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Age-related increase in blood levels of otolin-1 in humans

Ryan Tabtabai, **Laura Haynes, Ph.D.**[1,2], **George A. Kuchel, M.D.**[1], and **Kourosh Parham, M.D., Ph.D.**[3]

[1]Center on Aging, UCONN School of Medicine, Farmington, CT 06030

[2]Department of Immunology, UCONN School of Medicine, Farmington, CT 06030

[3]Department of Surgery, Division of Otolaryngology – Head & Neck Surgery, UCONN School of Medicine, Farmington, CT 06030

Abstract

Objective—To test the hypothesis that age-related demineralization of otoconia will result in an age-related increase in blood levels of otoconia matrix protein, otolin-1.

Study design—Cross-sectional observational clinical trial.

Setting—Clinical research center.

Patients—Seventy-nine men and women ranging in age from 22 to 95 years old.

Interventions—Diagnostic.

Main outcome measures—Blood levels of otolin-1 in relation to age.

Results—Levels of otolin-1 of subjects divided into four age groups (1: 20–30 [n=20], 2: 50–65 [n=20], 3: 66–80 [n=20], 4: 81–95 [n=19] years old) demonstrated an increasing trend with age The difference between otolin levels of groups 2 and 3, as well as, (P=0.04) and 2 and 4 (P=0.031) were statistically significant, but there was no significant difference between the two oldest groups.

Conclusions—Otolin-1 blood levels are significantly higher in patients older than 65 years of age. This is consistent with previous scanning electron microscopy findings of age-related otoconia degeneration and increased prevalence of benign paroxysmal positional vertigo (BPPV) with age. Normative data provided here can serve as important reference values against which levels from BPPV patients can be compared to further evaluate otolin-1 as a circulatory biomarker for otoconia degeneration.

Introduction

Biomarkers in circulation are powerful indicators of normal and pathological biological processes, as well as, response to pharmacological treatments[1]. Until recently no specific biomarker for inner ear diseases was available. The inner ear has been found to possess a number of unique proteins that mediate its specialized functions. We have demonstrated that

Correspondence: Kourosh Parham, 263 Farmington Ave, UCONN Health, Farmington, CT 06030-8083, parham@uchc.edu, Tel: 860-679-3372, Fax: 860-679-6564.

one of these proteins, otolin-1, has increased levels in circulation in patients with benign paroxysmal positional vertigo (BPPV), thus providing proof of concept for use of inner ear proteins as biomarkers[2,3]. Otolin-1 is a secreted glycoprotein whose 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[4]. Its functions include interaction with other specialized inner ear proteins, such as otoconin 90, to form and maintain otoconia[4–6]. Before otologic serological biomarkers such as otolin-1, can be adopted into clinical practice, the characteristics of otolin-1 in circulation need to be defined. Here we take the initial step toward this objective. To guide our work, we have maintained our focus on BPPV. BPPV increases in prevalence with age[7]. This characteristic of the disease appears to be related to age-related demineralization of otoconia[6,8]. Towards the goal of evaluating the potential of otolin-1 as a serological biomarker, here we test the hypothesis that with demineralization of otoconia, otolin-1 levels in blood will increase with age.

Methods

Subjects

This study was approved by the Institutional Review Board (15-006-3). Subjects were recruited as a part of an ongoing study conducted by the UConn Center of Aging on examining the impact of aging on the response to flu vaccination. With a recruitment goal of 20 subjects, participants were enrolled into the following age groups: young (20–30 years old), middle aged (50–65 years old), old (66–80 years old) and the oldest old (81–95 years old). We successfully met the recruitment goal for all groups except the oldest old which had 19 subjects. Inclusion criteria were male and female gender. Exclusion criterion for the youngest three age groups was major health conditions with the exception common chronic conditions such as hypertension, hypothyroidism, hypercholesterolemia, and reflux. For the oldest old, the only exclusion criterion was the inability to consent. Major health conditions did not serve as an exclusion criterion in order to increase recruitment. In this group, frailty was quantified using the Rockwood Frailty Index[9]. No vulnerable populations (trainees and prisoners) were recruited.

Data Collection

Non-fasting blood samples (‘green tops’) were collected from all subjects. Collected specimens were spun at 1,300G for 10 minutes. Plasma was then removed and frozen at −80° C until time of assay. Otolin-1 was measured in the plasma using the Human-OTOL1 enzyme-linked immunosorbent assay (ELISA) kit (MyBiosource.com, San Diego, Ca) as described in the manufacturer’s instruction manual. A 1:5 dilution was prepared, and each plasma 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).

Statistics

SPSS v 22 was used for statistical analyses. Normality of distributions was assessed with the Shapiro-Wilk test. Non-parametric tests were carried out when distributions departed from

normal. Correlation analysis was performed using Spearman's rho (r_s). Group comparisons were performed using Mann Whitney U tests of independent samples. Level of significance was set at $p < 0.05$, two tailed. According to convention, undetectable values were defined as being one-half the minimal detectable concentration for the assay.

Results

Seventy-nine subjects, 57 female and 22 male, participated in this study. In the oldest group the Frailty Index scores ranged from 1 (very fit) to 7 (severely frail), with mode of the distribution being 3 (multiple medical problems, but managing well).

Figure 1 shows otolin-1 levels as a function of age and gender. In the 20–30-year-old group, most subjects had levels below 200 pg/mL, but there were a few outliers with otolin-1 levels between 300–500 pg/mL. Similarly, in the older groups, two clusters were present, with the majority being below 350 pg/mL and a minority between 500–900 pg/mL. Among the subset of subjects with high values, female subjects outnumbered the males by 8 to 1.

In the older group, using a conservative index for outliers ($g=2.2$)[10,11], all values greater than 354 pg/mL were considered statistical outliers. These subjects likely represent a distinct entity and are deserving of focused attention. In considering the trends in the majority pool, to avoid an outlier bias in the statistical testing, these outliers were not included in the subsequent analyses. The Shapiro-Wilk tests of normality were performed on the four separate age groups after removal of the outliers. The distributions of the young- and middle-aged-groups were both statistically significantly different from normal distributions ($p = 0.023$ and $p = 0.01$, respectively). Therefore, nonparametric testing will be utilized to compare the four age groups.

Table 1 shows the results of correlation of otolin-1 levels and age. The male subgroups are underpowered and will not be discussed in detail, although their results are reported for completeness. Among, females, there were moderate to strong correlations noted between age and otolin-1 levels, however, they were not statistically significant. A weak, positive, but statistically significant, correlation was found between age and otolin-1 level ($r_s = 0.3$, $p = 0.01$) when all subjects were combined. Analysis by age groups showed a moderate, positive correlation between age and otolin-1 level ($r_s = 0.57$, $p = 0.02$) in the oldest old. Using a linear regression model for the oldest old, otolin-1 level could be predicted from age by the following formula: otolin-1 level = $-744.6 + \text{age} \times 10$ (i.e., for every year increase in age above 81, otolin-1 level increased by 10 pg/mL).

Figure 2 shows the median otolin-1 levels with interquartile ranges for the four age groups. The median of the two youngest groups were below 60 pg/mL while those of the two older groups were near or greater than 100 pg/mL. Mann Whitney U tests of independent samples demonstrated statistically significant differences between the middle aged and the old ($p = 0.04$), as well as, the middle aged and the oldest old ($p = 0.031$) groups. Besides age, the difference between the two oldest groups is that the oldest old included vulnerable and frail subjects. The absence of a difference in otolin-1 levels in these two groups implies that

frailty had no significant impact. This is further supported by the absence of a correlation between frailty index and otolin-1 level.

Discussion

Although current tools for investigating the inner ear are very helpful, there remain significant constraints on our ability to gain a full understanding of inner ear function and pathophysiology. Biomarkers represent an under explored avenue which could aid a better understanding of inner ear pathophysiology. Highly specialized inner ear functions are made possible by unique proteins which upon disruption of function or cellular injury may be released into circulation where they could provide useful information. Specifically, we have proposed otolin-1[2] and prestin[12] as a serological biomarkers and provided proof of concept in BPPV[2] and noise-induced hearing loss[13], respectively. Paving the path to practical application of inner ear biomarkers, however, requires additional validation. Toward that goal, in this study, we hypothesized that with the known age-related demineralization of otoconia, otolin-1 levels in plasma should increase with age. Our results support this hypothesis, as subjects above age 65 have higher blood levels of otolin-1 than those less than 65. The design of the current study, a cross-sectional observational human trial, is a limitation in that it only permits the association between otolin-1 levels in circulation and demineralization of otoconia by inference. Nevertheless, given current state of knowledge, this is a very strong inference. Certainly, future animal studies could be useful in further strengthening our conclusions. Animal models which have demonstrated changes in the morphometry and distributional pattern of otoconia include those in aged rats [14] and in an ovariectomized rat model of osteoporosis[15].

Comparison to BPPV subjects - Dizziness was not a criterion for participation in the study. We invited participants without major health conditions to participate in this study for the age groups below 80 years of age. We placed no restriction on older participants so as not to limit recruitment. Therefore, whether any subjects in this study had BPPV is unknown.

It is, however, instructive to compare the present results to those of subjects with known BPPV. In female, post-menopausal BPPV subjects, otolin-1 levels averaged around 600 pg/mL (range: 324.7–1002.3 pg/mL), while those of age-matched control subjects with osteoporosis averaged around 450 pg/mL (range: 86.3–607 pg/mL)[2]. The otolin-1 levels for both BPPV and osteoporosis groups were much higher than those reported in the present study. This further highlights the potential of otolin-1 as a biomarker for otolithic degeneration and BPPV and suggests that upper normal range could be 300–350 pg/mL.

In our sample, we had subjects in both the young and older age groups that were outliers. The outliers in the older groups, had otolin-1 levels comparable to those of BPPV subjects in our previous study[2]. It is tempting to speculate that the outliers have either undiagnosed BPPV or severe otolithic degeneration which places them at high risk of developing BPPV. Five out of 59 subjects older than 50 in the present study had very high otolin-1 levels, making up 8.5% of our sample. This is comparable to the 9% rate of undiagnosed BPPV in the geriatric population [16]. However, we emphasize that an elevated otolin-1 level is not necessarily equivalent to a diagnosis of BPPV, keeping in mind the intraoperative

observation that presence of particulate matter in the membranous portion of the posterior semicircular canal does not necessarily coincide with a diagnosis of BPPV[17]. We have also observed otolin-1 level over 600 pg/mL in post-menopausal, osteoporotic women who did not have BPPV[2].

Alternative interpretation

All of our subjects were healthy, with the exception of the oldest old. Since age-related changes were first noted in the old group, age-related decline in clearance mechanisms (e.g., renal), are unlikely to account for our observation of an age-related increase in blood otolin-1. Above we suggested an increase in circulating otolin-1 with age was consistent with age-related degeneration of otoconia. An alternative, but related, interpretation is that presence of otolin-1 in the blood is consistent with the notion that there is turnover in otoconia [18–20]. In line with this interpretation, persistent elevation of blood otolin-1 level could be the product of increased turnover with degeneration out pacing deposition/repair in the older subjects. This interpretation would be analogous to disrupted bone turnover in favor of bone resorption in osteoporosis, which has been shown to be strongly associated with BPPV [18–24].

Sex differences

Women are two times more likely to suffer from BPPV[25]. Based on this epidemiological knowledge we expected to find significant differences in otolin-1 levels between male and female subjects in our sample. However, the number of male participants in this study was much smaller than females and therefore, comparisons between male and females were underpowered. Larger samples of both male and females will be needed to elucidate gender-related trends.

How can an otolithic degeneration biomarker be useful - Since otolithic degeneration is a hallmark of BPPV, a biomarker for otolithic degeneration can be used to promote better understanding of BPPV. Majority of BPPV cases have no known precipitating etiology (75% are idiopathic). Even when there is a precipitating cause (e.g., URI or head trauma), it is not clear why some suffer from BPPV, but most don’t.

This work represents the early phase of biomarker evaluation. Biomarker evaluation consists of analytical validation of the assay, quantification and assessment of associations between the biomarker and disease states and contextual analysis of utilization.[1] Additional studies to determine if our methods are biomarker-specific and reliable enough or alternative methodologies are needed to utilize otolin-1 and other possible biomarkers for otologic diseases.

Aging is a risk factor for a majority of chronic diseases and pathologies which were previously thought to be disparate, but are now understood to be connected[26]. Understanding how aging enables chronic disease and developing novel multi-disease preventive and therapeutic approaches, i.e., geroscience, is an emerging priority[26,27]. Recent recognition of strong associations between BPPV, osteopenia/osteoporosis and vitamin D deficiency[21,23,24,28–31] are changing the way we think of BPPV: a BPPV episode may be manifestation of a progressive, chronic disorder involving otolithic degeneration. A thorough

understanding of such a progressive condition would be inherently limited, if we focus on characteristics of episodic manifestations (i.e., during BPPV attack) without acknowledgement of coexisting morbidities. By extension, clinical management of BPPV is suboptimal if management is directed at relieving individual BPPV episodes without attending to chronic predisposing factors. Easy to measure biomarkers in circulation that inform on the status of otoconia could provide useful insights into a structure which would otherwise be difficult to evaluate.

Otolin-1 can also be convenient tools for assessing effectiveness of novel pharmaceutical treatment strategies. For example, as our understanding of the associations of BPPV and osteopenia/osteoporosis and vitamin D deficiency translates into therapeutic strategies, otolin-1 level in circulation could be one measure to assess their effectiveness. Also, given the important role of structural proteins such as otolin-1, novel pharmaceuticals that stabilize the structure of otoconia could be explored and monitored to prevent BPPV attacks.

Finally, a biomarker in circulation could be a convenient tool to aid diagnosis of challenging presentations suspected of being BPPV. These include cases of subjective BPPV, multicanal or bilateral disease and when it is difficult to perform diagnostic positional maneuvers such as in kyphotic, frail elderly.

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Figure 1.
Otolin-1 concentration of male (squares) and female (circles) subjects as a function of age.

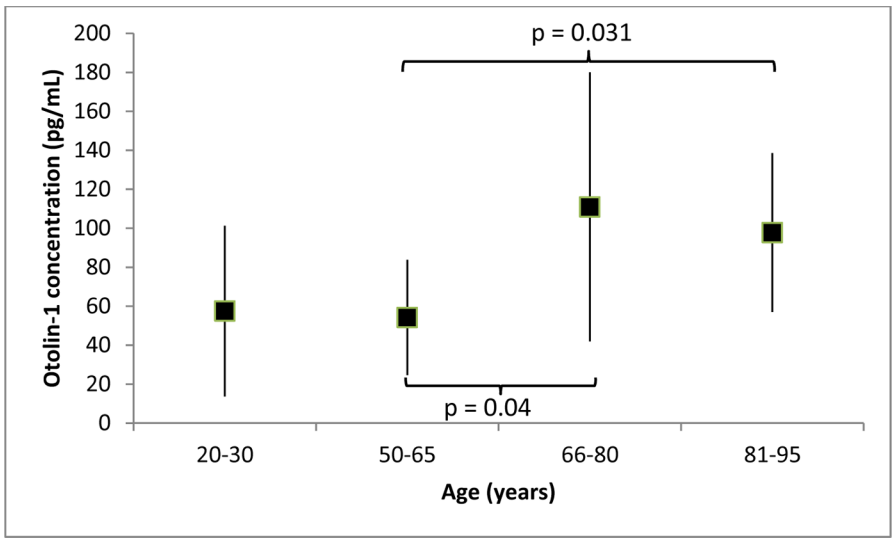

Figure 2.

Otolin-1 medians for the four age groups after removal of outliers. The number of subjects in each group was: young = 17, middle aged = 19, old = 19 and oldest old = 16. Error bars represent interquartile range.

Table 1

Spearman rho (r_s) correlation coefficient of otolin-1 concentrations with as a function of gender

Age (years)	All (n)	Male (n)	Female (n)
20–30	0.17 (17)	−0.41 (5)	0.26 (12)
50–65	−0.06 (18)	0.5 (3)	−0.19 (15)
66–80	0.012 (19)	0.07 (8)	0.46 (11)
81–95	0.57 * (16)	0.72 (5)	0.51 (11)
All	0.3 ** (70)	0.03 (21)	0.37 (49)

* p = 0.02;

** p = 0.012