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Estradiol Deficiency Is a Risk Factor for Idiopathic Benign Paroxysmal Positional Vertigo in Postmenopausal Female Patients

Hualan Yang, MD; Huanhuan Gu, MD; Wenjing Sun, MD; Yanpeng Li, PhD; Huijuan Wu, PhD; Molorerdene Burnee, MD; Jianhua Zhuang, PhD

Objectives/Hypothesis: Although it is generally considered that benign paroxysmal positional vertigo (BPPV) is associated with changes in female sex hormone levels, no direct data have been reported until now. The purpose of this article was to provide direct data showing the distinct relationship between female sex hormone fluctuations and BPPV in postmenopausal female patients.

Study Design: Prospective analysis in humans and basic research in animals.

Methods: Blood samples were analyzed to determine the levels of estradiol, progesterone, follicle-stimulating hormone, and luteinizing hormone in 50- to 80-year-old postmenopausal female patients newly diagnosed with idiopathic BPPV based on history compatible with BPPV and positive provocative maneuvers. Animal models of bilateral ovariectomy and female sex hormone replacement therapy were used to further confirm the relationship between BPPV and female sex hormone levels by determining the expression levels of otoconin 90, the protein suggested as essential in the dislocation of otoconia.

Results: Statistically significant differences between the estradiol level of BPPV patients and the control group were found ($P < .001$). Moreover, in bilateral ovariectomy in rats, 17-$\beta$-estradiol replacement reversed the decrease of otoconin 90 levels.

Conclusions: Our results suggest that estradiol deficiency may be an important risk factor for idiopathic benign paroxysmal positional vertigo in postmenopausal female patients.

Key Words: Benign paroxysmal positional vertigo, postmenopausal, estradiol, otoconin 90, ovariectomy, hormone replacement therapy.

Level of Evidence: NA.

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INTRODUCTION

Benign paroxysmal positional vertigo (BPPV) is the most common form of vertigo, and is believed to be caused by dislocation of otoconia into the semicircular canals, which renders the canals sensitive to gravity.\(^1,2\) This condition occurs more frequently in elderly persons with a history of trauma or surgery in the head or neck area, serious mental anxiety,\(^3\) viral neurulabyrinthitis,\(^4\) and diseases of the inner ear. However, approximately 80% of the cases are idiopathic. Until now, the triggers of otoconial degeneration and detachment of otoconia from the otoconial beds have not been elucidated. The male-to-female ratio of the individuals diagnosed with this disease is 1:2,\(^4\) and the incidence peak is between 40 and 60 years of age,\(^5\) almost consistent with the age range of menopause in women. In elderly women, BPPV is closely related to osteoporosis and osteopenia.\(^6\) Estradiol (E2) is essential for bone growth and for the development and maintenance of bone health in adulthood.\(^7,8\) Therefore, it is suggested that BPPV might be caused by decreased E2 secretion. However, limited clinical data have been reported so far. In an animal study conducted in 2008, Vibert et al. performed a morphometric analysis that clearly revealed that the otoconia in rats receiving bilateral ovariectomy (OVX) were larger and had lower density than those in the sham group.\(^9\) This result is a further corroborative of the hypothesis that the decrease of female sex hormone levels might be associated with the development of BPPV in postmenopausal women.

However, Vibert et al.’s study did not investigate otoconia structural proteins. Otoconia consist of biocrystals of calcium carbonate and otoconial proteins. Although there are few otoconial proteins, they are essential for the development of otoconia. Otoconin 90 (OC90) is known as the main protein in mammalian otoolith matrix and is considered an important factor for the maintenance of the normal morphology and growth of otoconia.\(^10–12\) In a previous investigation in OC90-null mice, otoconia were found to have loose morphology and were occasionally displaced into the semicircular canals.\(^13\) In our study, we tested the hypotheses that BPPV patient would have lower levels of E2, and that E2...
deficiency will reduce expression of OC90. We compared the changes in the levels of peripheral blood female sex hormones in postmenopausal women (age range: 50–80 years) with BPPV (BPPV group) and without BPPV (non-BPPV group). We eliminated the influence of causative factors, such as mechanical injury and other diseases related to BPPV incidence, as described in the Materials and Methods section. In addition, to determine the effect of female sex hormone levels on otoconial degeneration and detachment, we examined the expression of OC90 in rat models after ovary removal with and without hormone replacement therapy. The knowledge obtained on the risk factors for BPPV development is of important theoretical significance for further studies of the etiology and pathogenesis of BPPV.

MATERIALS AND METHODS

Patients and Clinical Study

A total of 228 patients presented at the Neurology Outpatient Clinic of Changzheng Hospital from September 1, 2015 to October 1, 2016 who were first diagnosed with idiopathic BPPV before the rats were randomly assigned to five groups. Then, a chemical luminescence method was used for data analysis. A value of \( P < 0.05 \) was considered statistically significant for all parameters.

RESULTS

Clinical Study

Baseline characteristics. To investigate the factors related to the risk of idiopathic BPPV in postmenopausal female patients with BPPV, we employed strict inclusion and exclusion criteria as described in detail in the Materials and Methods section. After screening, a total of 102 cases (52 in the control and 50 in the BPPV group) that met the diagnostic and inclusion criteria

Ovariectomy and Hormone Replacement Therapy

Twenty-five adult female Sprague-Dawley rats with a body weight of 200 to 250 g were housed in standard animal facilities. The animal protocol was approved by the Institutional Animal Care and Use Committee of the Second Military Medical University. Blood samples were drawn from the antecubital vein from 08:00 AM to 9:00 AM. Electrochemical luminescence was employed to detect E2, progesterone (P), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). A chemical luminescence method was used to detect 25-hydroxyvitamin D 25(OH)D3 by using the ADVIA Centaur XP Immunoassay System (Siemens Healthcare GmbH, Erlangen, Germany). The clinical protocol of this study was reviewed and approved by the ethics committee of Changzheng Hospital, Second Military Medical University, Shanghai, China. After fully explaining the procedure and the aims of the investigation, all subjects signed informed consent forms.

Western Blotting

Immunoblot analysis of OC90 was performed on the whole tissue of the inner ear. Each sample was ground in liquid nitrogen and homogenized in a lysis buffer containing 1% protease inhibitor. Protein concentrations were determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). Each blot was incubated with anti-otoconin-90 antibody (sc-376855, 1:1000; Santa Cruz Biotechnology, Dallas, TX) or a monoclonal anti-\( \beta \)-actin antibody. Each sample came from one rat with two cochleas. We repeated the Western blot and real-time reverse transcription-polymerase chain reaction (RT-PCR) experiments in five rats.

Quantitative RT-PCR for the Assessment of Messenger RNA

Total RNA was extracted from tissue using Rneasy MiNi Kit from Qiagen (Valencia, CA). Quantitative RT-PCR of the OC90 messenger RNA (mRNA) was performed using SYBR Premix Ex Taq TM II (TaKaRa, Dalian, China) through 40 PCR cycles (95°C for 10 seconds, 60°C for 25 seconds, and 72°C for 20 seconds). The primer sequences of OC90 were F: AATGGTTTTG-GATGTTGTCGCAA and R: GCACCATCATTTCCACGAGC.

Statistical Analysis

Statistical analysis of the results was performed using SPSS 16.0 for Windows (IBM, Armonk, NY). In the clinical analysis, quantitative data are presented as mean ± standard deviation, and the Student t test was used for comparison between the quantitative data of the two groups. Qualitative data were analyzed by \( \chi^2 \) test. At the 0.05 level, the data of animal studies were normally distributed, so the Student t test was used for data analysis. A value of \( P < 0.05 \) was considered statistically significant for all parameters.
TABLE I.
Baseline Parameters of the Control Subjects and BPPVPatients.

<table>
<thead>
<tr>
<th></th>
<th>BPPV Group, n = 50</th>
<th>Control Group, n = 52</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–60</td>
<td>19 (38%)</td>
<td>20 (38.5%)</td>
<td>.978</td>
</tr>
<tr>
<td>60–70</td>
<td>23 (46%)</td>
<td>23 (44.2%)</td>
<td></td>
</tr>
<tr>
<td>70–80</td>
<td>8 (16%)</td>
<td>9 (17.3%)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.62 ± 2.47</td>
<td>24.74 ± 12.7</td>
<td>.25</td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (54%)</td>
<td>29 (55.77%)</td>
<td>.858</td>
</tr>
<tr>
<td>Diabetes</td>
<td>18 (36%)</td>
<td>23 (44.23%)</td>
<td>.397</td>
</tr>
<tr>
<td>Dix–Hallpike</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP(+)</td>
<td>17 (34%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>RP(+)</td>
<td>21 (42%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Roll test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LH(+)</td>
<td>6 (12%)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>RH(+)</td>
<td>6 (12%)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean values ± standard deviation reported for the measurement data. Number (% ) are reported for the count data.

BMI = body mass index; BPPV = benign paroxysmal positional vertigo; LH = left horizontal semicircular canal; LP = left posterior semicircular canal; RH = right horizontal semicircular canal.

were included in the study. Of the 50 study patients, otocoria were dislocated into the left horizontal semicircular canal (LH-BPPV) in six (12%) patients, the right horizontal semicircular canal (RH-BPPV) in six (12%) patients, the left posterior semicircular canal (LP-BPPV) in 17 (34%) patients, and the right posterior semicircular canal (RP-BPPV) in 21 (42%) patients. There were no statistical difference in terms of age distribution, body mass index, and clinical history (P > .05) (Table I). Regression analysis revealed that age accounted for about 6% and 10% of the variability in E2 levels in the BPPV and control group, respectively. There was a statistically significant relationship between age and E2 values in the control group, but statistical significance was narrowly missed in the BPPV group.

**Decreased serum E2 and vitamin D levels in postmenopausal female patients with idiopathic BPPV.** The serum levels of the E2, P, FSH, LH, and 25(OH)D3 were measured in the studied subjects. We found that the level of E2 in the postmenopausal female patients with idiopathic BPPV was significantly lower than that in the control subjects (Table II) (P < .001). We also found the level of 25(OH)D3 in the postmenopausal female patients with idiopathic BPPV was significantly lower than that in the control subjects. However, there was no significant correlation between E2 and 25(OH)D3 by linear regression analysis.

**Animals Study**

**After female sex hormone replacement therapy, the levels of serum E2 and P were increased in OVX rats.** A postmenopausal model was induced by OVX, characterized by lower levels of E2 and P postmenopausal than the SHAM group. As expected, the administration of exogenous sex hormone treatment for 30 days resulted in a significant increase in the levels of E2 and P (Table III).

**E2 replacement therapy but not progesterone replacement can reverse the decrease of OC90 protein development after OVX.** In the present investigation, we examined the influence of sex hormone replacement therapy on the OC90 protein and mRNA levels in the inner ear of OVX rats using Western blot and RT-PCR. In the OVX group normalized by the SHAM group, the average percentages of OC90 protein and mRNA were 53.5% ± 5% and 43.7% ± 10.3%, respectively. Moreover, this reduction was reversed by 17β-estradiol (0.25 mL/kg, 10 μg/0.1 mL), but not by progesterone treatment (0.3 mL/kg/d, 10 mg/1 mL). No significant differences were found between the average percentages of OC90 protein in OVX + E2 (102.05% ± 3.58%) and OVX + E2 + P (112.05% ± 1.7%) groups, as well as between those of OC90 mRNA in OVX + E2 (89% ± 14.5%) and OVX + E2 + P (92.55% ± 11%) groups, and between OC90 protein and mRNA percentages in OVX and OVX + P groups (Fig. 1, n = 5, P < .05, compared to the SHAM group).

**DISCUSSION**

In our study, there were 150 female patients and 78 male patients diagnosed with BPPV, contributing to a ratio between males and females that was almost consistent with that of a previous report. The number of women aged 50 to 80 years was 118, including 50 female patients who met our criteria for inclusion in the trial group, with 21 RP-BPPV, 17 LP-BPPV, six RH-BPPV, and six LH-BPPV patients, which is also consistent with characteristics of the patients investigated in the earlier examination, indicating that posterior semicircular canal BPPV accounted for 78% to 93%, horizontal semicircular canal BPPV accounted for 1.9% to 16.4%, and anterior semicircular canal BPPV accounted for 1.2% to 3%. The pathogenesis of BPPV has remained unclear until now. Symptomatic maneuver therapy is effective, but the average recurrence rate in patients after its administration of exogenous sex hormone treatment for 30 days resulted in a significant increase in the levels of E2 and P (Table III).

**TABLE II.**

Serum Female Sex Hormone and 25(OH)D3 Levels in the Control Subjects and BPPV Patients.

<table>
<thead>
<tr>
<th></th>
<th>BPPV Group, n = 50</th>
<th>Control Group, n = 52</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2 (pg/mL)</td>
<td>17.33 ± 8.5</td>
<td>35.97 ± 17.6</td>
<td>&lt;.001*</td>
</tr>
<tr>
<td>P (ng/mL)</td>
<td>0.65 ± 0.60</td>
<td>0.56 ± 0.42</td>
<td>.41</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>27.77 ± 10.8</td>
<td>32.97 ± 18.6</td>
<td>.09</td>
</tr>
<tr>
<td>FSH (mIU/mL)</td>
<td>64.17 ± 21.12</td>
<td>61.95 ± 27.82</td>
<td>.65</td>
</tr>
<tr>
<td>25(OH)D3 (ng/mL)</td>
<td>23.13 ± 6.11</td>
<td>26.85 ± 5.92</td>
<td>.026†</td>
</tr>
</tbody>
</table>

Data are reported as mean values ± standard deviation for all parameters.

*BPPVs compared to controls when P < .001.
†BPPVs compared to controls when P < .05 (Student t test).
application is up to 50%, with a mean follow-up of 10 years. Thus, identifying the pathogenesis-related factors is particularly important. Our study was the first to provide clinical data indicating a possible association between female hormone-related factors and the development of BPPV in postmenopausal patients. Moreover, we further confirmed this relationship at the molecular level, evidencing that E2 is a key risk factor for BPPV in the postmenopausal period. Therefore, further research on effective measures for the prevention and treatment of BPPV is of critical significance.

The peak age for incidence of BPPV is consistent with the age range of menopause. E2 is involved in the prevention of bone loss by changing the natural regulators of bone mass and maintaining the production of osteoprotegerin. The decline of estrogen levels decreases the rate of bone turnover and consequently causes bone loss. One study observed morphologic changes and altered calcium content in otoconia in rats after simulated weightlessness, and demonstrated that the formation of bone and otoconia shared similar mechanisms. Another investigation showed that the prevalence of decreased bone mass density among BPPV subjects was 81%, and the prevalence of BPPV among osteopenia/osteoporosis subjects was 31%. An association between idiopathic BPPV and bone turnover disorders was also found in this examination by determination of serum markers of bone turnover. A morphometric analysis conducted earlier revealed that the otoconia in ovariectomized rats were larger and with a lower density than those in the SHAM group, suggesting that the decrease of female sex hormones is associated with the development and recurrence of BPPV. All of these studies indicate that the decline in estrogen function may be associated with BPPV occurrence. However, no direct clinical data supporting this implication are available to date.

In our study, we found that the level of E2 in postmenopausal women with BPPV was significantly lower than that of the controls. Furthermore, there was no significant difference in the levels of P, LH, and FSH.
between the BPPV and control group. Although we found the level of 25(OH)D3 in the postmenopausal female patients with idiopathic BPPV was significantly lower than that in the control subjects, the regression analysis tell us that there is no significant correlation between E2 and 25(OH)D3. We are in agreement with a previous study suggesting that vitamin D is a risk factor for BPPV, so we suppose vitamin D and E2 deficiency are two separate risk factors of BPPV in postmenopausal women.

To avoid the multitude of possible undesirable side effects that hormone therapy might cause in human patients, we conducted a further animal study to elucidate the effect of E2 on BPPV occurrence and to uncover the molecular etiology of BPPV. In the present study, middle-aged ovariectomized rats were used as a model of human menopause and received 30-day exogenous sex hormone treatment. As expected, E2 and P levels were significantly increased after the treatment, which enabled us to use the rats as good models to investigate the relationship between E2 levels and idiopathic BPPV occurrence.

It is generally accepted that OC90 is required for the development and growth of otocochlia. A study conducted by Zhao et al. showed that OC90 was essential for the formation of the otocochlia organic matrix that controls CaCO3 crystal growth and morphology, and OC90 recruited otolin to form the organic matrix of otocochlia. Furthermore, compared with wild-type mice, otocochlia in OC90-null mice were 20 to 50 times larger and looser, which might contribute to detachment. The investigation of Yang et al. revealed that OC90 interacts with both domains of otolin to form the otocochlia matrix framework, and this complex sequesters Ca2+ in the extracellular matrix for efficient calcification. All of these results suggest that OC90 deficiency would directly promote the dislocation of otocochlia. In our study, the expression levels of OC90 protein and mRNA were decreased after OVX, and E2 replacement therapy was able to reverse these changes. This result suggests that the deficiency of E2 might play an important role in otocochlia dislocation, which is consistent with the result of our clinical study. However, reduced expression of OC90 in rats is not equivalent to displacement of otocochlia into semicircular canals. It is meaningful and challenging to tie the current animal findings with the human findings. Recent reports suggested that otolin-1, an inner ear collagen, could be a serological biomarker for otocochlia degeneration/BPPV. Although OC90 decays rapidly in blood samples, it may be possible to explore serum levels of otolin-1, Sc1, keratan sulfate proteoglycans, and other minor otocochlia to diagnose and monitor otologic disorders.

CONCLUSION
There seems to be an association between the lower E2 levels and idiopathic BPPV occurrence in postmenopausal female patients. E2 deficiency can be considered a risk factor for BPPV in postmenopausal female patients.

Acknowledgments
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BIBLIOGRAPHY


