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Original article

Prestin autoantibodies screening in idiopathic sudden sensorineural hearing loss

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ABSTRACT

Objectives: To define the clinical association of serum prestin autoantibodies and their impact on prognosis, as specific serum diagnostic markers in patients with idiopathic sudden sensorineural hearing loss (ISSNHL).
Design: Sera from 63 patients with ISSNHL were screened prospectively for the presence of prestin autoantibodies by an enzyme-linked immunosorbent assay (Elisa) test. Serum was assayed for anti-prestin IgG antibodies using recombinant human prestin (SLC26 A5). Demographic, clinical, and audiometric variables were analyzed.
Results: Two patients (3.17%) had demonstrable anti-prestin antibodies in serum (exact 95% CI: −1.16% to 7.5%). No statistically significant association was found between prestin autoantibodies and demographic or audiologic parameters.
Conclusions: This preliminary and novel study does not support the presence of an active humoral immune reaction against prestin in ISSNHL.

1. Introduction

Idiopathic sudden sensorineural hearing loss (ISSNHL) represents a frequently encountered otological disease of unknown etiology. This has been defined as sensorineural hearing loss of at least 30 decibels in 3 contiguous frequencies over a period of 3 days or less. Whether this phenomenon represents a single pathophysiologic entity or is the common end result of several, varied pathologic processes still remains unknown. Different causes such as viral infection of the labyrinth or cochlear nerve, an ischemic event, and immune system-mediated mechanisms have been hypothesized [1].

Although most of the evidence suggests a multifactorial etiology for ISSNHL, few studies have implied immunologic mechanisms as possible mediators of ISSNHL. The immunologic hypothesis is based on the theory that circulating antibodies cross-react with inner ear antigens or activated T cells, thereby damaging the inner ear [2,3]. Such antibodies may be triggered by viruses or other agents. A number of inner ear antigens have been proposed as targets for autoantibodies, including type-2 collagen, Beta-actin, Cochlin and Beta-tectorin [4–7].

One such antigen is the prestin protein, which is believed to be specific for the inner ear. Prestin, a membrane protein that is highly and almost exclusively expressed in the outer hair cells (OHCs) of the cochlea, is a motor protein, which senses membrane potential and drives rapid length, changes in OHCs [8]. Because of its exclusive expression in the OHCs, prestin is an attractive molecular marker for the integrity of auditory hair cells. The relationship between prestin and sensorineural hearing loss was described before in non-syndromic hearing loss [9]. It is also known that salicylic acid causes a reversible sensorineural hearing loss due to its effect on prestin [10,11].

The presence of antibodies to prestin in inner ear diseases such as ISSNHL has not yet been evaluated. We have chosen prestin as a potential antigen in ISSNHL because prestin is an outer hair cell-specific protein located in the inner ear, which modulates hearing, and may serve as target for autoantibodies. Whether the appearance of these antibodies represents a marker for an autoimmune process or whether they play an essential role in the outcome of ISSNHL remains unknown. The purpose of this preliminary and novel study is to evaluate the clinical association of anti-prestin antibodies, as specific serum diagnostic markers in ISSNHL patients, for better characterization of this clinical phenomenon.

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Table 1
Demographic and clinical characteristics of patients with ISSNHL ($n = 63$).

	Number (%)
Age (Years)	
Mean ± SD	47 ± 16
Range	18–77
Gender	
Female	37 (58.7)
Male	26 (41.3)
Affected side	
Right	28 (44.4)
Left	35 (55.6)
Presenting Symptoms	
Vertigo	22 (34.9)
Aural fullness	31 (49.2)
Tinnitus	54 (85.7)

ISSNHL: Idiopathic sudden sensorineural hearing loss.

2. Materials and Methods

2.1. Patients

To determine the relationships between ISSNHL and specific autoantibodies, 63 adult patients diagnosed and treated for ISSNHL in our hospital, were screened for the presence prestin autoantibodies, C3, C4, anti-nuclear antibodies (ANA), and anti-extractable nuclear antigen (ENA). Demographic and clinical characteristics of patients with ISSNHL are presented in Table 1. Patients were recruited according to the following criteria: Unilateral sudden sensorineural hearing loss with or without tinnitus and vertigo of unknown origin, and no involvement of cranial nerves other than the eighth cranial nerve. Exclusion criteria were known causes of hearing loss (noise induced, drug induced, Meniere's disease, otitis media, herpes zoster oticus, and trauma), familial deafness, and use of steroids or immunosuppressant therapy prior to serum collection. After obtaining an accurate clinical history from all patients, they underwent a complete objective ENT evaluation, pure tone audiometry (250 to 8,000 Hz), tympanometry, measurements of speech reception threshold and speech discrimination scores, recording of stapedial reflexes, auditory brainstem evoked responses, computedtomography, and magnetic resonance imaging (to rule out acoustic neurinoma, inner ear malformations, and other pathological processes).

2.2. Determination of prestin autoantibodies

Serum samples were drawn from patients and stored at −20 °C immediately. All samples of blood were obtained at the day of admission to the hospital and before starting the treatment. Sera were tested by enzyme-linked immunosorbent assay (Elisa) for reactivity against prestin (Abnova). Elisa plates (Nunc) were coated with the antigen target proteins. Antigen was diluted with coating buffer (Biolegend), 100 μl was divided to each well (40 ng protein/well) and incubated overnight at 4 °C. Wells were washed with excess wash buffer (Biolegend) containing tween 20%. Non-specific binding sites were blocked with assay diluent (Biolegend) containing 10% BSA. One hour later, blood samples diluted to 1:400 or 1:800 were added (100 μl/well) and incubated for another 24 hours. Wells were washed to discard excess serum and a second antibody, goat anti-human Ig (H + L) peroxidase (Jackson Labs) at 1:5000 dilution was added for 2 hours. The wells were washed and 90 μl of TMB (SouthernBiotech) was added, followed by 90 μl of STOP solution (SouthernBiotech), after a full blue color was developed. The plate was read in an Elisa reader (DTX 880) at 450 nm. The optic density (OD) readings from the wells containing patient sera but no antigen represented background binding to the Elisa plate and were subtracted to derive the most accurate reactivity resulting from antigen presence. The binding of prestin to the wells was specified by using a specific antibody to prestin (goat anti SLC26A5) (Abnova). In order to exclude non-specific GST binding the patient's serum was also examined against different protein — Aquaporin 4 GST.

2.3. Audiometric parameters

Specific types of hearing loss were determined by audiometric curve patterns: ascending (hearing loss greater at the lower frequencies), flat, descending (hearing loss greater at the higher frequencies), and "U-shaped" (hearing loss at mid-frequencies) curves. The mean pure tone-hearing threshold for 6 frequencies (mean-PT-HT-6F) (250 to 8,000 Hz) was analyzed for each patient upon admission. We used the following scale of degree of hearing loss: mild >20 to 40 dB hearing loss, moderate > 40 to 70 dB hearing loss, severe > 70 to 90 dB hearing loss, and profound > 90 dB hearing loss.

2.4. Statistical analysis

The data was analyzed using descriptive statistical methods. Continuous variables were reported as means with standard deviations. Proportions were reported in percentages and fractions in brackets, along with their 95% exact binomial confidence intervals (CI). Association between categorical variables such as anti-Prestin autoantibodies, audiogram type, degree of hearing loss, appearance of vertigo, and tinnitus and the clinical and laboratory results were evaluated with Fisher's exact test. The associations between continuous variables such as age, and mean pure tone hearing threshold for 6 frequencies, were tested with the t-test or with ANOVA when more than two groups were compared.

2.5. Ethics

The study was approved by the ethics committee of the Hadassah Medical Center (HMO-09-0106) and informed consent was obtained from all the participants.

3. Results

The audiometric characteristics of the ISSNHL patients are presented in Table 2. The degree of initial hearing loss was distributed from mild to severe. Moderate hearing loss was the most common degree of hearing loss (58.7%). The mean-PT-HT-6F was 54.38 ± 14.84 dB. Types of hearing loss in the pure tone audiogram were observed as ascending (17.5%), flat (47.6%) descending (28.6%), and U-shaped (6.3%).

Table 3 demonstrates the distribution of prestin autoantibodies and immunologic tests results among the unilateral SSNHL patients. The proportion of unilateral ISSNHL patients with positive anti-prestin antibodies by Elisa was 3.17% (2/63), exact 95% CI:

Table 2
Audiometric characteristics among ISSNHL patients ($n = 63$).

	n (%)
Degree of hearing loss	
Mild	13 (20.6)
Moderate	37 (58.7)
Severe	13 (20.6)
Profound	0
Mean 6 frequencies hearing loss (dB ± SD)	54.38 ± 14.84
Ascending	11 (17.5)
Flat	30 (47.6)
Descending	18 (28.6)
U-Shaped	4 (6.3)

ISSNHL: Idiopathic sudden sensorineural hearing loss.

Table 3
Serologic and immunologic test results among ISSNHL patients ($n = 63$).

Test	n (%)	Exact 95% CI
Anti-prestin	2 (3.17)	−1.16%–7.5%
ANA	16 (25.4)	
C3	2 (3.2)	
C4	2 (3.2)	
ENA	2 (3.2)	

ISSNHL: Idiopathic sudden sensorineural hearing loss; CI: confidence interval; ANA: anti-nuclear antibodies; ENA: anti-extractable nuclear antigen.

−1.16% to 7.5%. ANA was positive in 16 patients (25.4%). Low levels of C3 and C4 were demonstrated only in 2 (3.2%) patients. ENA was positive in 2 (3.2%) patients.

No statistically significant association was found between prestin autoantibodies and age, appearance of vertigo, tinnitus, audiogram type, degree of hearing loss, and mean-PT-HT-6F.

4. Discussion

The etiology and pathogenesis of ISSNHL are controversial. Extensive disagreement exists regarding the role of different autoantibodies in the onset of ISSNHL. The relationship between the inner ear and the immune system was originally established by McCabe, who described a clinical entity characterized by steroid-responsive, rapidly progressive sensorineural hearing loss [12]. Serological evidence supporting the involvement of the immune system in ISSHL was provided by Harris and Sharp, who found circulating antibodies against several cochlear antigens in patients with idiopathic, progressive, bilateral sensorineural hearing loss [13].

Immune processes of the inner ear are difficult to investigate due to inaccessibility of the tissue in the human diseases. In addition, the autoimmune process in the cochlea is difficult to confirm due to the absence of a specific test that could identify the inner ear antigen(s) at fault. Like other organ specific autoimmunity, specific inner ear antigens could become the target for the body's immune response following events such as infections, trauma or vascular events. Significant attempts have been made to elucidate the inner ear antigens that could be the target of the disease process. A candidate antigen in the autoimmune process of the inner ear is prestin. Specifically, prestin is localized to the lateral plasma membrane of OHCs, where electromotility occurs. Electromotility is thought to be the physical process that underlies the cochlear amplifier, which means that prestin plays a central role in cochlear sensitivity and tuning. Damage to OHCs is one of the earliest events that lead to hearing loss. Therefore, prestin is uniquely suited to serve as a biomarker of inner ear function, and possibly hearing loss.

The objective of this study was to determine the possible existence and prevalence of anti-prestin antibodies in unilateral ISSNHL patients, and to determine whether any relationships exist between the presence of these autoantibodies and certain clinical features. Our main finding was a very low rate of anti-prestin antibodies (3.17%) among ISSNHL patients. The result of this study does not support the presence of an active humoral immune reaction against prestin, an outer hair cell-specific antigen of the inner ear, in unilateral ISSNHL. With respect to prestin, to which only two patients had a detectable antibody response, there is an alternative possibility that cell-mediated rather than humoral immunity may be implicated in the induction of this response. This cohort study, for the first time, provided us with useful insight on prestin autoantibodies in ISSNHL. However, the small sample size limits the results and warrants further investigation in larger patient populations at multiple geographic sites.

In conclusion, although the etiology of ISSNHL is still unknown, this study showed non-significant appearance of anti-prestin antibodies in sera of patients with unilateral ISSNHL that does not offer an applicable explanation for the immune mediated mechanism of ISSNHL. Direct causal relation cannot be advocated at this point with relation to anti-prestin antibodies among patients with unilateral ISSNHL. 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 uni- and bilateral SSNHL.

Disclosure of interest

The authors declare that they have no competing interest.

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