The Characteristics of Audioscan and DPOAE Measures in Tinnitus Patients with Normal Hearing Thresholds

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[Abstract]

Objectives: to investigate auditory dysfunction in patients with tinnitus and normal hearing thresholds using two sensitive audiological measures.

Design: The study was designed to investigate the characteristics of Audioscan and DPOAE tests in tinnitus patients with normal hearing thresholds. Audioscan and DPOAE notches were analysed and compared. All tests were performed in a sound-treated chamber or in a sound-treated room. *Study sample:* 45 tinnitus patients with normal hearing thresholds were examined following a written clinical protocol. *Results:* The averaged hearing levels obtained from tinnitus participants were significantly worse at high frequencies than those derived from the normative data. There was a significantly higher prevalence of Audioscan and DPOAE notches, whose central frequencies matched tinnitus frequencies in the mid frequency regions, but not in the low and high frequency regions. A significant correlation was found between the centre frequencies of the Audioscan notches and the DPOAE notches from 500 to 4,000 Hz. *Conclusion:* Tinnitus in different frequency regions may be associated with different underlying mechanisms of tinnitus generation. Some negative results on the Audioscan and DPOAE notches matching tinnitus pitches may be due to a limited set of discrete frequencies used for the tinnitus pitch matching test.

Key Words: Audioscan, DPOAE, Notches, Tinnitus, Normal Hearing Thresholds

[Introduction]

Tinnitus is defined as a perception of sound from activity within the nervous system without any corresponding mechanical or vibratory activity within the cochlea and unrelated to external stimulation of any kind (Jastreboff & Hazell, 2004). Although it is regarded as a common phenomenon because almost all adults experience some form of temporary tinnitus at some point in their life, persistent tinnitus symptoms affect about 10-17% of adults in the United States and Europe. Of these, 2 – 7 % seek medical advice and treatment (Davis & El Refaie, 2000).

In patients attending an ENT/Audiology clinic for tinnitus some 10% to 15% are found, on pure-tone audiometry, to have hearing thresholds within normal limits (Schaette & McAlpine, 2011). Whilst relatively rare in comparison to tinnitus patients that do show abnormal audiometric findings, the absence of abnormal audiometric findings in this group leaves the clinician with a diagnostic dilemma and difficulty in patient management.

Various hypotheses have attempted to explain the pathophysiological mechanisms present in tinnitus subjects showing a normal pure tone audiogram. One suggestion is that a normal hearing threshold on pure tone audiogram does not prove normal auditory function (e.g., König *et al*, 2006; Sirimanna *et al*, 1996; Weisz *et al*, 2006; Zhao & Stephens, 1998). Other hypotheses include minor cochlear dysfunction or abnormality beyond cochlear level (e.g., Shiomi *et al*, 1997; Thabet, 2009; Zhao F *et al*, 2002). For example, Jastreboff's theory of discordant dysfunction of outer hair cells (OHCs) and inner hair cells (IHCs), indicates that a different level of dysfunction, where damage to OHCs is greater than IHCs is a possible peripheral mechanisms that can trigger tinnitus

(Jestreboff, 1990). In addition, when the OHCs or/and IHCs are damaged, such sensory deafferentation induces changes in the auditory nervous system, and subsequently generates the perception of tinnitus (Schaette & McAlpine, 2011; Weisz *et al*, 2006).

TEOAEs and DPOAEs have been used widely to test the hypothesis that OHC damage is responsible for tinnitus generation. Evidence demonstrates lower amplitudes of TEOAEs or/and DPOAEs in tinnitus patients with normal hearing thresholds than in non-tinnitus controls (e.g., Sirimanna *et al*, 1996; Ozimek *et al*, 2006; Ami *et al*, 2008; Paglialonga *et al*, 2010). Furthermore, Weisz et al. (2006) investigated IHC function in 11 chronic tinnitus patients with normal hearing thresholds. Using 'The Threshold Equalising Noise (TEN) test', which holds deafferentation as the underlying trigger of central reorganisation leading to phantom sound perception, they argued for the presence of altered IHCs and/or neuron activity in tinnitus patients with normal audiometric thresholds. This view is confirmed by Thabet (2009) who found a significantly higher percentage of abnormal TEOAE (85%) in tinnitus ears with normal hearing thresholds than in control ears (20%). Only three tinnitus ears showed abnormal inner ear function. These results suggest that tinnitus patients with normal hearing thresholds may have subtle damage to their OHCs, which is in line with Jastreboff's theory of discordant dysfunction of OHCs and IHCs. Also the findings support the view that normal hearing thresholds in patients with tinnitus do not necessarily indicate the absence of the deafferentation effect on the central auditory system leading to tinnitus.

In the present study, we employ two sensitive audiological measures, the Audioscan technique and DPOAE test to investigate mild auditory dysfunction in patients with tinnitus and normal hearing thresholds. The Audioscan is a form of high definition audiometry based on iso-hearing level frequency sweeps, which was developed by Meyer-Bisch (1996). Compared with traditional pure tone audiometry, it sweeps across the preset frequency range at a predetermined sweep rate and provides a continuous audiometric curve. As the device has a maximum frequency range of 125 Hz to 16,000 Hz with 64 frequencies per octave, the Audioscan offers accuracy and sensitivity. Another advantage is its capacity to detect mild audiometric deficits such as narrow notches situated between the frequencies normally tested. These could represent auditory lesions at a stage when they cannot be detected by routine audiological methods. The Audioscan method offers a detailed audiometric curve as well as indicators of mild auditory dysfunction. DPOAE testing is also used as an objective measure and can be compared with Audioscan findings to explore whether changes in the Audioscan relate to pathophysiological changes. Both tests could provide evidence for the clinical application of these techniques to the investigation of tinnitus patients with normal hearing thresholds.

MATERIALS AND METHODS

- ***Participants***

Audioscan and DPOAE tests were carried out on a total of 45 tinnitus patients with normal hearing thresholds seen at the Welsh Hearing Institute. Patients were selected based on the following criteria:

(1) they had sought clinical help for their problems with tinnitus;

(2) they had audiometrically " normal " hearing thresholds, defined as ≤ 20 dB HL at each octave frequency from 500 to 4,000 Hz in the poorer ear, with no hearing level at any frequency between 250 and 8,000 Hz exceeding 30 dB HL;

(3) they had no obvious cause, such as central nerve system (CNS) pathology or ototoxic drug intake for their problem. In accordance with departmental protocol, all tinnitus patients with normal hearing thresholds have an ABR to rule out possible CNS pathology. If abnormal ABR results were found or other findings that suggested retrocochlear pathology (e.g., absence of acoustic reflexes, typical symptoms for acoustic neuroma), then they were referred for Magnetic Resonance Imaging (MRI);

(4) otological examination and admittance testing showed no sign of conductive pathology.

The mean age of participants was 38.6 years (SD: 10.5), range 20 to 50 years. There were 21 males and 24 females.

- ***Procedures***

All participants followed a written clinical protocol, comprising a hearing problem questionnaire, otoscopic examination, pure tone audiometry, admittance test, tinnitus match test, Audioscan and distortion product otoacoustic emission (DPOAE) test. All tests were performed in a sound-treated chamber, and the DPOAE test was carried out in a sound-treated room.

1. Threshold determination

Pure tone hearing thresholds were measured for both ears at frequencies 250, 500, 1,000, 2,000, 3,000 4,000, 6,000 and 8,000 Hz, using a calibrated Kamplex AC 4 audiometer with TDH 39P headphones. The procedure used was that recommended by the British Society of Audiology.

2. Tinnitus pitch matching test

The procedure for tinnitus pitch matching testing was adapted from the study of Pan *et al*.

(2009). Tinnitus pitch matching tones were presented ipsilaterally to the ear with predominant or louder tinnitus if there was a difference between the two sides. If the tinnitus was equally loud on both sides or was localized in the head, the matching tones were given to the ear with the better hearing. Otherwise, the ear was chosen randomly if there was no difference between the acuity of the two ears.

During the tinnitus pitch matching measurements, clear instruction was given to the patients to:-

a) make a clear distinction between the tinnitus pitch perception and presented matching tones.

b) report verbally whether the matching tone needed to go higher or lower until the exact matching tone or a close approximation of their tinnitus was obtained.

c) the patients were reinstructed if they did not follow the correct procedure or were confused between the matching tone and their tinnitus as it was crucial for reliability and validity of the test.

The tinnitus matching test was conducted using a calibrated Kamplex AC 4 audiometer with TDH 39P headphones. Pure tones were presented at comfortable levels for the test tones at the frequencies of 250, 500, 1000, 2000, 3000, 4000, 6000 and 8000 Hz. Due to a limited set of discrete frequencies obtained from the tinnitus pitch matching test, for the purpose of comparing tinnitus pitch frequencies and findings in the Audioscan test and DPOAEs, the measured tinnitus frequencies were divided into three frequency bands (as in Pan *et al*, 2009), low-frequency (<2,000 Hz), mid-frequency (2,001-4,000 Hz) and high-frequency (4,001-8,000 Hz) tinnitus bands.

3. The Audioscan test:

The Audioscan test was carried out using the Audioscan program on an Essilor Audioscan audiometer with DT 48 headphones. Detailed information can be found in previous publications (Meyer-Bisch, 1996; Zhao *et al*, 2002; Zhao and Stephens, 1998). A brief description of its principle and procedure is summarised as follows:

The principle of the Audioscan technique is to provide a frequency sweep at a constant hearing level across frequencies instead of an intensity sweep through individual frequencies. Thus, the sweep becomes horizontal instead of vertical. The ranges of frequencies swept and levels explored are determined by a programmed system which can be preset by the tester (Meyer-Bisch, 1996). Because of testing at 1/64 octave intervals over the preset frequency range, the Audioscan is a high frequency resolution audiometric technique and provides high sensitivity in the exploration of auditory deficits. In the present study, Audioscan testing was performed over the frequency range 250 – 8,000 Hz with a sweep rate of 30 sec/octave, using a starting level of -5 dB HL and a step size of 5 dB, with pulsed stimuli.

During the Audioscan test, participants were instructed to press and hold the response button for as long as they could hear pulse tones, and release it when the tones disappear. This information is recorded, and then the programme produces stimuli of the next intensity and tests only in those parts of the frequency range at which there was no response. The stimulus level is subsequently further increased until the participants have responded throughout the defined frequency range. By using the current sweep rate, the entire testing time takes approximately 10-20 minutes.

4. DPOAE testing

The Otodynamics ILO88/92 software, hardware and probe noted above were used to record DPOAEs. The stimuli comprised pairs of equilevel primary pure tones at frequencies f1 and f2. The f2/f1 ratio was equal to 1.22. The primary levels were adopted at 70 dB SPL. Each DPOAE represented a sub-average of 16 measurements with each epoch lasting 80 ms being repeated three times. Only the 2f1-f2 DPOAE was studied and its level was determined as a function of the primary tone f2 (Zhao and Stephens, 1998). F1 and f2 were varied in 1/8 octave steps. The results are presented as a DP line chart, with the level of the 2f1-f2 DPOAE plotted against the f2 frequency from 700 to 6,123 Hz.

5. Analysis of the Audioscan notches and DPOAE notches

Audioscan and DPOAE notches were determined using the working definition described in previous studies (e.g., Meredith, 1991; Zhao and Stephens, 1998).

- ***The Audioscan notches:*** A significant notch was defined as being 15 dB or deeper than the surrounding frequencies with the notch width not entering into the criterion.
- ***DPOAE notches:*** A significant 'notch' in the context of this study was defined as a localized sharp decrease in the amplitude of a response, extending over a certain frequency range, followed by a quick increase in the amplitude. The deepest point of the DP notch must be below -10 dB SPL.

The rationale behind the definition of a significant notch was based on previous prevalence studies of Audioscan notches and DPOAE notches between control subjects and patients with auditory disorders, which demonstrated their possible use in distinguishing and detecting mild cochlear damage (Meredith, 1991; Zhao and Stephens, 1998).

The Audioscan and DPOAE notches were measured using their Centre Frequency (Fa), and Depth (Pa). The Fa of the Audioscan and DPOAE notches were determined as the frequency corresponding to the deepest point of the notch, whereas Pa was referred to as the difference between absolute value of the notch at the lower extremity, expressed in dB measured at Fa, and the best hearing threshold preceding the notch (Zhao and Stephens, 1998).

To compare the measured tinnitus frequencies with the centre frequencies of the notches obtained from the Audioscan and DPOAE tests, categorization of the frequency bands was used; low-frequency (<2,000 Hz), mid-frequency (2,001-4,000 Hz) and high-frequency (4,001-8,000 Hz) tinnitus bands.

6. Normative data

Normative data (for example, hearing thresholds, prevalence of the Audioscan notches and prevalence of DPOAE notches) were obtained from age- and gender-matched controls selected from our previous study at the Welsh Hearing Institute (Zhao, 1998). They were obtained on the basis of an opportunistic stratified sample of 70 normal hearing participants consisting of one person of each sex for each calendar year of age from 16 to 50 years.

7. Statistical design and analysis

Collected data was stored in an Excel database and all relevant analyses were carried out using the Statistical Package for Social Science (SPSS) version 14.0 for Windows software. Significance level was set at the conventional 5% level for all statistical tests. Demographic data was analysed using frequency distribution. Data from both the Audioscan and OAE was in a text format known as numerical data, and was exported into Excel (Office 2003), then the data was exported to SPSS for relevant analysis.

[Results]

1] General information on the tinnitus patients with normal hearing thresholds

45 tinnitus patients with normal hearing thresholds were included in the study. 21 had bilateral tinnitus (42 ears, 46.7%), 15 had tinnitus on the left side (15 ears, 33.3%) and 9 had tinnitus on the right side (9 ears, 20.0%) (Figure 1A). Thus, a total of 66 ears with tinnitus were analysed in this study. Table 1 shows the mean hearing levels over the frequency range from 250 to 8,000 Hz in the tinnitus patients. Student *t*-test analysis showed the pure tone thresholds at individual frequencies in tinnitus patients with normal hearing thresholds were significantly poorer at high frequencies than those derived from the normative data, but this was not the case at low and mid frequencies.

INSERT TABLE1 NEAR HERE

INSERT FIGURES 1A, 1B AND 1C NEAR HERE

The results obtained from the tinnitus pitch matching test showed 36 tinnitus patients were able to find pure tones to match their own tinnitus perception, whereas 9 participants were unable to match their perceived tinnitus pitch using the frequency range 250 to 8,000 Hz. Figure 2 shows that the distribution of tinnitus frequencies ranged from 250 to 8,000 Hz in tinnitus patients with normal hearing thresholds. The major tinnitus pitch was in the high frequency region with a peak around 6,000 Hz. The average tinnitus pitch in the present study was 4,826 Hz (SD: 2,089 Hz).

INSERT FIGURE 2 NEAR HERE

2] Prevalence of Audioscan and DPOAE notches

Audioscan notches were present in 40 tinnitus patients with normal hearing thresholds (40/45, 88.9%) within the frequency band 250 and 8,000 Hz. In 10 participants (22.2%) they were in the right ear only, 8 (17.8%) in the left and 22 (48.9%) in both ears (Figure 1B). DPOAE notches were present in 28 of the 45 tinnitus patients with normal hearing thresholds (62.2%) within the frequency band 250 - 8,000 Hz. Of these, 24.4% of the participants had notches in the right ear only, 11.1% in the left and 26.7% in both ears (Figure 1C).

Compared with age- and gender-matched controls selected from our previous study, significant Audioscan notches between 250 and 8,000 Hz were found in only 12 normal hearing control subjects (12/45; 26.7%), whilst significant DPOAE notches were present in 8 normal hearing control subjects (17.8%). Chi-square analysis showed a significantly higher prevalence of Audioscan and DP notches in the tinnitus patients with normal hearing thresholds ($x^2_{\text{Audioscan}}$ =9.9, p<0.005; notches: x^2_{DPOAE} =8.1, p<0.005).

3] Audioscan and DPOAE notches in relation to tinnitus

The reported tinnitus was compared with Audioscan notches by ear. As shown in Table 2, approximately 87% of tinnitus patients had Audioscan notches on the same ear as the self-reported tinnitus. Only 6 patients (13%) had tinnitus on one side and did not have any Audioscan notches on this side. Further analyses were performed to compare the relationship between the centre frequencies of the Audioscan notches and tinnitus frequencies measured using the tinnitus matching test. The tinnitus frequencies matched well with the centre frequencies of the Audioscan notches in the mid frequency band (9/10, 90%), but not in the low frequency band (1/6, 16.7%); the centre frequencies of the Audioscan notches matched only 50% of the tinnitus frequencies in the high frequency band (10/20) (Figure 3A).

INSERT TABLE2 NEAR HERE

INSERT FIGURES 3A AND 3B NEAR HERE

Furthermore, comparing the reported tinnitus with the DPOAE notches by ear in the tinnitus patients with normal hearing thresholds, as shown in Table 3, 27 tinnitus patients (60%) had DPOAE notches and tinnitus in the same ear, whereas 18 tinnitus patients (40.0%) had tinnitus in one ear but did not have any DPOAE notches on this side. Further analysis compared the relationship between the centre frequencies of the DPOAE notches and tinnitus frequencies measured using the tinnitus matching test in the same three frequency bands. There was a good match in the mid frequency region (7/10, 70%), but not in the low and high frequency regions (Figure 3B). Only 3 of the centre frequencies of the DPOAE notches matched with the tinnitus frequencies in the high frequency region (3/20, 15%), and none of the centre frequencies of the DPOAE notches had corresponding tinnitus frequencies in the low frequency region.

INSERT TABLE3 NEAR HERE

4] Comparison of the notches found on Audioscan and DPOAE testing

The comparability between the frequencies of the Audioscan notches and the DPOAE notches varied in the different frequency regions. Figures 4A and 4B show an example of tinnitus patients having a good match between the centre frequencies of the Audioscan notches and the DPOAE notches at the low and mid frequencies, together with an example of tinnitus patients having a reasonable match between centre frequencies of the Audioscan notches and the DPOAE notches at low and mid frequencies but no match at high frequencies. A Pearson correlation analysis showed a low but significant correlation between the centre frequencies of the Audioscan notches and the DPOAE notches (r= 0.62, p<0.0005). Further analysis demonstrated a significant correlation between the centre frequencies of the Audioscan notches and the DPOAE notches from 500 to 4,000 Hz (r= 0.70, p<0.0005), but no significant correlations at the low and high frequencies (Figure 5).

INSERT FIGURES 4A and 4B NEAR HERE

INSERT FIGURE 5 NEAR HERE

[Discussion]

Various studies have shown that hearing thresholds within the normal audiometric range (e.g., <=20 dB HL) do not necessarily indicate normal function along the auditory pathway (König *et al*, 2006; Weisz *et al*, 2006; Zhao & Stephens, 1998; Ami et al, 2008). In the present study, although tinnitus participants had normal hearing thresholds on the audiogram, their averaged hearing levels at high frequencies were significantly worse than those derived from the normative data. This result is in keeping with the findings in several previous studies (e.g., Ami et al, 2008). This phenomenon of elevation of hearing levels may imply the beginning of hearing deterioration in tinnitus patients with normal hearing thresholds. However, it should be interpreted cautiously as evidence of mild auditory dysfunction because of the difficulty experienced by tinnitus subjects in differentiating between the pure tones and their own tinnitus pitches (Levi & Chisin, 1987).

Although OAEs have been used to investigate cochlear mechanisms in patients with tinnitus over the past 20 years, many discrepancies have been found in previous studies. For example, spontaneous otoacoustic emissions (SOAEs) were initially thought to be an objective form of tinnitus because SOAEs are of cochlear origin and emitted by the cochlea without any acoustic stimulation. However, the prevalence of SOAE related tinnitus is very low (<10%) and varies widely between studies (Norton *et al*, 1990). Moreover, the correlation between tinnitus and evoked otoacoustic emissions (i.e., TEOAEs and DPOAEs) is not uniform in investigating altered cochlear mechanisms in patients with tinnitus. Some evidence shows that TEOAEs and DPOAEs do reflect specific changes in cochlear mechanics being related to tinnitus.

In some studies, researchers have found a significantly higher prevalence of absent or abnormal TEOAEs and DPOAEs in tinnitus patients with normal hearing thresholds (e.g., Sirimanna *et al*, 1996; Shiomi *et al*, 1997; Ozimek *et al*, 2006; Ami *et al*, 2008; Paglialonga *et al*, 2010; Granjeiro *et al*, 2008; Zhou *et al*, 2011). For example, Granjeiro *et al.* (2008) investigated the prevalence of TEOAE and DPOAE test results in tinnitus patients with and without hearing loss. They found that the percentage of individuals with abnormal TEOAEs and DPOAEs was significantly higher in tinnitus patients with normal hearing thresholds than those obtained from the non-tinnitus normal hearing control group. In addition, a significant reduction in DPOAE and TEOAE amplitude in normal hearing thresholds with tinnitus was found when compared with non-tinnitus control subjects (Shiomi *et al*, 1997; Ozimek *et al*, 2006; Ami *et al*, 2008; Paglialonga *et al*, 2010; Granjeiro *et al*, 2008). Further frequency analysis showed a significant decrease in DPOAE amplitude mainly at the f2 frequency in mid and high frequency ranges. Therefore, the decrease either in overall averaged DPOAE amplitudes or DPOAEs over a limited frequency range in DP-gram found in tinnitus patients with normal hearing thresholds indicates minor OHC damage, which is most likely to reflect cochlear pathological changes underlying tinnitus generation in tinnitus ears with normal audiometric thresholds.

The DPOAE findings in the present study are in line with the hypothesis of gain adaptation as a mechanism of tinnitus generation (Parra & Pearlmutter, 2007; Zhou *et al*, 2011). In these studies, the modeling simulation and psychoacoustic results indicate that tinnitus may be associated with reduced cochlear nonlinearity, resulting in the perception of phantom sound at the notched frequency after listening to a notched broadband signal. Therefore, these studies proposed that auditory gain adaptation plays an important role in coping with reduced nonlinear cochlear compression, and consequently enhances internal noise at a frequency band perceived as resembling tinnitus, which would otherwise be silent because of hearing loss. Moreover, Zhou *et al* (2011) showed that subjects with tinnitus not only had elevated thresholds and reduced DPOAE, but also increased slope of the DPOAE input-output function in the high frequency region, indicating reduced nonlinear mechanical compression of the cochlea. This result provides evidence to support gain-adaptation hypothesis underlying tinnitus generation.

By contrast, in other cases, increased TEOAE and DPOAE levels with increasing hearing loss were found in the ears with tinnitus (Schaette & McAlpine, 2011; Norton *et al*, 1990; Gouveris *et al*, 2005). For example, Gouveris *et al.* (2005) observed a significant increase in DPOAE amplitudes over the 4 - 6 kHz frequency range in a group of tinnitus patients. They suggested that it could be the result of reduced afferent input to the central auditory system due to cochlear lesions, thus resulting in a decreased OHC efferent activity and ultimately leading to OHC hypersensitivity. This appears to support a different hypothesis, that a reinforced mechanical distortion generated by cochlear hyperactivity could be the source of the tinnitus.

Such inconsistent DPOAE results together with a wide intersubject variation in amplitudes of DPOAEs, suggest that the abnormality of a tinnitus patient's responses may not in practice be easily interpreted by comparing them with the normative data (Ozimek *et al*, 2006; Granjeiro *et al*, 2008). Therefore, analysis of characteristics of the DP-gram may contribute to clinical application of DPOAEs as it provides a significant indicator of subclinical lesions in the cochlea. In the present study, the characteristics of the DPOAEs in terms of notches showed a significantly higher percentage of notches on the DPOAE test in tinnitus patients with normal hearing thresholds than in controls. Such notches on the DP-gram may be recognised as indicators of subclinical cochlear (outer hair cell) damage, supporting the hypothesis of peripheral tinnitus generation.

The DPOAE is generally accepted as having a certain degree of "frequency specificity" (Lonsbury-Martin *et al,* 1990). The implication is that each part of the cochlea can be characterised by examining the amplitude of the DPOAEs. Although accurate estimation of hearing threshold levels is not yet possible with DPOAEs, it is usually possible to differentiate normal hearing thresholds vs. mild sensory hearing impairment at discrete frequencies using this technique. For example, the frequency-specific reductions of DPOAE amplitudes in frequency regions of behaviorally measured noise-induced permanent hearing impairment have been demonstrated by Lonsbury-Martin *et al*. (1990). They are absent in frequency regions with a moderate or severe cochlear hearing loss. At the same time, DPOAEs can still be present in adjacent frequency regions, within the same ear, where hearing sensitivity is normal. Thus, DPOAE measurement where there are subclinical changes in the cochlea and pure-tone sensitivity is still within normal limits, could provide important information on the functional state of the cochlea.

Previous studies have noted that one of the great advantages of Audioscan is its capacity to detect even the narrowest notches situated in the frequency range 250 to 16,000 Hz. The significance of Audioscan notches has been confirmed for detection of hearing deficiency in the screening of noise-induced hearing impairment, in carriers of genes for hearing impairment (Meredith 1991; Zhao *et al*, 2002), for example King-Kopetzky syndrome, in which an individual complains of difficulties in understanding speech in the presence of background noise but has normal hearing thresholds on pure tone audiometry (Zhao & Stephens, 1998).

Based on a systematic review of the Audioscan test and its clinical application (Zhao *et al*, 2002), only one previous tinnitus study by Sirimanna *et al.* (1996) has included the Audioscan test within the test battery. These authors recruited 26 tinnitus patients who had PTA thresholds less than 25 dBHL at frequencies of 0.25 to 8 kHz and who were able to match their tinnitus to pure tone. The participants underwent a series of audiological tests that including PTA, spontaneous otoacoustic emission (SOAE) and click-evoked otoacoustic emissions (TEOAEs). They found that 24 (96%) had Audioscan notches and 22 (85%) had either poor or absent emissions in the high frequency region. A high prevalence of Audioscan notches (89%) found in the present study is broadly in keeping with their study. It may suggest that this tinnitus population has a very high prevalence of localised damage to the cochlea shown as indicators in the Audioscan test.

It is noteworthy that reliability and validity have been questioned because Audioscan measurement takes longer to complete than other audiological tests (e.g. PTA). This may reduce subjects' concentration level and, consequently, produce unreliable results. A systematic review by Zhao *et al*. (2002) suggested that Audioscan notches were found less frequently in a group of subjects when tested with faster sweep rates than at slower sweep rates, and the test-retest reliability of hearing thresholds obtained from Audioscan was affected by sweep speed, with the reliability at the slower sweep speed being slightly better than that at a faster sweep rate. In a recent study of test-retest reliability and validity of the Audioscan using a fast sweep rate (15 s per octave), the results showed that 86% of participants had hearing threshold variability within 5 dB at most frequencies except at 1,500 Hz (80%) and 4,000 Hz (81%), while more than 90% of participants had variability of hearing threshold of less than 10 dB at all frequencies (Ishak *et al*, 2011). This suggests that the Audioscan can be used as a reliable and valid method of hearing threshold determination, even using a fast sweep rate.

A significant correlation between the Audioscan and DPOAE notches found in the frequency areas from 500 to 4,000 Hz. enhances the diagnostic value and credibility of both measures. However, a discrepancy in the central frequencies between the DPOAE notches and the Audioscan notches confines the significance of their clinical strength, particularly in the high frequency area. The mechanism is not clear. One reason may be attributed to the limitations of DPOAE recording in testing OHC functions at high frequencies, i.e. (1) errors arising from standing-wave nodes in the levels of primary tones and (2) DPOAEs expected at high frequency regions may underlie a greater variability (Lonsbury-Martin *et al*, 1990; Zhao & Stephens, 1998).

Furthermore, it is noteworthy that a small percentage of the Audioscan and DPOAE notches was found in non-tinnitus ears. The precise nature of this finding is at present unknown. A previous review hypothesized that the notches found in controls subjects with normal hearing thresholds may be associated with genetic background and environmental factors (Zhao *et al*, 2002), indicating a more susceptible zone related to damage or the site of degenerative processes, and represent evidence of early minor cochlear pathology. Long-term follow-up studies should be pursued to reveal whether these non-tinnitus ears with such notches show progression of their symptoms. If this proves to be the case, it would provide valuable information for further understanding the pathogenesis in tinnitus patients with normal hearing thresholds.

Previous studies have explored the relationship between individual audiometric profile and perceived tinnitus pitch in tinnitus patients both with and without hearing loss (e.g., Roberts *et al*, 2008; Pan *et al*, 2009; Zhou *et al*. 2011; Sereha *et al*, 2011; Schechlmann *et al*, 2012). Some studies found significant correlations between tinnitus pitch and the edge frequency of hearing impairment or the area corresponding to the frequency of the maximum hearing loss (Roberts *et al*, 2008; Sereha *et al*, 2011; Schechlmann *et al*, 2012), whereas others failed to demonstrated any correlation of audiogram shape and tinnitus pitch (Pan *et al*, 2009). Such inconsistent results have been linked to the small sample sizes and lack of sub-group analysis because it is inappropriate to pool all possibly heterogeneous tinnitus patients together for statistical analysis. A few specific types of perceptual tinnitus have been proposed to be associated with particular audiological characteristics. For example, König *et al*. (2006) demonstrated an association between tinnitus pitch and edge frequency in a subgroup of patients with noise-induced hearing loss who reported that their tinnitus was tone-like, but not in the subgroup of patients who reported tinnitus with broad spectral features. Although the study by Pan *et al*. (2009) claimed to fail to find a relationship between tinnitus pitch and the edge of hearing loss or two other measures of hearing loss (average of the hearing thresholds across frequencies and frequency of the maximum hearing loss), they noted that some individuals did exhibit a pitch at the low-frequency edge of hearing loss (Figure 8 in Pan *et al*, 2009).

More recently, several studies have further investigated the relationship between audiometric profile and tinnitus pitch. In the studies by Sereha *et al*. (2011) and Schechlmann *et al*. (2012), both suggest a relationship between pitch and audiometric profile in a certain subgroup of people with particular perceptual characteristics of tinnitus. Sereha *et al*. (2011) found that the pitch of the tinnitus in a subgroup of patients with a narrow tinnitus bandwidth was associated with the audiometric edge frequency, while Schechlmann *et al*. (2012) confirmed a relationship between tinnitus pitch and maximum hearing loss, which was affected by audiometric steepness and the side of ear. Moreover, Roberts *et al*. (2009) and Zhou *et al*. (2011) measured ‘tinnitus likeness spectrum’ by rating the similarity of tinnitus to tones at various frequencies. Roberts *et al.* (2009) found that tinnitus spectra increased with the amount of threshold shift over the region of hearing impairment, and the tinnitus spectrum shifted towards higher frequencies when the audiometric edge frequencies increased. These results were confirmed in the study by Zhou *et al.* (2011), showing significant correlation between tinnitus spectrum and hearing thresholds.

In the present study, by comparison, the central frequencies of the Audioscan notches and DPOAE notches only had a good match with tinnitus frequencies in the mid frequency region, but failed to match with tinnitus frequencies at low and high frequency regions. Such negative results may be due mainly to a limited set of discrete frequencies used for the tinnitus pitch matching test. Moreover, it is noteworthy that there is a large variability of tinnitus pitches measured using conventional tinnitus frequency matching tests. A recent study showed that the within-subject variability was about within one octave of the average pitch match frequency (Pan et al, 2009). This is mainly due to the difficulties in obtaining a consistent tinnitus match because the tinnitus percept is often more complex than a single discrete frequency (Noreña *et al*, 2002). Therefore caution should be exercised in interpreting the relationship between audiometric profile and tinnitus pitch. The ‘tinnitus likeness test’, by rating the similarity of their tinnitus to tones at the various frequencies mentioned above, seems to provide a more reliable and appropriate tinnitus pitch test, potentially for future clinical application.

[Conclusion]

A significantly higher prevalence of Audioscan and DPOAE notches has been found in the present study and provides evidence of indicators of cochlear damage in tinnitus patients with normal hearing thresholds before gross behavioural threshold reduction can be demonstrated. Although a significant correlation between the Audioscan and DPOAE notches found in frequency ranges from 500 to 4,000 Hz enhances the diagnostic value and credibility, a discrepancy in the high central frequencies between DPOAE notches and Audioscan notches confines the significance of their clinical strength. This may be attributed to the limitations of DPOAE recording for testing OHC functions at high frequencies. Furthermore, central frequencies of the notches obtained from the Audioscan and DPOAEs matched tinnitus frequencies only in the mid frequency region, but not in the low and high frequency regions. This may indicate that tinnitus in different frequency regions may be associated with different underlying mechanisms of tinnitus generation. It is noteworthy that a limited set of discrete frequencies was used for the tinnitus pitch matching test. Further studies should consider more detailed analysis of perceptual tinnitus characteristics when evaluating the relationship between characteristics of Audioscan and DPAOEs and tinnitus pitch.

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[CONFLICT OF INTEREST]

No conflicts of interest.

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Captions of Figures and Tables

Figure 1: Prevalence of tinnitus, Audioscan and DPOAE notches by the ears

Figure 2: The distribution of tinnitus frequencies ranged from 250 to 8,000 Hz in the present study

Figure 3: Audioscan and DPOAE notches in relation to tinnitus frequency band

[A] Percentage of the centre frequencies of the Audioscan notches matching with tinnitus frequency bands;

[B] Percentage of the centre frequencies of DPOAE notches matching with tinnitus frequency bands.

Figure 4: Examples of good match and no match on Audioscan and DPOAE testing

Figure Legend:

The red area indicates the noise floor;

The blue line indicates the DPOAE responses across different frequencies

Figure 5: The correlations between the notch centre frequency on Audioscan and DPOAE testing

Figure Legend:

The solid regression line indicates a correlation including the frequencies from 250 to 8,000 between the centre frequencies of the Audioscan notches and the DPOAE notches (R^2=0.39, $p<0.0005$);

The dash regression line indicates a correlation only including the frequencies from 500 to 4,000 Hz between the centre frequencies of the Audioscan notches and the DPOAE notches (R^2=0.49, $p<0.0005$);

Table 1: Comparing the mean thresholds at individual frequencies in tinnitus patients with normal hearing thresholds with the normative data obtained from age- and gender-matched controls

Table 2: Comparison between tinnitus and the Audioscan notches by ears

Table 3: Comparison between tinnitus and DPOAE notches by ears

Table 1

Frequency *(Hz)*	**Tinnitus ears** *Mean ± SD (dB HL)*	**Controls** *Mean ± SD (dB HL)*	***t* value**	***p* value**
250	6.5 ± 6.2	5.0 ± 4.0	1.53	=0.13
500	5.9 ± 6.1	4.7 ± 2.6	1.30	=0.20
1,000	3.8 ± 6.0	4.5 ± 3.4	-0.78	=0.44
2,000	6.6 ± 6.7	5.0 ± 3.4	1.55	=0.12
4,000	10.0 ± 6.4	5.4 ± 3.4	4.60	<0.0005
8,000	13.9 ± 9.9	7.9 ± 5.6	3.86	<0.0005

Table 2

Tinnitus Side	**Audioscan Notches**			
	No Notches	***Right Ear***	***Left Ear***	***Both Ears***
Right Ear (n=9)	0	7	0	2
Left Ear (n=15)	2*	1*	7	5
Both Ears (n=21)	3*	2	1	15

***** Tinnitus patients had tinnitus on one side of ear but not having any Audioscan notches on this side.

Table 3

Tinnitus Side	**DPOAE Notches**			
	No Notches	***Right Ear***	***Left Ear***	***Both Ears***
Right Ear (n=9)	1*	7	0	1
Left Ear (n=15)	8*	1*	3	3
Both Ears (n=21)	8*	3	2	8

***** Tinnitus patients had tinnitus on one side of ear but not having any DPOAE notches on this side.

Figure 1

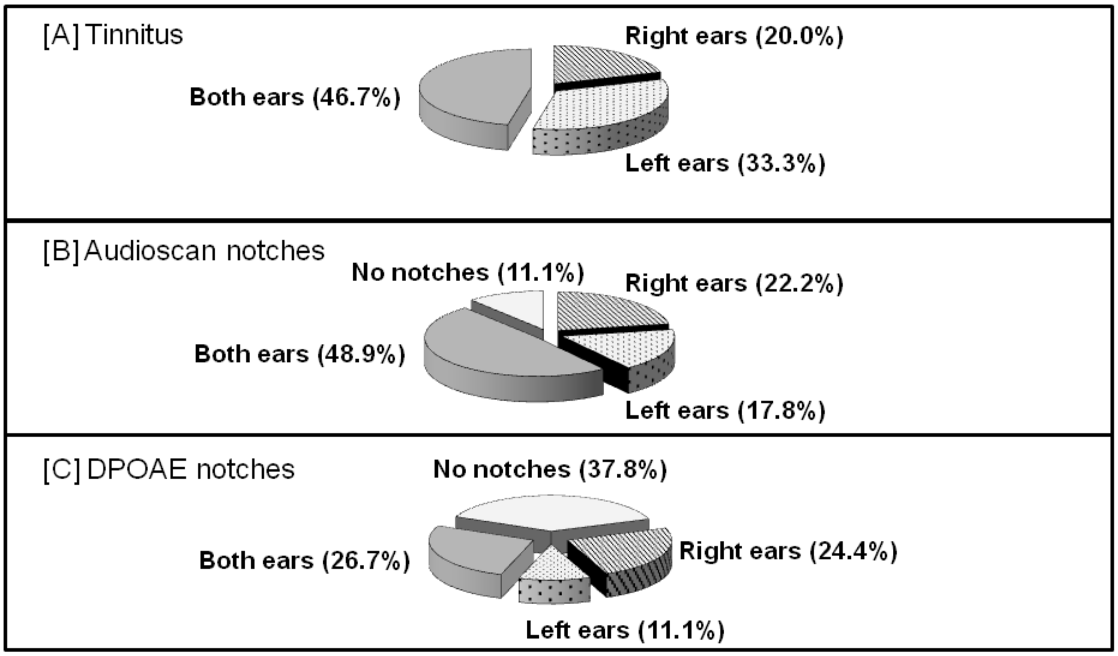

[A] Tinnitus
Right ears (20.0%)
Both ears (46.7%)
Left ears (33.3%)
[B] Audioscan notches
No notches (11.1%)
Right ears (22.2%)
Both ears (48.9%)
Left ears (17.8%)
[C] DPOAE notches
No notches (37.8%)
Both ears (26.7%)
Right ears (24.4%)
Left ears (11.1%)

Figure 2

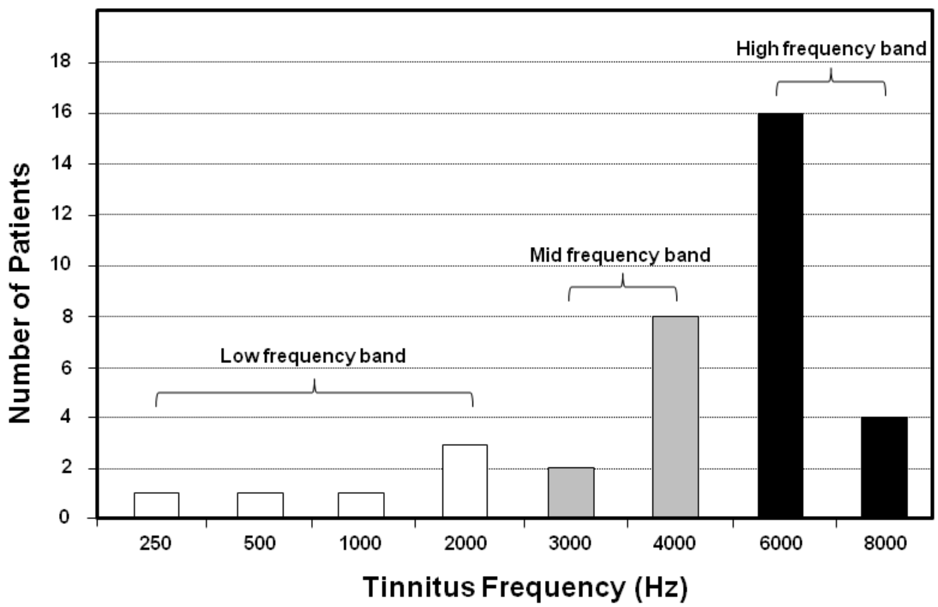
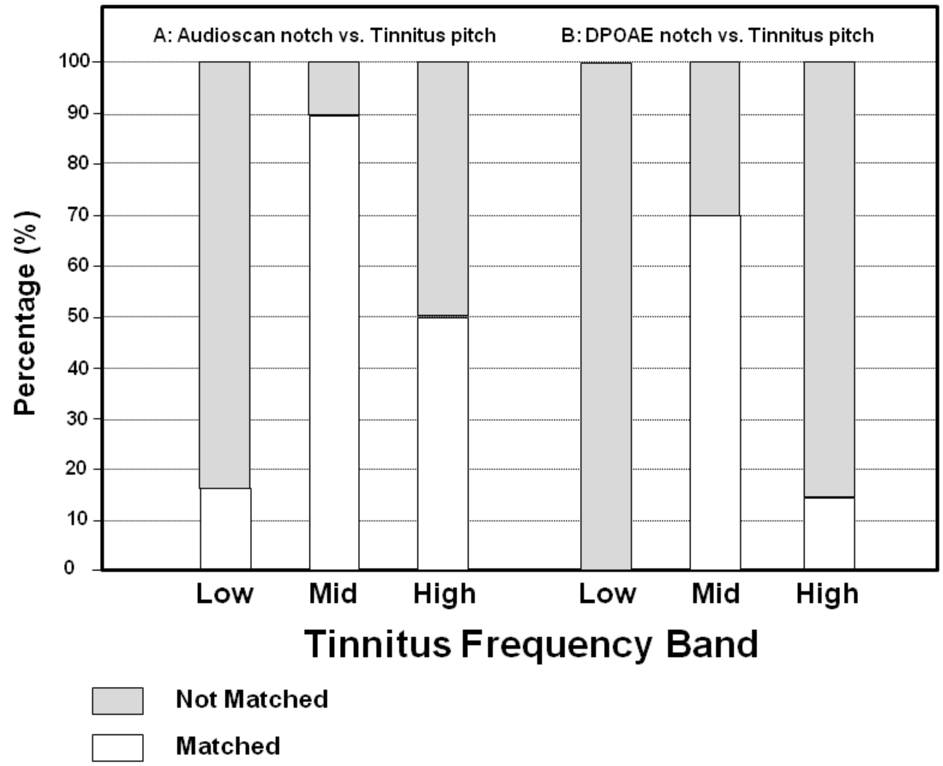

High frequency band
Mid frequency band
Low frequency band
Number of Patients
18
16
14
12
10
8
6
4
2
0
250
500
1000
2000
3000
4000
6000
8000
Tinnitus Frequency (Hz)

Figure 3



A: Audioscan notch vs. Tinnitus pitch
B: DPOAE notch vs. Tinnitus pitch
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
Low
Mid
High
Low
Mid
High
Tinnitus Frequency Band
Not Matched
Matched

Figure 4

Example 1 – Good match at the mid frequency **Example 2 – No match at the high frequency**

A B

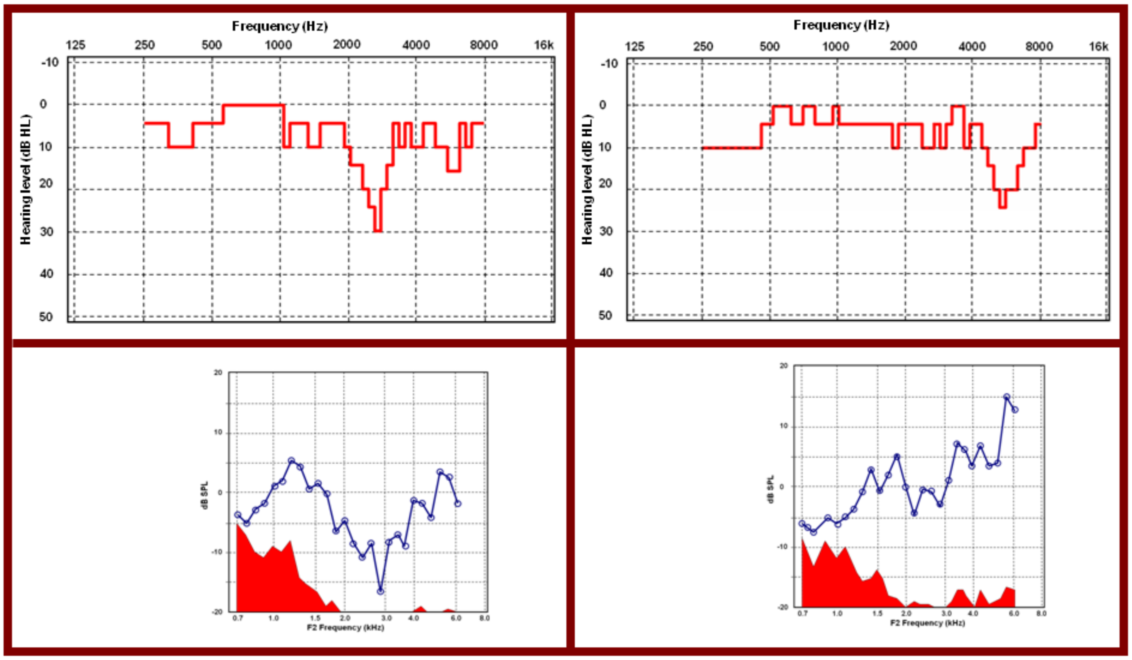
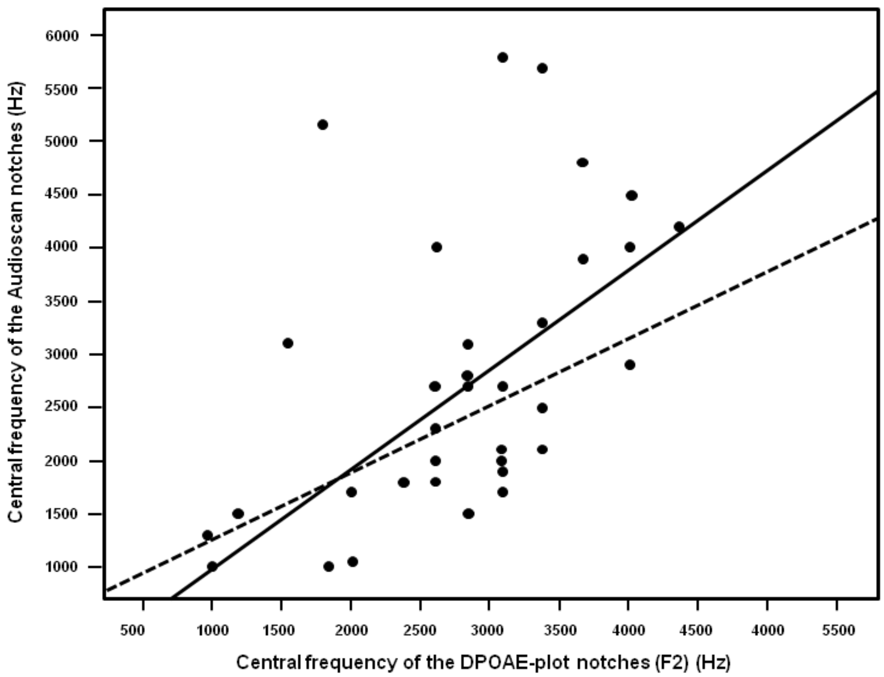


Figure 5


6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
Central frequency of the Audioscan notches (Hz)
500
1000
1500
2000
2500
3000
3500
4000
4500
4000
5500
Central frequency of the DPOAE-plot notches (F2) (Hz)