INTRODUCTION

Tinnitus can be defined as an auditory illusion or a phantom sensation of sound in the absence of external stimulation, i.e., the perception of a sound in the absence of any objective physical sound source (ANSI, 1969). In fact, tinnitus is a symptom that may cause decreased quality of life, somatization disorders, or depression. Regardless of the source of tinnitus, it is perceived in the auditory cortex [1]. The incidence of tinnitus has been reported to be 10–15% in adult population all over the world.

Various attempts have been made to understand the mechanism of tinnitus, especially as related to cochlear function. Spontaneous OAEs have frequently been reported in studies related to tinnitus. Many studies have shown that TEOAE and DPOAE tests can be used as methods to detect early hearing loss (HL), without it being seen in an audiogram [2, 3]. Reported OAE-results related to tinnitus were contradictory. Although lower TEOAE or DPOAE amplitudes in subjects with tinnitus was reported in some studies [3, 4], some others reported increased amplitudes of TEOAEs or DPOAEs in subjects with tinnitus [5, 6]. It could be concluded that OAE finding related to tinnitus demonstrate altered OHC functions.

Another test that used to evaluate cochlear functions especially inner hair cells function in tinnitus is Threshold equalizing noise (TEN) tests [7]. Similar to OAE, there are also contradictory results for TEN tests. Although Weisz et al. (2006) reported that there were cochlear dead region (DR) in 8 tinnitus subjects out of 11 (72.7%) [8], Thabet et al. (2009) found DR in only 3 of 20 (15%) subjects [9]. Buzo & Carvallo (2014) reported significantly increased thresholds in TEN in tinnitus patients with normal hearing even if there was no DR [10].

Although it is difficult to determine the pathophysiology of tinnitus, it is important to test the peripheral hearing correctly in order to decide on the treatment approach. As a result our
hypothesis was that the TEN and OAE test results of tinnitus patients with normal hearing would be different from the control group. Therefore, the aim of the current study was to evaluate cochlear functions in tinnitus patient with or without hearing loss (HL). In this study, while OAE was used to evaluate outer hair cell function OAE, TEN test was used to evaluate inner hair cells activity in the cochlear region.

METHODS
The study was approved by Hacettepe University Clinical Research Ethics Committee (KA-180134/ Date: 10.01.2019). Informed consents were given to all subjects. This study is based on first author’s master dissertation.

Subjects
Subjects with at least six-month of tinnitus at least one ear were included in the study. The subjects were divided into two study groups according to the presence of HL. The first study group (SG-I) included subjects with tinnitus accompanying sensorineural HL, and the second study group (SG-II) included subjects with tinnitus with normal-hearing thresholds. Normal-hearing subjects without tinnitus were included as the control group. The age range of the subjects was 18 to 45 years. Retro-cochlear pathology, neurologic problems, psychological problems, temporomandibular joint problems, pulsatile tinnitus, or conductive HL were regarded as exclusion criteria. Tinnitus Handicap of Inventory was used to decide tinnitus discomfort levels and define subjects’ profiles.

Audiological Evaluation
A detailed anamnnesis was obtained from all subjects. Air-conduction thresholds were measured between 125 and 12,000 Hz through TDH-39 headphones and bone-conduction thresholds between 500 and 4000 Hz through B71 bone vibrator with the Grason Stadler (GSI) Audiostar. Warble tone stimulus was used for testing. ANSI (1969) standards were used to classified the degrees of HL

Evaluation of Tinnitus
The subject’s history was used to determine tinnitus localization. To define frequency and pitch of tinnitus, the method proposed by Vernon was used [11]. In this method, two sounds of different frequencies are presented to the patient (f1 and f2, f1<f2) and the patient is asked which of these two sounds is closer to the tinnitus. In loudness matching, the patient is presented with the sound in the determined tinnitus frequency range and by asking the patient whether it is louder or softer, this range is narrowed and the closest loudness level is matched. Accordingly, while evaluation begins with the worse ear in cases of bilateral tinnitus; when tinnitus is perceived equally in both ears, then the left ear was the first ear to evaluate [12]. In case of unilateral tinnitus, just one ear was tested. If the subject defines tinnitus as perceived in side of head, these cases accepted as bilateral tinnitus. The Turkish version of the Tinnitus Handicap Inventory (THI) was used to determine subjects discomfort level. The THI includes 25 items with three answers. The discomfort level of tinnitus is determined according to the total score.

Threshold Equalizing Noise Test
For all subjects, the TEN was applied at 500, 1000, 2000, and 4000 Hz frequencies using a Grason Stadler (GSI) Audio Star Pro audiometer device and TDH39 headphones. In subjects with bilateral tinnitus, TEN were conducted in both ears. In case of unilateral tinnitus, only affected ear was tested.

TEN levels were determined based on the hearing thresholds of the subjects, as described by Brian Moore et al (2000) [7]. When hearing thresholds were better than 25 dB, in order to prevent false positive responses, the TEN was set at 50 dB; when hearing thresholds were between 25 and 60 dB, the TEN was 70 dB, and for thresholds worse than 60 dB, the TEN was set 10 dB higher than the threshold. The TEN should adjusted maximum 90 dB, due to cochlear sensitivity [13]. Additionally, researchers proposed that TEN could performed at different severity levels [8, 9].

Otoacoustic Emission Tests
TEOAE and DPOAEs were measured for all subjects in the study using 26.41.0 version number Autodynamics EZ Screen. TEOAEs were measured in ears with tinnitus in quick screen mode using a 260 sweep at 84 dB SPL at 80 msec. TEOAEs from each stimulus were recorded at 1000 Hz, 1414 Hz, 2000 Hz, 2828 Hz, 4000 Hz and both the signal-to-noise ratios (SNR) and the amplitudes of the response were recorded. For DPOAEs, amplitudes and SNRs recorded responses generated at 1001, 2002, 3003, 4004, 6006, 7996 Hz frequencies (2f1-f2) ratio and L1/L2-65/55 dB SPL intensity levels;) with average of 500 sweeps.

Statistical Analysis
Statistical analyses were carried out using the Windows-based SPSS version 23.00 package program. The obtained results were analyzed by visual (histogram and scatter charts) and statistical (Kolmogorov Smirnov-Shapiro Wilks) methods. Descriptive analysis was conducted and median and interquartile ranges were used for non-normally distributed variables; the mean (X) and standard deviation (SD) were used for this. A median and interquartile range were used in the analysis along with Kruskal-Wallis test. A Bonferroni test was used for double post-hoc comparisons. In the comparisons between the two groups, an independent sample t test was used when the data showed normal distribution. values below 0.05 were considered significant.
RESULTS
Demographic Data
In the study groups, there were 31 subjects in total; 16 (7F, 9M) with tinnitus and HL in SG-I and 15 (6F, 9M) with only tinnitus in SG-II. In CG, there were 21 (12F, 9M) subjects with neither tinnitus nor hearing loss. The mean age for SG-I was 37.2 and in for SG-II was 28.73; the mean age for CG was 24.42. There was no age difference between the male and female (p>0.05). (see Table 1).

Tinnitus discomfort level on the THI showed variations from 1 to 5 for both SG-I and SG-II. There was no significant difference between the THI levels of the two groups (p: 0.14) (p> 0.05). In SG-II tinnitus frequencies showed variations, they mostly focus on high frequencies (4-12 kHz). In the SG-I group, tinnitus was generally detected at frequencies with hearing loss.

The HL configurations were sloping in 11 participants, flat in 4 participants, and 6 kHz notch in 1 participant. According to Pure Tone Averages (PTA), moderately severe HL was detected in 5 individual, moderate HL in 4 individual, and mild HL in 3 individuals. While PTAs of other individuals were within normal limits, SNHL was present at high frequencies. (See Figure 1). When the patient’s anamnesis was examined, there was no chronic disease and none of the hearing losses were congenital.

TEN Test Results
In the current study, the accepted criteria for a DR was that the masked threshold should be at least 10 dB worse than the unmasked threshold. Threshold changes in the presence of TEN at all frequencies were analyzed. In SG-II and CG, there were no subjects DRs. In contrast and as expected in SG-I, there were two subjects with DRs. One of the subjects (#32) was a 39-year-old with sloping bilateral SN hearing loss and tinnitus. All hearing threshold were increased with TEN and DR was detected at 4 kHz in the right ear. The other subject (#56) was 25-year-old with bilateral flat, moderately severe SN hearing loss and tinnitus. In this subject, DRs were observed bilaterally at 0.5 kHz, 1 kHz and 4 kHz.

Unlike the control group, it was observed that the all thresholds in study group increased with TEN. The difference was significantly different between groups at all frequencies (see Table 2). Threshold increases in SG-I were significantly different from thresholds in the control group and SG-II. When SG-II was compared with CG, there were significant differences at 0.5 kHz (p<0.05); however, there was no significant difference at other frequencies (p>0.05) (see Table 2).

OAE Tests Results
For TEOAE and DPAOE tests, SNR and emission response amplitudes were evaluated. Because the subjects in the SG-I had hearing loss, either minimum OAE amplitudes were observed or no OAE was observed. Only 5 participants in this group responded in OAE tests with amplitudes ranging from -12.5 to 8.6 dB. Therefore, only SG-II and CG were compared.

There was no significant difference between SG-II and CG in relation to SNR and amplitudes at 1000Hz, 1414Hz, 2228Hz, and 4000Hz. When compared the SNRs in DPOAE test, similarly with TEOAE, there was no significant difference between SG-II and CG at 1, 1.5, 2, 3, 4, 6, and 8 kHz. However, emission response amplitudes at 6 kHz and 8 kHz were significantly decreased in SG-II (respectively p: 0.030; p: 0.015 p<0.05).

Table 1. Profiles of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Number of Subjects</th>
<th>Mean Age</th>
<th>Localization of Tinnitus</th>
<th>Hearing Loss</th>
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<td></td>
<td></td>
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<td>Unilateral</td>
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</tr>
<tr>
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</tr>
<tr>
<td><strong>Control Group</strong></td>
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<td>24.42</td>
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<td>–</td>
</tr>
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</table>

F: Female, M: Male
DISCUSSION

It is widely accepted that severe damage to the auditory pathways can cause tinnitus-related hearing loss [14]. As it is known, the destructive effects that cause hearing loss and tinnitus first damage OHC [15]. However, evaluation with conventional audiometer does not provide the opportunity to determine whether the damage is OHC or IHC. Therefore, in this study with HL and tinnitus patients who had normal thresholds on the audiogram were evaluated with tests evaluating OHC and IHC functions. In this way, the widely accepted theory was tested.

TEN Test: Dead Regions and Elevated Thresholds

In the current study it was hypothesized that tinnitus patient with HL (SG-I) would have increased hearing thresholds in the TEN when compared to tinnitus patient with normal-hearing (SG-II). The TEN test results of SG-I were significantly different from SG-II and CG. The TEN showed that hearing thresholds were increased in all subjects with tinnitus in SG-I. Additionally, in SG-I, there were two subjects who had DRs. Especially in SG-I, OAE was absent or minimally observed. The sensorineural-based tinnitus model explains tinnitus as damage to hair cells, degenerated spiral ganglia, and decreased outputs on the frequency-specific nerve endings of the auditory nerve [16]. This damage to SNHL may have occurred in both OHC and IHC. For example, if the total hearing loss at a given frequency is 60 dB, 40 dB of that loss might be due to OHC damage and 20 dB to IHC damage [7]. Absence or minimal presence of OAEs in SG-I indicates a decrease or loss of function of OHC at the tested frequency, while an increase in TEN thresholds indicates loss of function of IHC. In the literature, only hearing loss due to OHC damage is stated to be no more than about 50 dB at low frequencies and 65 dB at high frequencies [15,17]. However, even though the HL levels in this group were lower than the specified levels, the increase in the thresholds in the TEN test made us think that the damage may have reached the IHC even if there was no DR.

Also, when while taking subjects’ history in SG-I, they reported that their first compliant with hearing loss and then they suffer from tinnitus appeared. It is known that auditory deprivation could cause hyperactivity in nerve fibers and auditory nuclei and, as a result, the plasticity in the central auditory level increases. Input deprivation in the nervous system is the strongest factor that can activate neural plasticity [18]. In cases of auditory plasticity, changes can alter the processing of sounds and cause hyperactivity that can promote tinnitus and hyperacusis in the CNS. In particular, cochlear degeneration, which eliminates the neural output of the cochlea, can induce or intensify the phantom sound perception of tinnitus [14].

In the TEN test results of the tinnitus patients with normal hearing, significantly lower thresholds were found at all frequencies except 500 Hz compared to the tinnitus patients with HL; compared to the control group, a higher threshold was obtained at all frequencies and a significant difference was obtained only at 500 Hz. Conflicting results have been obtained.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Frequency (kHz)</th>
<th>Min. (dB)</th>
<th>Max (dB)</th>
<th>Mean (dB)</th>
<th>Median (dB)</th>
<th>IQR</th>
<th>P values</th>
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<td>0.5</td>
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<td>5.0</td>
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</tbody>
</table>

kHz: Kilohertz, IQR: Inter Quantile Range, SG-I: Study group 1, SG-II: Study group 2, CG: Control group, N: Number of tested ears, Min: Minimum, Max: Maximum *: p<0.05
in studies in the literature [8-10, 19]. Moore et al. (2000) stated that during the TEN tests, if there is a 10 dB or more increase in hearing thresholds in the presence of noise, it is accepted that there is a “dead region” at that frequency [7]. However, if this increase in threshold is less than 10 dB, there may be a degeneration in that frequency region; even if there is no specific DR, there may be a disruption in the central pathways of hearing or auditory neuropathy [7, 20]. In the current study, even though there was no detected DR in normal-hearing subjects with tinnitus, an increase in hearing thresholds presence of TEN could be accepted as sign of degeneration in the cochlea and may also be seen as the early sign of hidden hearing loss. The elevation of thresholds in presence of TEN was explained as poor processing efficiency either caused by a reduction of neural synchrony or by synaptopathy in literature [21]. In order for IHCs to be damaged, stronger disrupting factors are needed than OHCs. It was reported that even if up to 80% of IHC is damaged, in very rare cases, audiometric thresholds may not be affected [22]. In the literature on hidden hearing loss, there are some studies that have found that a DR could be observed in normal-hearing subjects with tinnitus [8]. Obtaining different results in studies may be related to the variability of the underlying etiologies of tinnitus.

OAEs
In addition to TEN, OAE also were used to evaluate use of cochlear function, especially in OHC. OAEs in the normal-hearing subjects with tinnitus (SG-II) were slightly different from control group. TEOAE amplitudes and SNRs were similar in both groups. However, DPOAE results showed significant decreases in emission amplitudes at 6 and 8 kHz in normal-hearing subjects with tinnitus. Fabijanska et al. reported significantly reduced amplitudes in DPOAE in normal-hearing subjects with tinnitus [3]. It is known that destructive effects of noise, ototoxic effects, metabolic imbalances on the hair cells starting from the basal region of the cochlea [23]. Degeneration on the basal region result in decrement in OAE amplitudes. The decrease in input in this region may have triggered the tinnitus. However, TEN test was limited to 4000Hz and so there is no chance to test this statement. Our results could be a guide for future studies.

Tinnitus Handicap Inventory
THI was applied to all subjects with tinnitus in the study group to determine and evaluate the tinnitus discomfort level. THI evaluates the perceived effect of tinnitus on a subject’s life quality. Ratnayake S et al. (2009) reported that THI was affected by hearing loss and thus, hearing-loss subjects with tinnitus had higher scores [24]. However, Newman CW et al. (1996) reported no significant difference on THI scores between normal-hearing subjects and subjects with hearing loss [25]. Similar to Newman et al’s results, there was no significant difference in THI scores between the study groups in the current study.

Limitations
To determine audiometric thresholds, TDH-39 headphones were used and only thresholds up to 12 kHz were measured. Not including higher frequencies is the main limitations of the study.

CONCLUSION
In conclusion, when tinnitus occurs with hearing loss, both the TEN and OAE produce different results, supporting the theory of hair cell degeneration in the cochlea in both IHC and OHC. Evaluations with conventional audiometers are insufficient to evaluate tinnitus individuals with normal hearing; high frequency thresholds should be tested and TEN and similar tests should be used for IHC functions.

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Conflict of Interest: The authors have no conflict of interest to declare.

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Author Contributions: Concept – MC, BCÇ; Design – MC, BCÇ; Supervision – BCÇ, MDB; Resources – MDB; Materials – MC, MDB; Data Collection and/or Processing – MC; Analysis and/or Interpretation – BCÇ; Literature Search – MC; Writing Manuscript – MC, BCÇ, MDB; Critical Review – BCÇ, MDB.

Ethics Committee Approval: The study was approved by Hacettepe University Clinical Research Ethics Committee (KA-180134/ Date: 10.01.2019). Informed consents were given to all the subjects. This study is based on first author's master dissertation.

REFERENCES


