Inner Ear Protein as a Biomarker in Circulation?

Kourosh Parham, MD, PhD, Daniel Sacks, MD, Catherine Bixby, MS, and Pamela Fall, MS

Abstract

Serum biomarkers detect the earliest events in disease, monitor management, and provide insight into disease pathogenesis. At this time, there are no biomarkers available for otologic disorders. Otolin-1 is a scaffolding protein exclusively expressed in otoconia and cells of the vestibule and the cochlea; therefore, it may be a biomarker candidate for assessing the health of the inner ear. As a proof of concept, we used serum samples from controls without otologic history and subjects with a history of benign paroxysmal positional vertigo (BPPV), performed enzyme-linked immunosorbent assay for otolin-1, and measured the optical density of the substrate. Otolin-1 was detectable and quantifiable in all subjects, indicating that this inner ear protein crosses the blood-labyrinthine barrier. Furthermore, subjects with BPPV had significantly higher levels, with about one-third being above the control range. This promising preliminary result suggests that inner ear-specific proteins have the potential to serve as biomarkers for otologic disease processes.

Keywords

otolin, biomarker, inner ear, otoconia, stria vascularis, benign paroxysmal positional vertigo

Received January 21, 2014; revised August 1, 2014; accepted August 22, 2014.

Biomarkers represent measurable products of biological processes, thus making assessment of those processes more practical. In disease states, because biomarkers are often measured from body fluids, they eliminate the need for costly modalities to diagnose and monitor progression. Examples of widely used biomarkers include cardiac enzymes and bone turnover markers used to evaluate myocardial infarction and osteoporosis, respectively.¹

To date, no specific biomarkers for inner ear diseases have been used. The inner ear has been found to possess a number of unique proteins that mediate its specialized function. Otolin-1 is a secreted glycoprotein whose messenger RNA (mRNA) expression is restricted to the inner ear, specifically the support cells of the vestibular maculae, semicircular canal cristae, organ of Corti, and marginal cells of the stria vascularis.² Its functions include interaction with other specialized inner ear proteins, such as otoconin-90, to form and maintain otoconia.²,³

There are several prerequisites for an effective biomarker. First, they have to be specific enough to be able to hone in on one organ or disease process. By virtue of its unique expression in the inner ear, otolin represents an excellent biomarker candidate of the inner ear. Second, the biomarker should be present in easily accessible body fluids (eg, serum), and third, it has to be quantifiable. Fourth, at this early stage of work, the biomarker has to show potential to distinguish between normal and disease states. There are no reports in the literature on whether otolin-1 is detectable in the serum and, if so, whether it is quantifiable or has diagnostic potential.

Methods

This study was approved by our institutional review board. Serum samples from 14 postmenopausal women with a history of posterior canal benign paroxysmal positional vertigo (BPPV) and 10 postmenopausal women who had no history of otologic disorders were used. This approach allowed us to keep the subject population uniform and limit confounding effects of sex and hormonal influences. Additional information on subjects and procedural details for verification of diagnoses are described elsewhere.⁵

Morning fasting blood samples were collected from all subjects. Collected specimens were allowed to clot and spun at 3000 rpm for 15 minutes at 4°C. Serum was then removed and frozen at −80°C until time of assay. Otolin-1 was measured in the serum using the Human-OTOL1
enzyme-linked immunosorbent assay (ELISA) kit (QAYEE-BIO, Atlanta, Georgia) as described in the manufacturer’s instruction manual. A 1:5 dilution was prepared, and each serum sample was assayed in triplicate. The optical density in the wells of the ELISA microplate was measured at 450 nm using a BioTek ELx808 plate reader, and data were compiled using the KCJunior software package (BioTek Instruments, Winooski, Vermont).

Results

Otolin was detected in the serum samples of all subjects. Figure 1 shows the distribution of serum otolin concentration for the 2 groups. In control subjects, otolin levels ranged from 86.3 to 607 pg/mL, with an average of 443.1 ± 45.2 (mean ± SEM) pg/mL. Among subjects with BPPV, levels ranged from 324.7 to 1002.3 pg/mL, with an average concentration of 590.3 ± 45 (mean ± SEM) pg/mL. One-third of subjects with BPPV had levels above the control range. On average, the BPPV group had higher serum levels by 150 pg/mL (Figure 2). A 2-tailed t test demonstrated that the difference between the 2 groups was statistically significant (P = .036).

Discussion

Biomarkers assist in screening and risk assessment prior to diagnosis, staging and treatment during diagnosis, and monitoring treatment efficacy and disease reoccurrence.6 Biomarkers are powerful indicators of normal biological and pathological processes and pharmacological response.7

Our data show that the inner ear scaffolding protein, otolin-1, can be detected and quantified in serum. Since otolin-1 can be measured in serum and current evidence suggests its expression is specific to the inner ear, it may serve as a biomarker to potentially diagnose and monitor inner ear disorders. Detection of otolin in serum is important because it proves that this inner ear protein can exit the endolymph, pass through the labyrinth-blood barrier, and enter the systemic circulation.

Criteria that apply to an ideal biomarker include specificity, detectability, and measurability. A fourth criterion is that a biomarker has to be meaningful. Benign paroxysmal positional vertigo, which involves fragmentation of otoconia, is convenient for proof of concept because its diagnosis can be objectively verified using the Dix-Hallpike maneuver. Otolin-1 forms the scaffolding on which the organic and inorganic matrix of the otoconia forms, as well as the fibrils that interconnect the otoconia.2-4 From a practical perspective, otolin-1 levels may be used, for example, in up to 30% of BPPV cases where diagnosis and management is challenging (eg, lateral, superior, or multiple/bilateral canal involvement).

Only one-third of subjects with BPPV had serum otolin-1 values above control range. The absence of 100% differentiation may reflect that subjects with BPPV had their sentinel/most recent episode up to 2 years prior to enrollment into the study. Assessment of otolin-1 levels during or shortly after an acute BPPV episode should be considered in future studies.

Future experimental and clinical studies can explore serum levels of otolin-1 and other inner ear biomarkers (eg, otoconin-90, prestin) for compelling purposes, such as monitoring otoxicity or identifying patients with idiopathic sudden sensorineural hearing loss who are likely to respond to steroid therapy. The purpose of the present preliminary report is to stimulate interest in the concept of otologic biomarkers and raises the possibility of measuring otolin-1 as a biomarker for BPPV and other inner ear diseases, but additional work is needed to establish its value.

Author Contributions

Kourosh Parham, conceived hypothesis, design and execution of the study, data analysis, and primary author of manuscript; Daniel Sacks, data analysis and contributed to writing manuscript; Catherine Bixby, data analysis and contributed to writing manuscript; Pamela Fall, data analysis and contributed to writing manuscript.
Disclosures
Competing interests: None.
Sponsorships: None.
Funding source: M01RR006192/RR/NCRR NIH and the Division of Otolaryngology/Head and Neck Surgery, UCONN School of Medicine.

References