Oxidative status in patients with benign paroxysmal positional vertigo

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OBJECTIVE: Benign paroxysmal positional vertigo (BPPV) is the most frequently peripheral vestibular disorder, especially in older patients suffering from vertigo. The brief vertigo attacks and imbalance symptoms of BPPV are caused by freely floating otoconia within the semicircular canals. The aim of this prospective study was to evaluate the role of oxidative stress, using native thiol/disulfide (SH/SS) homeostasis as a novel indicator, in the etiology of BPPV.

MATERIALS and METHODS: The 62 participants in the study included 31 patients with BPPV and, as the control group, 31 healthy individuals without any cochleovestibular disorders.

RESULTS: Patients with BPPV initially had significantly lower native SH levels and significantly lower SH/total thiol (TT) ratios, as well as significantly higher SS/SH and SS/TT ratios than the control group. After successful treatment of their vertigo, confirmed based on the results of the second blood sample, BPPV patients still had lower SH levels, and SH/TT ratios, and significantly higher SS/SH and SS/TT ratios compared with the control group.

CONCLUSION: Our results suggest a role for oxidative stress in the development of BPPV, through both calcium metabolism and the direct toxic effects of free oxygen radicals, including the triggering of apoptosis.

KEYWORDS: Benign paroxysmal positional vertigo, oxidative stress, thiol, disulfide

INTRODUCTION
Benign paroxysmal positional vertigo (BPPV) is the most frequently peripheral vestibular disorder, especially in older patients suffering from vertigo (1). The brief vertigo attacks and imbalance symptomatic of BPPV are caused by otoconia freely floating within the semicircular canals (2). Vertigo occurs after specific head movements and has the characteristics of nystagmus, including with respect to latency time, fatigability and transiency. Canalithiasis (3) and cupulolithiasis (4) are the most probable mechanisms underlying BPPV. Although any of the three semicircular canals may be involved, canalithiasis of the posterior semicircular canal is the underlying cause in at least 85% of patients (3).

Oxidative stress, defined as excess production of reactive oxygen species (ROS) that is not counterbalanced by adequate endogenous and exogenous antioxidant defenses, causes cellular dysfunction and is a risk factor for microvascular injury (8). Several studies have shown an elevation of oxidative stress levels in different pathologies, with higher than control levels of biomarkers such as modified lipids, proteins, and nucleic acids, reductions in antioxidant capacity, and increased ROS production by leukocytes. In otolaryngology practice, the relationship between oxidative stress and laryngeal cancer, hearing loss, rhinosinusitis, otitis media, chronic tonsillitis, and other conditions has been investigated (7-10). In a recent study, it was revealed that calcium metabolism and its relationship with oxidative stress may play a role in the development of BPPV (7).

The aim of this prospective study was to evaluate the role of oxidative stress, using thiol/disulfide homeostasis as a novel indicator, in the etiology of BPPV.
MATERIALS and METHODS

Patients and controls
This prospective two-center study was conducted from June 2017 to July 2017 at the Department of Otolaryngology Head and Neck Surgery of the Umranie Training and Research Hospital, and at the Department of Otolaryngology Head and Neck Surgery of Icercenkoy Hospital, Bayındır Health Care group. The study was approved by the Ethics Committee of Umranie Training and Research Hospital (number 0.01-76). Written informed consent was obtained from all participants recruited to the study.

The 62 participants consisted of 31 patients with BPPV and, as the control group, 31 healthy individuals without any cochleovestibular disorders.

Patients with hearing loss, a history of otologic surgery, neurologic disorders, smoking, malignancy, autoimmune disorders, hypertension, endocrine disorders including diabetes mellitus, hypothyroidism, cardiovascular disease, a history of antiaggregant therapy, infectious disease, or other inflammatory condition were excluded.

Vestibular and acoustic evaluation
All participants underwent a complete otorhinolaryngologic examination, including a neuro-otologic examination. The otologic examination included otomicroscopy, to visualize the tympanic membrane for vesicles attributable to herpes zoster infection, and to determine the presence of chronic ear disease or retraction pockets with cholesteatoma. The Valsalva maneuver and pushing on the tragus cartilage (fistula test) were performed; whether either induced a vertigo attack, which would have suggested perilymphatic fistula, was evaluated. All participants underwent pure tone audiometry. The neurologic examination focused on gait, balance, and coordination. Gait and balance were assessed using Romberg’s sign and the Fukuda stepping test. The presence of cerebellar signs was evaluated to exclude central pathologies.

For the diagnosis of BPPV in patients whose symptoms worsened with sudden head movements, videonystagmography-assisted Dix-Halpike and supine-roll tests (Pagnini- McClure supine roll test) were performed. The characteristic pattern of BPPV nystagmus and the history of the disease were recorded for all patients in the study group. Only those with posterior canal BPPV were included. Patients in the study group had geotropic, torsional nystagmus beating towards the undermost ear. The nystagmus duration was < 60 s, with a latency of a few seconds and a decline in response upon repetitive maneuvers. Epley’s repositioning maneuver was performed in the treatment of the BPPV patients. The Dix-Halpike maneuver was repeated for all BPPV patients 2 days after the diagnosis of posterior canal BPPV; Epley’s repositioning maneuver was repeated if the patient complained of vertigo, nausea, or vomiting, or if nystagmus was determined during the Dix-Halpike test. The latter test was performed in control individuals 21 days after first hospital admission.

Blood sample collection
Peripheral venous blood samples were obtained from the BPPV patients upon first hospital admission (during the vertigo attack) and 21 days after treatment. Blood samples were obtained from the healthy controls during routine medical examination. Plain tubes were used to collect blood from the patients and controls. The samples were centrifuged for 10 min at 1,500 g, after which the serum was separated and stored at −80 °C until analysis. Serum native thiol (SH), total thiol (TT), and disulfide (SS) levels were analyzed in these samples, to collect blood from the patients and controls. The samples were centrifuged for 10 min at 1,500 g, after which the serum was separated and stored at −80 °C until analysis. Serum native thiol (SH), total thiol (TT), and disulfide (SS) levels were analyzed in these samples, and the SS/SH, SS/TT, and SH/TT ratios were calculated according to the method of Erel and Neselioglu [11].

Statistical analysis
The NCSS 2007 program (NCSS, Kaysville, Utah, USA) was used for statistical evaluation of the data. Mean, standard deviation, median, minimum, maximum, frequency, and percentage values served as descriptive statistics. Normally distributed data were compared between groups with the independent samples t test. The paired samples t test was used for within-group analysis of normally distributed data, and the Pearson chi-squared test was applied to evaluate qualitative data. A P value < 0.05 was considered to indicate statistical significance. The 95% confidence intervals were also determined.

RESULTS
There was no statistically significant difference between the study and control group with respect to mean age (59.65 ± 10.97 and 58.35 ± 4.75 years, respectively) or the male/female ratio (14:17 and 14:17, respectively) (Table 1). The demographics of the BPPV patients and healthy controls are shown in Table 1.

However, the differences between the oxidative parameters of the BPPV and control groups were statistically significant. At baseline, patients with BPPV had significantly lower SH levels and SH/TT ratios, and significantly higher SS/SH and SS/TT ratios, than the control group. After treatment for vertigo, the BPPV patients still had lower SH levels and SH/TT ratios, and significantly higher SS/SH and SS/TT ratios, than the control group (Figures 1 and 2, Table 2).

DISCUSSION
To the best of our knowledge, this is the first study to investigate SH/ SS homeostasis as a novel marker of oxidative stress in patients with BPPV. Specifically, we examined SH/SS homeostasis in patients with BPPV and a control group of healthy individuals. Our study showed a significant difference between the groups.

Free oxygen radicals are naturally generated during every reaction in the body. Normally, these unstable electron-laden chemicals are largely destroyed or removed by the body’s natural antioxidant defense systems. Oxidative stress occurs due to an inadequate response to the formation of free radicals. Among the major non-enzymatic
antioxidants able to eliminate oxidative stress in the cell are sulfhydryl groups (-SH) containing SHs [11]. Circulating blood albumin binds SH (sulfhydryl) groups via albumin cysteine residues. Reversible disulfide bonds form with the cysteine residues located at the active sites of the protein, thereby reducing the toxicity of ROS [12-13]. TT levels in the cell remain constant to ensure continuous SH/SS homeostasis, reflecting the turnover between SSs and SHs [11].

A global plasma oxidative stress index was recently developed and validated in several diseases. Oxidative stress is related to cardiovascular diseases and their risk factors, such as diabetes, hypertension, and obesity, all of which are highly prevalent in numerous countries throughout the world. A relationship between a decline in thiol levels and several systemic diseases has been demonstrated in many studies [14-15]. While SH/SS homeostasis could previously only be measured by measuring the levels of the individual components, Erel and Neselioglu [11] described a new method that allows for measurement of the levels of these compounds both individually and cumulatively.

Episodes of dizziness are common in the elderly and significantly increase the risk of falls. The incidence of BPPV increases with age. Peripheral vestibular dysfunction, including BPPV, is one of the most common causes of dizziness in the elderly and one of the most frequent diseases seen in dizziness clinics. The results of our study indicated that oxidative stress may be one of the etiologic factor in the development of BPPV. However, in this study, while SH and TH levels were significantly lower in BPPV patients than in the control group, SS levels did not differ significantly between the groups. Patients with BPPV were treated with Epley’s repositioning maneuver and then followed-up (outpatient visits) until nystagmus was resolved. SH and TH levels increased after treatment, but not statistically significantly. The SS/SH, SS/TT, and SH/TT ratios, which represent corrected values and predominantly indicate oxidative homeostasis, differed significantly between the control and BPPV groups: the BPPV patients had significantly higher SS/SH and SS/TT ratios and lower SH/TT ratios. These findings are consistent with an increase in oxidative stress in BPPV patients. However, there was no significant difference in oxidative stress parameters in the BPPV patients before versus after treatment. Although oxidative stress may play a role in the development of BPPV, the increase of oxidative stress did not respond to the treatments that we administered to our patients. Gucluturk et al. [16] studied the levels of the antioxidant paraoxonase in BPPV patients before and after treatment and reported results similar to ours.

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Figure 1. Native Thiol Levels

Figure 2. Total Thiol Levels

Table 2. Serum native thiol, disulphide and total thiol levels

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>BPPV group (At admission)</th>
<th>BPPV group (after treatment)</th>
<th>p1</th>
<th>p2</th>
<th>p1^2</th>
<th>p2^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SH Min-Max</td>
<td>280.30-454.80</td>
<td>128.60-395.30</td>
<td>218.90-394.20</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>375.95±38.95</td>
<td>305.22±59.70</td>
<td>308.60±45.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT Min-Max</td>
<td>329.80-514.50</td>
<td>162.27-456.51</td>
<td>248.68-451.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean±SD</td>
<td>417.98±40.92</td>
<td>347.03±66.03</td>
<td>352.88±47.90</td>
<td></td>
<td></td>
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<tr>
<td>SS Min-Max</td>
<td>15.70-34.45</td>
<td>1.10-38.71</td>
<td>7.97-36.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean±SD</td>
<td>21.01±4.87</td>
<td>20.91±9.77</td>
<td>22.14±7.96</td>
<td>0.956</td>
<td>0.504</td>
<td>0.545</td>
<td></td>
</tr>
<tr>
<td>SS/SH % Min-Max</td>
<td>4.02-10.78</td>
<td>0.45-18.97</td>
<td>3.52-22.32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>5.76±1.72</td>
<td>10.23±4.81</td>
<td>10.67±4.45</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>0.671</td>
<td></td>
</tr>
<tr>
<td>SS/TT % Min-Max</td>
<td>3.72-8.87</td>
<td>0.44-13.75</td>
<td>3.29-15.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>5.12±1.32</td>
<td>8.23±3.42</td>
<td>8.59±2.91</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>0.630</td>
<td></td>
</tr>
<tr>
<td>SH/TT % Min-Max</td>
<td>82.26-92.56</td>
<td>72.49-99.11</td>
<td>69.14-93.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>89.75±2.65</td>
<td>83.54±6.84</td>
<td>82.83±5.83</td>
<td>&lt;0.001**</td>
<td>&lt;0.001**</td>
<td>0.630</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.01

SD: Standard deviation; SH: Native Thiol; TT: Total Thiol; SS: Disulphide
In the semicircular canals, the presence of otoconial debris originating from the utricle and saccule causes BPPV when the head position is changed quickly and suddenly. Otoconia consist of calcium, a mixture of calcium and carbonates. The structure of otoconia differs from that of the teeth and bones because of its carbonate, rather than phosphate, composition. In the inner ear, calcium and carbonate levels are under the control of the calcium channel transport system. Normal levels of calcium and carbonate are important for the maintenance of otoconial function. Vibert et al. designed an osteoporosis model in ovariectomized rats and studied the ultrastructure of their otoconia. The otoconia of these rats were less dense and contained less calcium; similar mechanisms may underlie the pathogenesis of BPPV. Talaat et al. found that patients with recurrent or non-recurrent BPPV had significantly lower levels of vitamin D than a control group, while the patients with recurrent BPPV had significantly lower vitamin D levels than those with non-recurrent BPPV. In another study, Talaat et al. found an association between the recovery of serum 25-hydroxyvitamin D3 levels and a significant reduction in the rate of BPPV relapse.

Oxidative stress is related to calcium metabolism, with the endoplasmic reticulum being the most important cellular site for calcium storage and protein folding. In the presence of cell stress, the endoplasmic reticulum may initiate an increase in cellular calcium levels, causing rupture of the mitochondrial membrane and apoptosis. Recent studies suggested that oxidative stress and inner ear diseases are related. Brosel et al. reported a strong link between oxidative stress, the related apoptosis of cochlear cells, and age-related hearing loss. Dinc et al. found significant differences in SH/SS homeostasis between patients with sudden sensorineural hearing loss and a control group. Tsai et al. reported increased levels of oxidative stress markers in blood samples from patients with BPPV.

Iwasaki et al. showed a correlation between an age-related decrease in vestibular function and age-related decline in vestibular hair cells and neurons. The underlying mechanism of age-related cell loss in the vestibular end-organ is not known but the cumulative effect of a genetic predisposition and oxidative stress may play an important role. The authors recommended further studies on the protective effect of antioxidant therapies with respect to vestibular function during aging.

These studies, together with the present results, indicate a role for oxidative stress in the development of BPPV, through both calcium metabolism and the direct toxic effects of free oxygen radicals, which trigger apoptosis. These mechanisms may also have a synergistic effect.

CONCLUSION
Our study was conducted on a small patient group. Further studies with larger samples are needed to evaluate and compare other oxidative stress markers, such as paraoxonase and arylesterase, as well as the total antioxidant status in BPPV patients.

REFERENCES

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