Normal Serum Levels of Otolin-1 in Patients with Meniere Disease in Remission

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Int Arch Otorhinolaryngol 2023;27(3):e440–e444.

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Abstract

Introduction Degenerative changes in the otolithic organs have been theorized to be caused by the mechanical obstruction to endolymphatic flow, possibly resulting in endolymphatic hydrops (ELH). Otolin-1 is an otoconial matrix protein that crosses the blood labyrinth barrier and has been found in the serum of healthy and diseased patients.

Objective To measure the serum levels of Otolin-1 in Meniere disease (MD) patients and compared them with the healthy individuals.

Methods This pilot, cross-sectional study was performed at our tertiary care referral center to compare the serum Otolin-1 levels of healthy individuals with those of MD patients. The blood samples were obtained during patients' visit to the vertigo clinic following remission of an acute episode. The data was analyzed using the Stata/SE version 12.0 (StataCorp. College Station, TX, USA). Comparison between the serum Otolin-1 levels in the two groups was performed using the unpaired *t*-test. A *p*-value of 0.05 was considered to be statistically significant.

Results The participants were divided into two groups, with 31 MD patients, and 30 age and gender-matched members of the control group. The serum levels of Otolin-1 in MD patients (247.6, $\pm$44.2 pg/ml) were not found to be significantly different from those of the control group (236.2, $\pm$43.5 pg/ml) ($p = 0.31$).

Conclusion The current study reveals that the serum levels of Otolin-1 are not significantly different between the patients with MD in the interictal phase and the control group's healthy ones.

Keywords
- Meniere disease
- otolithic membrane
- endolymph drainage
- biomarkers

Introduction

The etiology and pathophysiology of Meniere disease (MD) remain obscure despite its description more than one and a half-century back by Prosper Meniere. Various etiologic theories include autoimmune, traumatic, or viral causes. The mechanical configurations of the endolymphatic duct and sac have been revealed to be narrower and shorter in patients with MD than in normal individuals.[1] A genetic predisposition has been recently demonstrated in a minority

received
September 18, 2021
accepted after revision
April 17, 2022

DOI https://doi.org/10.1055/s-0042-1749390.
ISSN 1809-9777.

Thieme Revinter Publicações Ltda., Rua do Matoso 170, Rio de Janeiro, RJ, CEP 20270-135, Brazil

of the patient population.[2] The delicate configuration of the deeply situated inner ear, with its small size harboring a few microliters of endolymphatic fluid, makes it difficult to visualize the real-time changes in the endolymphatic compartment in various diseases.

Otolithic organs, specifically the utricle and saccule, have a complex arrangement of calcium carbonate nanocrystals embedded in an organic matrix. Otolin-1 is a collagenous matrix protein constituting the organic matrix scaffold, being responsible for the stable assembly of various proteins and otoconia in forming otolith organs.[3,4] It has been observed to cross the blood labyrinth barrier and was detected in peripheral blood.[5] The levels have been shown to increase in conditions causing traumatic or degenerative insult to the otolithic organs, for example, in age-associated otolithic degeneration,[6] Benign paroxysmal positional vertigo (BPPV),[5,7] and even in trauma associated with drilling during mastoidectomy.[8] A more recent school of thought hypothesizes MD to be a result of blockage of the communicating channels between various endolymph compartments filled with free-floating particles, that is to say otoconial debris, leading to an alteration in the endolymph's circulation dynamics.[9,10]

Given the small size and hidden location of the inner ear, the use of biomarkers to investigate various diseases is a recent, evolving field. To date, no reliable biomarkers have been validated to study patients with MD. In this study we evaluated the serum levels of Otolin-1 in patients with MD to understand the dynamics of inner ear degeneration. Furthermore, we investigated the hypothesis that Otolin-1 serum levels should be elevated in individuals with MD, in cases where otoconial degeneration with the resulting blockage in circulation of endolymph by otoconial debris is the underlying causative factor leading to hydrops.

Materials and Methods

This study was a case-control study following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines, performed at the Department of Otolaryngology and Head and Neck surgery at our tertiary care referral center. The study was performed after obtaining clearance from the Institutional Ethical Committee (IEC-506/05–10–2018, RP-14/2018) and in accordance with the principles of the declaration of Helsinki, revised in 2013, on human investigations.

Given the lack of prior studies on the levels of Otolin-1 in MD patients, we planned a pilot study involving 30 patients of MD and compared them with an equal number of age and gender-matched individuals. Consecutive patients, aged from 18 to 75 years, with a definite MD diagnostic using the American Academy of Otolaryngology and Head and Neck Surgery (AAO-HNS) 1995 criteria for MD,[11] with no history of any other otologic/neurotologic disorder or surgery, who were treated in our vertigo clinic for one year, from October 2018 to October 2019, were prospectively enrolled in the study after obtaining an informed and written consent form. Pure tone audiograms were obtained for all the patients, and the disease staging was performed based on the AAO-HNS guidelines on MD (1995).[11] When appropriate, additional tests like impedance audiometry, brainstem evoked response audiometry (BERA), and contrast-enhanced magnetic resonance imaging (MRI) of the brain were performed to rule out a middle ear or retrocochlear pathology. The blood samples for the measurement of serum Otolin-1 were drawn on the patients' first visit to the vertigo clinic. Finally, we recruited age and gender-matched individuals who consented on being part of the study's control group; the members of this group had no otologic, neurotologic or systemic diseases.

Determination of Serum Otolin-1 Levels

Otolin-1 levels were assessed in serum samples using Human Otolin-1 Enzyme-Linked Immunosorbent Assay kits (MyBioSource, Inc. San Diego, CA, USA), which employs the principle of biotin double antibody sandwich technology. A series of solutions containing a precisely known concentration of Otolin-1 (0 pg/ml, 50 pg/ml,100pg/ml, 200pg/ml, 400 pg/ml, 600 pg/ml, 800 pg/ml, and 1000 pg/ml) were used to prepare a standard graph. Standards and samples were loaded on antibody-coated plates, and the conjugate reagent was added to the wells and incubated at 37°C for 60 minutes. After the incubation, the plates were washed multiple times. The color was developed by adding 50µl of the chromogen solution to the wells and incubating for 15 minutes at 37°C in the dark. The absorbance, also known as optical density (OD), of the plate was measured at 450 nm wavelength. According to the standard solution's concentration values (known values mentioned above) and the corresponding OD values, the standard curve's linear regression equation was calculated. According to the OD value of the samples, the concentration of each corresponding sample was calculated.

Statistical Analysis

The data were collected on a pre-designed proforma and gathered on the Microsoft Excel 2016 (Microsoft Corp. Redmond, WA, USA), version 15.26, for Mac. The data were analyzed using the Stata/SE software (StataCorp. College Station, TX, USA), version 12. Based on the data distribution, continuous variables were summarized as mean ($\pm$ standard deviation; range) or median (interquartile range). Comparison between the groups was conducted using unpaired *t*-test for normally distributed data and using the rank-sum test for skewed data. Categorical data were summarized as proportions and 95% confidence interval of the estimates, and compared using the Chi-square test or the Fisher exact test. A *p*-value of < 0.05 was considered statistically significant.

Results

A total of 61 individuals participated in the study (MD-31, Control-30). There was no significant difference in terms of mean age and gender distribution between the two groups. Among the patients with MD, 16 had disease involvement on the right side, and 15 on the left. The demographic characteristics are summarized in ►**Table 1**. The majority of patients

Table 1 Demographic characteristics of the study participants

	Age (years)		Gender distribution**		Symptom duration (months)	
	Mean* (SD)	Range	Males	Females	Mean (SD)	Range
Control group ($n = 30$)	47 (11.5)	27–68	14	16	NA	NA
MD ($n = 31$)	45.7 (11.3)	28–71	18	13	19.7 (19.6)	4–84

Abbreviations: MD, Meniere disease; *n*, no. of patients; NA, not applicable; SD, standard deviation. **Notes:** * Mean difference (95% confidence interval) = 1.32 (-4.5,7.2); $p = 0.65$. **$p = 0.37$

Table 2 Stage wise distribution of the MD patients

MD stage*	No. of patients (%)
1 ($\leq$ 25 dB)	21 (67.8)
2 (26–40 dB)	7 (22.6)
3 (41–70 dB)	3 (9.8%)
4 ($\geq$ 70 dB)	0

Abbreviations: dB, decibel; MD, Meniere disease. **Notes:** *Based on the AAO-HNS guidelines on MD (1995)[9]

Table 3 Serum levels of Otolin-1 in the study participants

	Otolin-1 (pg/ml)	
	Mean* (SD)	Range
Control group ($n = 30$)	236.2 (43.5)	189–370
MD ($n = 31$)	247.6 (44.2)	201–397

Abbreviations: MD, Meniere disease; pg, picograms; ml, milliliters; SD, standard deviation. **Notes:** * Mean difference (95% confidence interval) = -11.4 (-33.9,11.0); $p = 0.31$.

($\sim$ 68%) presented with MD Stage-1, while none of the patients had Stage-4 disease at the time of recruitment in the study (►**Table 2**).

The sampling for serum Otolin-1 was performed in the interictal phase of the disease, at the time of follow-up in the vertigo clinic. The patients had experienced the last active episode within the last one month. The total symptoms duration in patients with MD varied widely from four months to 84 months, with an average of around 20 months. The mean serum level of Otolin-1 in the control group participants was 236.2 ± 43.5, and in MD cases it was 247 ± 44.2. While the median (IQR) serum Otolin-1 levels in the control group participants was 225 (211.3 – 242), and in MD cases it was 234 (210 – 270), the difference mean was not statistically significant ($p = 0.31$). (►**Table 3**) (►**Figure 1**). The study related data are available as Supplementary file 1.

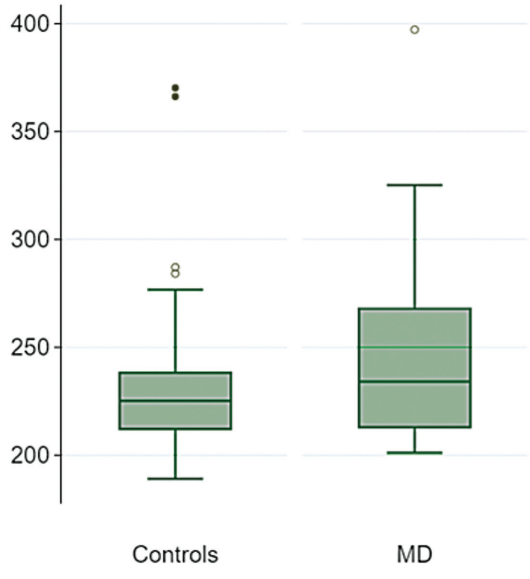

Fig. 1 Box and whisker plot of the levels of Otolin-1 in the Meniere disease patients and healthy control group participants.

Discussion

In the current study, we evaluated the serum levels of inner ear protein, Otolin-1, in patients with MD and compared them with healthy members of the control group. The mean serum levels of Otolin-1 were found to be statistically similar between the MD patients (247.6 pg/ml) and the control group (236.2 pg/ml). These findings are in contrast to the expected results with a degenerative process involving vestibular apparatus. Despite some controversies, the pathophysiological hallmark of MD is thought to be endolymphatic hydrops (ELH).[12] The hydrops may be a result of overproduction of endolymph or its compromised drainage. Recently proposed theories mentioned the endolymphatic outflow obstruction, possibly by the degenerated otoconial fragments, to be the underlying mechanism leading to ELH in MD patients.[9,10,13] We aimed to evaluate serum Otolin-1 values in the MD patients and control group members, having expected raised values in MD patients in view of possible otolithic degeneration causing outflow obstruction. However, the difference in the serum values between the two groups was not found to be statistically significant.

Phillip and Prinsley[9] hypothesized the obliteration of the communicating channels within the endolymphatic compartment with free-floating particles from the otolithic organs as a potential mechanism of altered endolymph drainage mechanics leading to hydrops. This theory was further supported by Yamane et al.,[10] who proposed that the occlusion of the ductus reuniens by the otoconial debris falling from the saccular otolithic organs results in

obstructed endolymphatic flow between cochlea and saccule. This obstruction results in hydrops in the cochlear compartment, a common observation in the initial stages of MD. Later, the obstruction may progress to involve distal outflow pathways of the saccular duct and endolymphatic sinus and duct, resulting in vestibular dysfunction. This exciting hypothesis was supported by the blunting of the grooves for the canals connecting various endolymphatic compartments (ductus reuniens, saccular duct, and endolymphatic sinus) observed on 3D-Cone Beam CT scans of the participants with MD and healthy controls. The authors believed this blunting to be a result of the otoconial debris obliterating these communicating channels. Hornibrook[13] pointed toward the striking similarity between the age of affliction of BPPV and MD patients, and hypothesized this epidemiological correlation to be a possible pointer toward a common underlying etiology of the two diseases. Furthermore, BPPV has recently been shown to be associated with a rise in the serum levels of the Otolith matrix protein, Otolin-1.[5,7] The current study was undertaken to look for the evidence of inner ear degeneration using serum Otolin-1 as a biomarker. We found that the levels of Otolin-1 were comparable between the two groups. These findings contrast with our primary hypothesis of the otolithic organs' degeneration being responsible for the endolymphatic outflow obstruction resulting in MD.

Our current understanding of the temporal variation in the levels of inner ear proteins detectable in the serum through the course of a disease involving the inner ear is limited and needs to be explored in more detail. In our pilot study, the patients were evaluated for the serum Otolin-1 levels during the interictal stage, at the time of their presentation to the vertigo clinic during follow-up. Hence, the serum biomarker dynamics during the acute episodes could not be studied. Nevertheless, most of the patients were enrolled within a few weeks from the disease's acute episode. Also, there were no patients with Stage-4 MD.

In the postmenopausal BPPV patients studied by Parham et al.,[5] it was observed that even though the mean serum Otolin-1 level was significantly higher in BPPV patients, absolute levels of serum Otolin-1 were only higher than the control group in one-third of the BPPV patients. The authors explained this disparity possibly as a result of enrollment of the subjects up to two years from the BPPV episode. A more recent study performed by Wu et al.[7] in a larger cohort of BPPV patients during active episodes found the median serum values to be significantly higher when compared with the control group. Based on their data, a value of 299.45 pg/ml was found to distinguish BPPV patients from normal subjects with a sensitivity of 67.9% and specificity of 72.7%. To date, no studies have specifically looked at the inner ear-specific proteins as serum biomarkers in patients with MD. The current pilot study concludes that the serum levels of the Otolin-1 protein in MD patients in the interictal phase are comparable to those of healthy individuals. Future, larger-scale studies involving MD patients presenting with acute episodes should help clarify the relevance of disease activity on the levels of inner ear biomarkers in serum.

Conclusion

Evaluation of serum biomarkers is an area of research in evolution with the potential to unravel the pathophysiologic mechanisms underlying various inner ear pathologies. In this study, we found that the serum levels of Otolin-1 are not significantly different between the healthy controls and MD patients investigated in the interictal phase of the disease. Evaluation of serum biomarkers in a larger cohort of patients, including patients presenting during active episodes, may help illustrate the role of otolithic biomechanical disruption in greater detail.

Disclosures

The corresponding author has received research grant from the institutional research committee. Rest of the authors have no conflicts of interest to declare. To keep the anonymity, rest of the details are mentioned in title page (following double blind policy of the journal).

Ethical Considerations

The ethical clearance was obtained from the institutional Ethical committee (Ref No. - IEC 506/05.10.18, RP-14/2018; dated 18.10.2018) prior to commencing the study. The same has been uploaded with the rest of the files on author's page.

Data Availability Statement

The study related data are available as supplementary file-1 uploaded with the rest of the files.

Author Contribution

David Victor Kumar Irugu – Main researcher of this study, conception and design of the study, acquisition, analysis, and interpretation of data.
Anup Singh – Acquisition, analysis, and interpretation of data, drafting the article, final approval of the version to be published.
Harsha Yadav – Acquisition, analysis, and interpretation of data, drafting the article, final approval of the version to be published.
Hitesh Verma – Analysis and interpretation of data, drafting the article, final approval of the version to be published.
Kapil Sikka – Analysis and interpretation of data, drafting the article, final approval of the version to be published.
Ransi Ann Abraham – Analysis and interpretation of data, drafting the article, final approval of the version to be published.

Funding

Funded by Research Section, All India Institute of Medical Sciences, New Delhi (Project code: A-643, No.F.8-643/A-643/2018/RS, Dated 15-11-2018). Research grant

approval letter has been uploaded with the rest of the files on author's page.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

Authors express their sincere gratitude to the Research Section All India Institute of Medical Sciences, New Delhi, India, for their support in carrying out the project. We would also like to thank Dr. Ashish Upadhyay, Assistant Professor, Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India, for his statistical support for the study.

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