

Original Article

# Otolin-1, as a Potential Marker for Inner Ear Trauma after Mastoidectomy

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**OBJECTIVES:** The aim of this prospective clinical study was to examine the negative effect of drilling by measuring peripheral Otolin-1 levels as a potential biomarker.

**MATERIALS and METHODS:** Patients who underwent mastoidectomy due to chronic otitis media were included in the study. Otolin-1 levels were measured preoperatively and 6 h postoperatively, and total drilling time was noted. Preoperative serum Otolin-1 levels in 31 patients were compared with those in 31 age- and sex-matched healthy individuals. Pre- and postoperative serum Otolin-1 levels were also compared.

**RESULTS:** Tympanoplasty was performed through canal wall-down (n=17) and wall-up mastoidectomy (n=14) in our sample. The mean duration of drilling was  $52.7 \pm 13.8$  min. Preoperative serum Otolin-1 levels were significantly lower in patients than in healthy controls ( $21.0 \pm 3.0$  vs.  $23.5 \pm 3.9$  pg/mL,  $p=0.006$ ). We also found significantly higher postoperative serum Otolin-1 levels than preoperative levels ( $21.0 \pm 3.0$  vs.  $27.0 \pm 6.9$  pg/mL,  $p<0.001$ ). An increase in serum Otolin-1 levels during surgery was independently associated with drilling time in multivariate linear regression analysis ( $r=0.309$ ,  $p<0.001$ ).

**CONCLUSION:** A nearly postoperative increase in serum Otolin-1 levels after mastoidectomy was independently associated with drilling time. We show that serum Otolin-1 levels may be used to indicate inner ear trauma in clinical practice in the future.

**KEYWORDS:** Otolin-1, drilling, mastoidectomy, inner ear, biomarker

## INTRODUCTION

The surgical procedure for diseases of the ear is decided based on disease status and hearing acuity. Drilling is an important part of the surgical procedures performed to treat chronic otitis media<sup>[1]</sup>. The loudness generated during drilling of the mastoid bone reaches up to 110 dB<sup>[2]</sup>. Previous studies reported that such a level of energy can result in hearing loss, irrespective of the underlying disease<sup>[3]</sup>. Drilling can cause hearing loss through three different mechanisms: (1) by causing excessive vibration in the stapes footplate through direct ossicle chain contact, (2) by direct damage on the membranous labyrinth, and (3) by causing acoustic trauma due to high levels of drill noise<sup>[4]</sup>. Kylen et al.<sup>[5]</sup> reported the occurrence of transient hearing loss during ear surgeries involving drilling. Palva et al.<sup>[6]</sup> observed sensorineural hearing loss detected at high frequencies in 1.2%-4.5% of patients during the postoperative period. Various other researchers also reported that hearing loss may occur in patients exposed to drilling<sup>[7,8]</sup>.

Since optimal evaluation of hearing in the early postoperative period may be difficult, there is a need for new methods that allow easy, rapid and objective assessment of potential hearing loss after drilling. Inner ear-specific proteins could be used as a biomarker for detection of various inner ear pathologies. Biomarkers are circulating biochemical indicators of normal and/or pathological processes. They are widely used in the diagnosis and treatment of many pathological conditions, such as autoimmune diseases and myocardial infarction<sup>[9]</sup>. There is no universally accepted specific biomarker for inner ear damage that can be used in clinical practice.

Otolin-1 is a glycoprotein specifically secreted by the inner ear. It is found in the supportive cells of the vestibular macula, cristae of the semi-circular canal, marginal cells of the striavascularis, and organ of Corti. It is a vital component of the extracellular matrices



in contact with sensory hair cells, including the otoconial membrane of the vestibule and tectorial membrane of the cochlea. Therefore, Otolin-1 is necessary for hearing and vestibular function<sup>[10]</sup>. Otolin-1 can be detected and measured in serum, because it exits the endolymph, passes through the labyrinth-blood barrier, and enters the peripheral systemic blood circulation. Therefore, it might be a potential biomarker that can be used for diagnosis of inner ear pathologies, possibly including hearing loss. Serum levels of Otolin-1 were previously shown to be increased in patients diagnosed with benign paroxysmal positional vertigo<sup>[11]</sup>. However, the relationship of Otolin-1 to the drilling procedure, which is essential to ear surgeries, has not been examined previously.

The aim of the present study was to show the diagnostic role of serum Otolin-1 levels in inner ear damage due to drilling in patients who underwent mastoidectomy. We hypothesized that the drilling procedure during ear surgeries results in an increase in inner ear scaffolding protein serum Otolin-1 levels by causing disruption of the inner ear extracellular matrices. Therefore, we investigated the postoperative change in serum Otolin-1 levels of patients who underwent mastoidectomy for chronic otitis media.

## MATERIALS AND METHODS

This was a prospective clinical study. A total of 31 age- and sex-matched healthy subjects and 31 patients scheduled for mastoidectomy at Adnan Menderes University Otorhinolaryngology Department due to chronic otitis media between May 1, 2017 and November 1, 2017 were included in the study. The study methods were submitted to and approved by the Institutional Review Board of the local ethics committee in accordance with the Declaration of Helsinki. Informed consent was waived (Institutional Review Board research protocol 2017-1137). For patients <18 years old, informed consent was obtained from the patients and their parents.

Patients with a history of otologic surgery or head trauma were excluded from the study. Patients with Meniere's disease, ototoxic drug use, the presence of a chronic disorder, or medication use that impaired renal excretion were also excluded. Following the baseline measurements of Otolin-1 levels in preoperative patients and healthy controls, a second blood sample was obtained from patients at 6h after surgery. The duration of the drilling procedure was noted for each patient.

Serum specimens were stored at -80°C until the time of Otolin-1 ELISA tests. Anticoagulant-free tubes were centrifuged at 1000g for 10 min for 20-60 min. Otolin-1 levels in serum samples were determined via a commercial Human Otolin-1 ELISA kit (catalog no.SL2638 Hu; Sunlong Biotech Co., Ltd., Yuhang District, Hangzhou, Zhejiang, China). The test results were calculated by a bio elisa reader Elx800 (BioTek Instruments, Winooski, Vermont, USA) using a standard curve and measuring the absorbance at 450 nm. The limit of detection for Otolin-1 was given as 0.5pg/mL. The assay range was given as 1.6-100 pg/mL. Procedures were performed according to the manufacturer's instructions.

## Statistical Analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 22.0 (IBM Corp., Armonk, NY, USA). The

Kolmogorov-Smirnov test was used to investigate whether continuous variables fit the normal distribution. The Student's *t*-test was used for comparison of continuous variables. A paired *t*-test was used for comparison of preoperative and postoperative levels of serum Otolin-1. The chi-square test was used for comparison of categorical variables. The effects of different variables on the level of delta Otolin-1 were calculated in univariate analysis for each variable. The variables for which the unadjusted *p* value was <0.20 in linear regression analysis were identified as potential risk markers and included in the full model. We reduced the model by using backward elimination multivariate linear regression analyses and eliminated potential risk markers by using likelihood ratio tests. A *p* value of <0.05 was considered statistically significant (two-tailed test).

## RESULTS

A total of 31 patients (51.6% female) with chronic otitis media who underwent tympanoplasty and 31 age- and sex-matched healthy individuals (51.6% female) were included in the study (Table 1). The age of the patients was 45.0±17.5 years, and the age of healthy individuals was 44.9±16.3 years. Of the 31 patients, 17 (54.8%) underwent canal wall-down tympanoplasty with a diagnosis of cholesteatoma, whereas 14 (45.2%) without cholesteatoma underwent canal wall-up tympanoplasty. Surgery was performed on the right ear in 16 (51.6%) patients. The mean duration of drilling during surgery was 52.7±13.8 min. Table 1 demonstrates the comparison of baseline demographic and clinical characteristics of the patient and control groups. The mean preoperative serum Otolin-1 levels were significantly lower in the patient group than in healthy controls (21.0±3.0 vs. 23.5±3.9 pg/mL, *p*=0.006) (Figure 1). A paired *t*-test revealed that postoperative Otolin-1 levels were significantly higher than preoperative levels (21.0±3.0 vs. 27.0±6.9 pg/mL, *p*<0.001) (Figure 2). The effects of different variables, including the presence of cholesteatoma, type of operation (canal wall-up or down), operation site of the ear (right or left), and drilling time, on Otolin-1 levels were reduced by multivariate linear regression analyses. Accordingly, an increase in the serum Otolin-1 level with mastoidectomy was independently associated with drilling time during the procedure (*r*=0.309, *p*<0.001).

## DISCUSSION

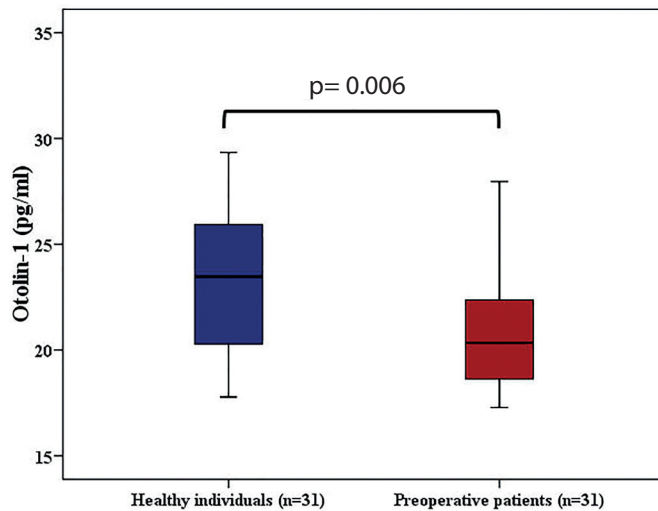
In the present study, we found that Otolin-1 levels significantly increased following tympanoplasty and were independently associated with the duration of drilling. In addition, the Otolin-1 level as a marker of the sensorineural hearing level is significantly lower in patients with chronic otitis media than in age- and sex-matched healthy individuals.

Biomarkers are significant and beneficial molecules for early diagnosis of several diseases and monitoring of the treatment period. An

**Table 1.** Baseline demographic and clinical characteristics of the patient and control groups

	Control (n=31)	Patients (n=31)	p
Age	44.9±16.3	45.0±17.5	0.982
Sex (female/male)	16/15	16/15	1.000
Otolin-1 (pg/mL)	23.5±3.9	21.0±2.9*	0.006

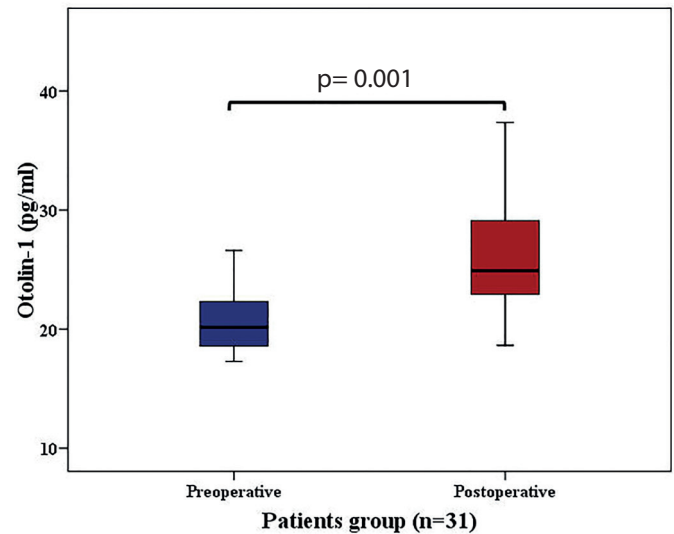
\*Preoperative.



**Figure 1.** Box-plot graph showing patients' preoperative otolin-1 levels and that of control group.

ideal biomarker should be tissue specific, detectable, and measurable. With the increasing understanding of the role of biomarkers in ear disease, we believe that new biomarkers will be defined for early detection of various adverse events. It was previously reported that the inner ear-specific proteins, prestin and Otolin-1, could be used as serum biomarkers for diagnosis and monitoring of inner ear diseases [11, 12]. Parham et al. [11] reported that Otolin-1 levels in patients with benign paroxysmal positional vertigo are significantly higher than those in healthy individuals. Tabatabai et al. [13] demonstrated that Otolin-1 levels increased due to age-related demineralization. For this purpose, we examined the role of Otolin-1 levels in detecting inner ear trauma after mastoidectomy. We found that Otolin-1 levels significantly increased with inner ear trauma due to surgery. In addition, the Otolin-1 level was independently associated with the duration of drilling, suggesting a close relationship between Otolin-1 and inner ear trauma. In a previous study, Palva et al. [14] reported a significant association between duration of drilling and postoperative hearing loss. To the best of our knowledge, there are no published data on a biomarker that shows this relationship between drilling and inner ear damage. We showed for the first time that Otolin-1 levels can be a useful marker to detect the early effects of trauma and the duration of the exposure to the inner ear.

A drill is a frequently used instrument in chronic otitis media surgeries. Several studies have shown transient and short-term hearing loss in the inner ear due to the direct and secondary effects of drilling [14-17]. In our study, preoperative Otolin-1 levels in the treatment group were lower than those in the control group. Otolin-1 is a vital component of the extracellular matrices in contact with sensory hair cells, including the otoconial membrane of the vestibule and tectorial membrane of the cochlea [10]. It is well established that chronic ear disease, especially chronic otitis media, is closely related to inner ear functions [18]. Therefore, we speculate that inner ear function is lower in patients with chronic otitis media than in healthy individuals. Accordingly, as a marker of inner ear function, it was significantly lower in the patient group than in healthy individuals. However, further studies are needed to confirm these findings.



**Figure 2.** Box-plot graph showing preoperative and postoperative otolin-1 levels of the patient group.

We found a significant increase in Otolin-1 levels at 6h after mastoidectomy. Moreover, this early increase in Otolin-1 levels was found to be related to the duration of drilling. We suggest that the use of a drill is associated with an increase in Otolin-1 levels via the enhancement of glycoprotein turnover due to trauma.

The present study has a few limitations. First, postoperative serum samples were only obtained at 6h. Measurement of Otolin-1 levels in larger samples is required for a more comprehensive investigation of the changes in serum Otolin-1 levels. Therefore, obtaining serum samples at later postoperative hours/days can provide more information about changes in Otolin-1 levels. Second, the lack of postoperative hearing assessment should be considered as another limitation. Several studies reported transient hearing loss due to drilling [1-8]. Since early hearing loss after surgery is short-term and transient, this loss cannot be clearly detected by conventional methods, such as pure tone audiometry. Moreover, factors, such as the presence of wound dressing and ear tamponade and the potential change in hearing thresholds caused by pain and the surgical procedure (in particular, the change in air conduction), decrease the reliability of audiologic evaluations. At this stage, conventional methods are insufficient, and test results would be interpreted subjectively. Therefore, as it biochemically reflects damage to the inner ear, Otolin-1 can provide more objective and reliable results by eliminating any potential errors caused by various factors. Therefore, it would be better to determine the spectral change in serum Otolin-1 levels, which are more often measured from the early to the late phase of the postoperative period, and examine its relationship to hearing thresholds.

## CONCLUSION

Hearing loss is an important health care problem that may cause adverse psychosocial effects. Early diagnosis and treatment of hearing loss to prevent permanent conditions are the main goals of clinicians. We showed that an increase in the serum Otolin-1 level is independently associated with drilling duration in patients who underwent mastoidectomy. Accordingly, serum Otolin-1 could be a useful marker to identify inner ear damage in various conditions. Further

prospective studies should be planned to determine the effects of not only drilling but also other conditions, such as toxic drugs and acoustic trauma, on serum Otolin-1 levels.

**Ethics Committee Approval:** The study method was approved by the Ethics Committee of Adnan Menderes University School of Medicine. (No: 2017-1137).

**Informed Consent:** A written informed consent was obtained from the patients agreed to be involved in the study. For patients <18 years old, informed consent was obtained from the patients and their parents.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - M.D., M.S.; Design - M.S., M.D., Y.K.; Supervision - M.D.; Data collection and/or processing - M.S., M.D., Y.K.; Analysis and/or interpretation - M.S., M.D., Y.K.; Literature search - M.D.; Writing of the manuscript - M.S., M.D.; Critical review - M.S., M.D., Y.K.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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