRP.

References

1. Tebo AE, Jaskowski TD, Keith LL, Litwin CM Hill HR: Comparison of immune assays for the detection of anti-hsp70 antibodies in patients with idiopathic sensorineural hearing loss. Clin. Chim. Acta, 2007; 381: 140-144.

2. Agrup C and Luxon LM. Immune-mediated inner-ear disorders in neuro-otology. Curr. Opin. Neurol, 2006; 19: 26-32.

3. Oh SH, Yu WS and Song BH: Expression of heat shock protein 70 in rat cochlea with cisplatin-induced acute oto toxicity. Acta. Otolaryngol; 2000; 120: 146-150.

4. Hughes GB: Sudden sensorineural hearing loss. Otolaryngol. Clin. North Am; 1996; 29: 393–405.

5. Boulassel M: COCHB5B2 is a target antigen of anti-inner ear antibodies in auto-immune inner ear diseases. Otolaryngol Neurotol; 2001; 22: 614-618.

6. Bonaguri C, Orsoni JG, Zavota L, Monica C, Manzotti F and Piazza F: Anti-68 KDa antibodies in autoimmune sensorineural hearing loss: are these autoantibodies really a diagnostic tool? Autoimmunity; 2007; 40: 73–78.

7. Bovo R, Ciorba A, Martini A: The diagnosis of autoimmune inner ear disease: evidence and critical pitfalls. Eur Arch Otorhinolaryngol; 2009; 266(1):37-40.

8. Hain TC: Autoimmune inner ear disease. Amer. Hear. Res. Found; 2003; 8: 115-117.

9. Matt B: Sudden sensorineural hearing loss. ENT Toda; 1: 1–15, 2006.

10. Berrocal GJR, Camacho RR, Arellano B and Vargas JA. Validity of the Western blot immunoassay for heat shock protein-70 in associated and isolated immunorelated inner ear disease. Laryngol; 2002; 112: 304-309.

11. DGross M, Eliashar R, Yaakov P, Ulmansky R and Elidan J: Prevalence and clinical significance of anti-cardiolipin, anti-beta(2)-glycoprotein-1, and anti-heat shock protein-70 autoantibodies in sudden sensorineural hearing loss. Audiol. Neurootol; 2008; 13: 231–238.

12. Harris JP and Sharp P: Inner ear autoantibodies in patients with rapidly progressive sensorineural hearing loss. Laryngo; 1990; 100: 516-524.

13. Moscicki RA, San Martin JE, Rauch SD, Nadol JB and Bloch KJ: Serum antibody to inner ear proteins in patients with progressive hearing loss. JAMA; 1994; 272: 611-616.

14. Naumann A, Hempel JM and Schorn K: Detection of humoral immune response to inner ear proteins in patients with sensorineural hearing loss. Laryngorhinootol; 2001; 80: 237-244.

15. Rauch S, Zurakowski D, Bloch D and Kurt J (2000): Anti-Heat shock protein 70 antibodies in Meniere`s disease. Laryngo; 110: 1516-1520.

16. Samuelsson AK, Hydén D, Roberg M and Skogh T (2003): Evaluation of anti-HSP70 antibody screening in sudden deafness. Ear Hear; 24: 233-235.

17. Suslu N, Yilmaz T and Gursel 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(2):341-346.

18. Zeitoun H, Beckman J, Arts H, El-Kashlan H, Telian SA, Denny D, Ramakrishnan A, Disher M, Sataloff R and Carey T: Corticosteroid response and supporting cell antibody in autoimmune hearing loss. Arch. Otolaryngol. Head Neck Surg; 2005; 131: 665–672.

19. Zou J, Bi A, Wu X, Yang W and Li Z: A novel method for screening anti-inner ear autoantibody in patients with autoimmune diseases. Zhonghua. Er Bi Yan Hou Ke Za Zhi; 2000; 35:113-116.

20. Hirose K, Wener MH and Duckert LG: Utility of laboratory testing in autoimmune inner ear disease. Laryngo; 2001; 111: 552-554.

21. McCabe BF and McCormick KJ: Tests for autoimmune disease in otology. Am. J. Otol; 1984; 5: 447-449.

22. Dayal. VS, Ellman M, Sweiss N: Autoimmune inner ear disease: clinical and laboratory findings and treatment outcome. J Otolaryngol Head Neck Surg; 2008; 37(4):591-596.

23. Toubi E, Ben-David J, Kessel A, Halas K, Sabo E and Luntz M: Immunemediated disorders associated with idiopathic sudden sensorineural hearing loss. Ann. Otol. Rhinol. Laryngol; 2004; 113: 445-449.

24. Westergren A: Studies of the suspension stability of the blood in pulmonary tuberculosis. Acta Med Scand; 1921; 54: 247–282.

25. MMafong DD, Shin EJ and Lalwani AK: Use of laboratory evaluation and radiologic imaging in the diagnostic evaluation of children with sensorineural hearing loss. Laryngo; 2002; 112: 1–7.