

Detection of serum IgA to HSV1 and its diagnostic role in sudden hearing loss

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SUMMARY

A viral etiology of sudden hearing loss has been hypothesized by many authors. HSV1 neurotropism and its involvement in sudden hearing loss has implicated HSV1 as one of the most accredited etiological agents. A non-invasive method such as the titration of HSV1-specific IgA was evaluated to determine the role of HSV1 as a possible cause of sudden hearing loss.

A prospective study was carried out by titration of serum IgA to HSV1 in 93 patients and in a control group of 50 healthy subjects and 35 subjects suffering from recent herpes labialis reactivation. Statistical analysis of the results disclosed that IgA titers to HSV1 higher than 1:80 are suggestive for the association of HSV1 infection and sudden hearing loss. Moreover, acyclovir therapy was effective in 81% of patients who showed high specific IgA titers.

Overall, the titration of specific serum IgA to HSV1 can be a useful tool to determine the viral etiology of certain cases of sudden hearing loss. This method is simple to perform and minimally invasive. It can lead to a rapid presumptive diagnosis and to prompt specific therapy, reducing the need for corticosteroids.

KEY WORDS: Sudden hearing loss, Herpes simplex 1, Serum IgA, Titration.

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INTRODUCTION

Sudden deafness is a clinical entity characterized by rapid onset of hearing loss or a progressive loss over 12 h with an average hearing loss of more than 30 dB on at least three contiguous frequencies. Hearing loss is generally unilateral and principally affects adult males (Grossan, 1999). Some studies show an increasing incidence of hearing loss with age (Wilson *et al.*, 1980; Moscovitz *et al.*, 1984; Byl, 1984; Mattox and Lyles, 1989).

Since there is no clear etiology of idiopathic sudden sensorineural hearing loss (ISSNHL), nu-

merous hypotheses have been formulated to define its etiopathogenesis. Because of the lack of a definition of ISSNHL, there is no standardized therapeutic protocol and this represents a controversial topic in the otorhinolaryngology literature (Mattox and Lyles, 1989). Because of the low incidence of ISSNHL, neither its etiology or adequate therapy can be defined with certainty. This leads to a symptomatic therapy that could improve the auditory performance of the patient as soon as possible instead of identifying the etiology. These factors lead to an underestimation of the possible role of infective agents in the genesis of ISSNHL (Mattox and Lyles, 1989). The most accepted hypotheses concerning the etiology of this disease are viral (Wilson, 1986; Real *et al.*, 1987; Pyykko *et al.*, 1993) and vascular (Yamasoba *et al.*, 1993; Biavati *et al.*, 1994).

A viral cause, based on a positive anamnesis for previous viral infections, has been hypothesized in about 35% of ISSNHL cases (Yoshida *et al.*,

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1996). Vasama and Linthicum (2000), using histological data, hypothesized a viral etiology for ISSNHL (Uri *et al.*, 2003). In addition, *in vivo* experiments demonstrated the induction of labyrinthitis processes by infecting Guinea pigs with HSV1 (Stokross *et al.*, 1999).

The action of neurotropic viruses in the induction of morpho-functional alterations of the cochleovestibular system (Bachor *et al.*, 1996) is now a universally accepted theory (Huges *et al.*, 1996).

Among neurotropic viruses, Alphaherpesvirinae have a pronounced neurotropism (Sabin, 1938) since they penetrate into the peripheral nerve endings after their multiplication in epithelial cells (Metterleiter, 2003). HSV1 is one of the most prevalent Alphaherpesvirinae in the world (Diefenback *et al.*, 2008). It penetrates the host through the superficial epithelium of the oronasal region and then invades the peripheral nervous system (PNS) to reach the central nervous system (CNS) by the olfactory route, trigeminal nerve, sympathetic nerve and parasympathetic nerve (Metterleiter, 2003). In sudden hearing loss the virus could act on particularly delicate structures such as the sensorineural cochlear and together with other causes could lead to irreversible anatomical-functional lesions.

The meager knowledge on the etiology, multifactorial pathogenicity, and worsening phenomenology are factors underlying the emergence of ISSNHL. These are conditions that need a multidisciplinary and rapid diagnostic and therapeutic approach.

On the basis of the above factors, it is important to determine the viral etiology, particularly concerning HSV1, of ISSNHL both for therapeutic reasons and for a better understanding of its epidemiology and clinical prognostics. A diagnostic method such as the titration of specific serum IgA to HSV1 can be a useful tool to determine the viral etiology of certain cases of sudden hear-

ing loss since it is simple to perform and minimally invasive. Thus, it can lead to a rapid presumptive diagnosis and to prompt specific therapy.

MATERIALS AND METHODS

Patients

Ninety-three ISSNHL patients, 7 of whom were also suffering from vertigo, 57 men (61%) and 36 women (39%), age range 8-67 years (mean 49.7 years, median 52), were analyzed between January 1997 and May 2007. The pathology evolved in a time range of a few hours to 5 days after the onset of the symptoms, and the patients had a clinical check-up at a few days or a few weeks after their first examination at our otorhinolaryngology unit. They underwent an audio-vestibular examination consisting of pure-tone audiometry, auditory brainstem response, impedance audiometry including tympanometry, caloric test, and nystagmographic evaluation. The clinical evaluation of these patients is summarized in table 1. None of them reported herpes labialis relapses within 8 months. They were usually discharged 5-7 days after admission and underwent a clinical check-up within one week after discharge and then weekly for two months. All patients started the complete therapy (Table 2) on the same day as clinical diagnosis. When laboratory results available within two days after hospital admission were suggestive of viral etiology, the treatment plan switched to acyclovir alone for 10 days, otherwise the complete therapy was continued. During hospitalization subjective variations in symptomatology and oto-functional modifications were evaluated and serological parameters were monitored.

Moreover, 85 volunteer subjects were enrolled as a control group: 35 of them presented current herpes labialis relapse or were symptomatic with-

TABLE 1 - Clinical evaluation (n. 93 patients).

Pure-Tone audiometry	Tympanometric examination	Stapedial reflex examination	Vestibular examination
Unilateral hearing loss 4,000÷8,000 Hz (n. 47) 2,000÷8,000 Hz (n. 19) 250÷8,000 Hz (n. 17)	"A" type tympanogram (n. 93)	- Absence of reflexes (n. 52) - Presence of reflexes (n. 41) - Positive Metz's test (23/41)	Ipsilateral vestibular hypofunction (n. 7)

TABLE 2 - *Clinical investigation: current methodological plan.*

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1. **Clinical history** defining characters for the onset of sudden hearing loss and the relative subjective symptomatology.

 2. **Instrument evaluation of hearing function:**
 - a. Pure-tone audiometry
 - b. Impedance audiometry
 - c. Examination of Auditory Brainstem Response (ABR)
 - d. Caloric test and nystagmo graphic evaluation

 3. **Evaluation of the supra-aortic vessel cerebral blood flow**

 4. **CT and/or MR**

 5. **Therapeutic treatment:**
 - a. Oral corticotherapy: metilprednisolone (1mg/kg/die) in 2 administrations/die for 6 days
 - b. Hypertonic therapy: mannitol (500 ml al 10%) in 6 h + KCl (2 gr)
 - c. Vasoactive therapy: mesoglycan (100 mg/die) in 2 administrations/die for 10 days
 - d. Empiric antiviral therapy (aciclovir 1000 mg/die in 5 administrations)

 6. **Laboratory diagnoses:**
 - i) *serology:*
 - CMV, VZV, HSV1 and HSV2 IgG, IgM and IgA
 - ii) *biological tests:*
 - a. Fasting glycemia
 - b. Lipid balance
 - c. Hemochrome using leukocyte formula
 - d. Platelet count
 - e. Index of platelet aggregation and adhesiveness
 - f. Prothrombin time
 - g. Thromboplastin time
 - h. D-dimer (index of thrombin activity)
-

in 14 days before the examination. The other 50 subjects had had no herpetic manifestations during the previous eight months, but all of them had a history of herpes labialis. All subjects included in the study were matched for age, geographical origin and socio-economic status. Sera from immigrants were excluded.

Serological investigation

The test of indirect immunofluorescence (IFA) for the detection of immunoglobulin A (IgA) was carried out with only minor modifications to the method described by Falaky *et al.* (1977). Briefly, for the preparation of the slides for the IFA tests, the McIntyre strain of HSV1 was propagated on

B-Vero cells in 199 medium with 2% fetal calf serum (FCS). The cells were scraped when a 60-80% cytopathic effect was observed and were washed three times in saline phosphate buffer (PBS), pH 7.2. The cells were then re-suspended in PBS and 10^4 cells were placed in each well of 10 well immunofluorescence slides (bioMérieux Italia, Rome, Italy) and fixed for 5 min in cold acetone. The slides were then stored at -80°C until use. The specific IgA assay was carried out by initially evaluating three dilutions in PBS from 1:40 to 1:160. In the cases in which the 1:160 dilution was still positive, a new test was carried out up to 1:1280 dilution. Ten microliters of each dilution were spotted onto each well. The slides were incubated for 30 min at 37°C in a moist atmosphere and then washed twice in PBS. Fluorescein-conjugated rabbit immunoglobulins to human IgA (DAKO A/S, Denmark) were diluted 1:20 in PBS supplemented with Evans Blue as counterstain. Ten microliters of diluted conjugate were spotted onto each well and the slides were again incubated for 30 min as above. After washing, the slides were dried and then observed under an epifluorescence microscope.

The detection of IgG and IgM to HSV1, HSV2, varicella-zoster virus (VZV) (Euroimmun Italia Diagnostica Medica s.r.l., Padua, Italy) and cytomegalovirus (CMV) (Vidas, BioMérieux, Marcy-l'Etoile, France) was carried out using commercial enzyme immunoassay (EIA) or enzyme linked fluorescent assay (ELFA) kits. Commercial EIA kits were also used for the detection of IgA to HSV2, VZV and CMV (BioChem Immuno Systems Italia S.p.A., Bologna, Italy). All tests were carried out blind.

In those cases in which there were associated signs of a viral pathology supported by the laboratory results, the treatment plan switched to the antiviral monotherapy for 10 days, while in the other cases the complete therapy was continued. During hospitalization subjective variations in symptomatology and any otofunctional modifications were evaluated and serological parameters were monitored.

RESULTS

All serum samples were negative for specific IgM to HSV1, HSV2, VZV and CMV. Fourteen (7.9%)

TABLE 3 - IgA titer distribution within the subject classes.

IgA titre	ISSNHL (%)	Relapses (%)	Healthy(%)	Total (%)
<1:40	22/93 (23.6)	4/35 (11.4)	33/50 (66.0)	59/178 (33.1)
1:40	31/93 (33.3)	13/35 (37.1)	17/50 (34.0)	61/178 (34.3)
1:80	9/93 (9.7)	18/35 (51.4)	0.0	27/178 (15.2)
1:160	22/93 (23.6)	0.0	0.0	22/178 (12.4)
1:320	9/93 (9.7)	0.0	0.0	9/178 (5.0)

sera were negative for specific IgG to HSV1, 163 (91.6%) to HSV2, 19 (10.7%) to VZV, and 44 (24.7%) to CMV. These data are comparable to the specific Italian seroprevalence (Condorelli *et al.*, 1993; Suligoi *et al.*, 2000; Gabutti *et al.*, 2008). Moreover, there were no significant differences in the IgG titers detected in both groups of subjects/patients included in this study. The IgG to HSV1 were slightly higher in patients who had recent herpes labialis relapse and in some ISSNHL patients. As regards specific IgA titers 59 patients had a titer lower than 1:40, 61 subjects had a titer of 1:40, 27 subjects had a titer of 1:80, 22 patients had a titer of 1:160 and 9 patients had a titer of 1:320 (Table 3).

Two (2.1%) of the 93 patients affected by sudden hearing loss and 5 (5.9%) of the 85 healthy subjects had a borderline IgA titer to HSV2; 18 subjects (19.3%) from the affected group and 14 (16.5%) from the control group showed a grey-zone CMV IgA value; VZV IgA borderline values were detected in 9 cases (9.7%) belonging to the ISSNHL patients, and 8 (9.4%) to the non-affected subjects group. Table 4 shows the HSV1 results aggregated in a 2x2 contingency table on the basis of the IgA titer ($\leq 1:80$ or $> 1:80$) and of the groups studied (affected or not affected). The cut-

TABLE 4 - Number of cases aggregated in a 2x2 contingency table.

IgA titre	ISSNHL	Control group	Total
$\leq 1:80$	62	85	147
$> 1:80$	31	0	31
Total	93	85	178

off titer 1:80 was calculated following the suggestions of Guglielmino *et al.* (1989) selected on the basis of the statistical inference from the analysis of the IgA titer distribution routinely detected in the laboratory from healthy subjects (unpublished data). The analysis of the results obtained indicates that none of the healthy subjects had HSV1 IgA titers higher than 1:40, while 51.4% of the patients belonging to the group of recently affected by HSV labialis relapse presented an IgA titer of 1:80. Titers higher than 1:80 were never detected in the control group subjects, whereas 33.3% of the ISSNHL patients had a titer of 1:160 or higher. Data analysis shows that the IgA titers $> 1:80$ detected in the affected patients' sera should be considered associated with sudden hearing loss ($\chi^2=34,308$; $p<0.001$).

The biological data from blood examinations showed that 39 patients (21.9% of the total sample) had lipid anomalies such as hyperlipemia and/or hypercholesterolemia and alterations of hematic viscosity, 13 of whom (7.3% of the total sample) also had modifications in cerebral blood flow. Regarding these parameters, there were no significant differences between the two groups of subjects. The CT/MR scans showed that 2 patients (1.1% of the total sample) had a neurinoma of the VIII cranial nerve; both of them had an IgA titer to HSV1 of 1:40.

Thanks to the results of the titration of the specific IgA, acyclovir treatment was started in the subjects in whom a viral infection was suspected, as mentioned above. In these patients, various otofunctional investigations showed that in 25 of them (80.6%) there was hearing improvement, from good to complete, while in the remaining 6 (19.3%) the restoration of hearing was poor or absent.

In the months after hospitalization audiologic monitoring showed no further significant oto-functional modifications.

DISCUSSION

Sudden hearing loss is a pathology lacking well-defined features both as regards etiopathogenetic determination and disease evolution. These factors that contribute to a “so-called” audiologic emergency (Pitkaranta and Julkunen, 1998).

There are many etiological agents, differing both in type and nature, which can potentially play a role in the onset of ISSHNL. For this reason it is hard to adopt a uniform and standardized approach to the disease and its therapy. The identification of the etiological agent for ISSHNL requires the study of a large number of potential infective and non-infective agents that can indicate, where possible, the causes of the disease (Hirano *et al.*, 1999).

Therefore, it would be important to introduce into the diagnostic course specific serologic evaluations aimed at identifying the etiology, which could be of great importance for a correct therapy. Among the possible causes of this pathology, viruses are the most accredited.

Virological diagnosis is usually carried out by direct or indirect techniques: the first detects the virus in its site of infection; the second evaluates the host immunological response to the infection by titration of specific antibodies. In sudden hearing loss, direct diagnosis is not possible due to the difficulty and/or inconvenience of obtaining samples because of the invasiveness of the sampling techniques. Therefore, an indirect diagnosis is often established based on the detection of antibodies of the M class to identify a primary infection that is usually the one most frequently responsible for overt disease. In HSV infection,

however, the pathology can be induced by an endogenous re-infection that, in most cases, would not give rise to IgM. This can lead to an underestimation of the role of herpes viruses as etiological agents in sudden hearing loss. IgM are usually not detectable after the first infection, while the presence of IgA might also be suggestive during re-infection, as previously described in other viral infections (Costanzo *et al.*, 2011). HSV1, for example, infects 40-80% of the world’s population depending on geographical area, socio-economic and public health conditions. It is distinguished by its capacity to infect the host organism and remain latent inside neurons, causing repeated infections (Diefenback *et al.*, 2008).

In order to study the role of HSV1 in the induction of ISSHNL, it can be important to find and validate a non-invasive diagnostic parameter that could give reliable indications in this type of diagnosis.

The causal link with the herpes simplex virus, hypothesized on the basis of our serological evaluations, is also supported by the observation, reported by various authors (Yoshida *et al.*, 1996; Vasama and Linthicum, 2000; Uri *et al.*, 2003), of histopathological cochlear samples similar both in sudden hearing loss and experimental labyrinthitis induced by herpesvirus infection. In both circumstances degeneration of the vascular stria, alterations of the tectoria membrane, and destruction of the organ of Corti were observed (Gulya, 1996).

On the basis of such etiological pleomorphism a therapeutic protocol was designed including drugs also able to act on the viral etiopathogenic element of this pathology.

The results obtained (Table 5) show a good improvement in 81% of patients with high specific IgA titers when acyclovir monotherapy was adopted. Nevertheless, the effectiveness of this treatment also depends on factors intrinsic to

TABLE 5 - ISSHNL patients’ outcome after therapy based on laboratory diagnosis.

Outcome	HSV1 IgA>1:80 (n. 31) (aciclovir monotherapy)	HSV1 IgA<1:80 (n. 62) (complete therapy)
Complete recovery	16.1% (5/31)	4.8% (3/62)
Good recovery (>25db)	64.5% (20/31)	35.5% (22/62)
No improvement	19.4% (6/31)	59.6% (37/62)

ISSNHL, such as the initial level of hearing deficit, the morphological characteristics of the curve and the time between the onset of the symptoms and the start of therapy.

In agreement with the literature (Schweinfurth *et al.*, 1996), we observed a greater otofunctional improvement in those subjects with hearing loss lower than 70 dB HL (28% of the cases) and with audiometric curves of the flat or ascending type. This finding could be explained by a better evolution of the ISSNHL forms in which the damage is localized in the apical turns of the cochlea with respect to those forms where the alterations are in the basal area.

In the absence of guidelines for the treatment of ISSNHL, it would be opportune to adopt a therapeutic strategy supported by laboratory analyses. Conlin and Parnes reported that there are no differences in the outcome of ISSNHL patients using steroids vs placebo or an antiviral plus steroids vs placebo plus steroids therapy (Conlin and Parnes, 2007; Conlin and Parnes 2007). On the basis of the results obtained using specific antiviral therapy in suspected HSV1 patients, it seems useful to perform a serologic diagnosis of HSV1 activation by titration of the specific IgA. This would provide a rapid and, when possible, adequate and correct pharmacological approach. The synergy of these factors appears to be of fundamental importance in order to optimize and stabilize the otofunctional performance of these patients.

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