A Study design/planning B Data collection/entry C Data analysis/statistics

- D Data interpretation
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EVALUATION OF WIDEBAND ABSORBANCE **TYMPANOMETRY IN ADULTS WITH** ABNORMAL POSITIVE AND NEGATIVE MIDDLE EAR PRESSURE

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Abstract

Background: Middle ear pressure plays a vital role in the transmission of sound to the inner ear. However, limited research data exists to understand the effect of abnormal middle ear pressure on wideband absorbance (WBA) tympanometry. The purpose of the study was to evaluate WBA at peak pressure and ambient pressure in adults with abnormal positive and negative middle ear pressure and compare them with normal adult ears having normal middle ear pressure.

Material and methods: Three groups of adults - normal middle ear pressure group (56 ears), negative middle ear pressure group (30 ears), and positive middle ear pressure group (15 ears) - in the age range 22 to 50 years were considered. WBA was measured at peak and ambient pressures across the frequencies from 250 to 8000 Hz.

Results: WBA at peak pressure was observed to be higher than at ambient pressure in all the groups, with the difference seen mostly at low and mid-frequencies up to 2000 Hz. The negative middle ear pressure group showed the most considerable difference in mean WBA, seen between 600 Hz and 1000 Hz, followed by the positive middle ear pressure group, with a negligible difference for the normal middle ear pressure group.

Conclusions: The study highlighted the importance of measuring WBA at peak pressure and ambient pressure. The results suggest that the comparison of WBA at peak and ambient pressures, especially from lower to mid-frequencies up to 2000 Hz, would help in differentiating abnormal negative/positive pressure from normal middle ear pressure and also between ears having negative and positive pressure.

Key words: absorbance • peak pressure • middle ear pressure • ambient pressure

OCENA ABSORBANCJI SZEROKOPASMOWEJ U DOROSŁYCH Z PODCIŚNIENIEM I NADCIŚNIENIEM W UCHU ŚRODKOWYM

Streszczenie

Wstęp: Ciśnienie w uchu środkowym odgrywa istotną rolę w przekazywaniu dźwięku do ucha wewnętrznego. Jednak istnieją ograniczone dane badawcze pozwalające zrozumieć wpływ nieprawidłowego ciśnienia w uchu środkowym na tympanometrię absorbancji szerokopasmowej (WBA). Celem pracy była ocena WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia u dorosłych z podciśnieniem i nadciśnieniem w uchu środkowym i porównanie ich z wynikami u dorosłych z prawidłowym ciśnieniem w uchu środkowym.

Materiał i metody: W badaniu udział wzięły trzy grupy dorosłych w wieku od 22 do 50 lat: grupa z normalnym ciśnieniem w uchu środkowym (56 uszu), grupa z podciśnieniem w uchu środkowym (30 uszu) i grupa z nadciśnieniem w uchu środkowym (15 uszu). WBA mierzono przy ciśnieniu szczytowym i ciśnieniu otoczenia w zakresie częstotliwości od 250 Hz do 8000 Hz.

Wyniki: Zaobserwowano, że WBA przy ciśnieniu szczytowym jest wyższe niż przy ciśnieniu otoczenia we wszystkich grupach, z różnicą obserwowana głównie przy częstotliwościach niskich i średnich do 2000 Hz. Grupa z podciśnieniem w uchu środkowym wykazała najwieksza różnicę w średnim WBA, obserwowaną między 600 Hz a 1000 Hz. Następna była grupa z nadciśnieniem w uchu środkowym, natomiast w grupie z normalnym ciśnieniem w uchu środkowym różnica była nieistotna.

Wnioski: Badanie wskazuje na znaczenie oceny WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia. Wyniki sugerują, że porównanie WBA przy ciśnieniu szczytowym i ciśnieniu otoczenia, zwłaszcza w przedziale od niskich do średnich częstotliwości do 2000 Hz, pomogłoby w odróżnieniu podciśnienia/nadciśnienia od normalnego ciśnienia w uchu środkowym, a także rozróżnieniu podciśnienia i nadciśnienia pomiedzy obojgiem uszu.

Słowa kluczowe: absorbancja • ciśnienie szczytowe • ciśnienie w uchu środkowym • ciśnienie otoczenia

Introduction

Conventional tympanometry is carried out using either a single probe tone frequency or multiple frequencies by varying air pressure in the ear canal to measure the admittance of the middle ear. The transmission of sound energy is usually maximum at the level where the pressure is equal on both sides of the tympanic membrane [1].

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In individuals with a healthy middle ear, the maximum energy flows into the middle ear at a pressure closest to that of the atmosphere [1]. Any deviation of middle ear pressure (MEP) from atmospheric pressure is likely to hinder the effective transmission of sound to the inner ear. Abnormal MEP is indicative of some abnormality in the middle ear, perhaps leading to middle ear disorders. Thus, air pressure plays a significant role in the transmission of sound to the middle ear.

Recent studies have indicated that wideband absorbance (WBA) is a sensitive tool in assessing middle ear function compared to conventional tympanometry [2] and in the differential diagnosis of middle ear disorders [3,4]. WBA is a non-invasive middle ear analysis technique that measures either the absorbance or reflectance of sound energy across a wide range of frequencies between 200 to 8000 Hz [5]. Studies have shown a distinct pattern of WBA for various pathological conditions such as otosclerosis, ossicular chain discontinuity, tympanic membrane perforation, and fluid in the middle ear cavity [2,3,6].

The most common occurring middle ear pathology that affects the transmission of sound to the inner ear is abnormal negative or positive MEP [7]. In the condition of Eustachian tube dysfunction (ETD), maximum energy flows at a negative pressure; on the other hand a positive pressure range is usually seen in the early stages of acute otitis media without effusion, where there is a bulging tympanic membrane [1,8]. Such abnormal pressure characteristics can be effectively studied by measuring middle ear absorbance across frequency, and also at different pressures, improving the accuracy with which middle ear disorders can be diagnosed.

Few efforts have been made to study the effect of abnormal MEP on WBA measurements [7,9–11], and most of these studies were based on simulated conditions in healthy individuals or cadavers. Studies have shown that any alteration in MEP increases energy reflectance (decreases absorbance) below 3 to 4 kHz and decreases reflectance (increases absorbance) at frequencies beyond 4 kHz [12]. On the other hand, studies on young children who had tympanometric peak pressure (TPP) more negative than –100 daPa have shown increased energy reflectance (decreased absorbance) for frequencies up to 4 to 5 kHz [9,10]. Thus, there is an apparent inconsistency in the findings of WBA under altered MEP conditions, especially at higher frequencies.

Further, the effect of pressure variations (ambient and peak) on WBA tympanometry readings has indicated a reduction in absorbance below 2 kHz under ambient pressure conditions compared to WBA at peak pressure [4,7,13]. To date, only one study has evaluated the difference in pressure variation on children with ETD and compared the result in children with normal ears [11]. Interestingly, the study showed a differential absorbance pattern with significant lower WBA only at ambient pressure for children with ETD, compared to normal ears [11]. On the other hand, no attempts have been made to use WBA tympanometry to evaluate the effect of positive MEP.

It is well known that a variation in MEP induces physiological changes in middle ear structures and thereby affects transmission of sound to the inner ear. Thus, it is crucial to understand the effect of MEP on WBA across frequency and to quantify the effects of positive and negative MEP. Also, to the best of our knowledge, there has been no previous report of WBA findings on an adult population who have abnormal positive or negative MEP. Thus, the purpose of this study was to examine WBA tympanometry findings on adults who had abnormal negative or positive MEP (as indicated by conventional tympanometry). The study also sought to determine the differences in WBA between peak and ambient pressures in those abnormal MEP subjects compared with the results from subjects with normal MEP.

Materials and methods

A total of three groups of adult subjects in the age range 22 to 50 years (mean age 36.0 ± 10.1 years) were considered for the study. The control group (normal MEP group) consisted of 37 healthy individuals (56 ears) having normal MEP of +50 to -100 daPa (mean -10.89 ± 12.28 daPa; range -32 to 12 daPa) as given by Jerger [14]. This middle ear pressure was obtained using conventional 226 Hz probe tone tympanometry by inserting a probe into the ear canal. The ear canal pressure at which the peak of the tympanogram occurred was considered as the TPP/MEP [28]. All participants in this group had normal hearing thresholds of less than 15 dB HL at all octave frequencies, static admittance of <1.6 mmho (mean 0.73 ± 0.34 ; range 0.22 to 1.54) with the presence of normal acoustic reflex thresholds, and the presence of transient evoked otoacoustic emission (≥ 3 dB SNR).

The clinical study group consisted of two sub-groups that included adults having 'negative middle ear pressure' more negative than -100 daPa (MEP_N) and a 'positive middle ear pressure' group having pressure greater than 50 daPa (MEP_P), measured using conventional 226 Hz probe tone tympanometry [14]. The MEP_N group included 25 participants (30 ears) with a mean static admittance of 0.70 mmho ± 0.41 SD (range 0.16 to 1.87) and mean TPP of $-207.0 \text{ daPa} \pm 99.9 \text{ SD}$ (range -353 to -108 daPa). MEP_P consisted of 14 participants (15 ears) who had mean TPP of 156.9 daPa ±43.6 SD (range 75 to 168 daPa) and mean static admittance of 0.58 mmho \pm 0.24 SD (range 0.15 to 1.17 daPa). Both study groups had hearing thresholds that ranged between minimal to mild conductive hearing loss, with elevated or absent acoustic reflex thresholds. However, all groups had an intact tympanic membrane without any perforation or active ear discharge. The institutional Ethics committee of bio-behavioural research involving human subjects reviewed and approved this study (No. WOF-0404/2014-15 with effect from 04.06.14). Informed consent was obtained from each of the subjects before enrolling in the present study.

Wideband absorbance measurement was performed using the Interacoustics Titan IMP/WBT 440 equipment and it measured absorbance values under two conditions: at peak pressure (WBA_{PP}) and ambient (0 daPa) pressure (WBA₀). The peak pressure is the ear canal pressure that was obtained using the wideband average tympanometry (i.e. average of WBA from 375 to 2000 Hz) that was automatically generated during WBA measurement in the Titan equipment. However, the mean TPP obtained from WBA measurement and the conventional 226 Hz probe tympanometry were identical. The probe was inserted into the ear canal, and a click stimulus of 100 dB peSPL (approx. 65 dBnHL) at a rate of 21.5 Hz was delivered. WBA was measured across frequencies (250–8000 Hz) while ear canal pressure was swept from +200 to –400 daPa at a rate of 200 daPa/sec. Before administrating the test, daily calibration of the WBA equipment was performed using four couplers as recommended by the manufacturer. The study extracted WBA values at 1/3rd octave frequencies (16 frequencies) at peak pressure and ambient pressure which were placed in an Excel spreadsheet and transferred to SPSS version 21.0 for statistical analysis.

Statistical analysis

Data were analysed using descriptive and inferential statistics. Parametric analysis was performed as the absorbance data followed a normal distribution as indicted by a Shapiro–Wilk normality test (p>0.05). A mixedmodel ANOVA was carried out to analyse WBA between the groups (normal MEP, MEP_N, and MEP_P) and pressure conditions (peak, ambient) across 16 frequencies (250 to 8000 Hz). A Greenhouse–Geisser approach [15] was used to compensate for violation of compound symmetricity and sphericity. A post hoc Tukey's Honestly Significant Difference (HSD) was administered for multiple pair-wise comparisons with a *p*-value of 0.05 as the level of significance. A paired-sample *T*-test was performed within the subject groups to compare peak and ambient pressure across the frequencies.

Results

Descriptive statistical results (mean and standard error) for WBA_{PP} and WBA_0 measured across frequencies for the three groups are illustrated in Figure 1. It can be observed that in the normal MEP group, at both peak and ambient pressure, WBA increases gradually as the frequency increases from 250 Hz, reaches a maximum at 2000 Hz, and reduces thereon to a minimum at 6000 Hz. At peak pressure, the

 MEP_P group showed a biphasic pattern, i.e., an increase then plateau at mid-frequencies, followed by a rise to a maximum at 2500 Hz, and then a further decline. At peak pressure the MEP_N group showed a similar pattern to the normal MEP group with a lower absorbance from frequencies between 250 Hz and 3000 Hz. However, at ambient pressure, all three groups showed a similar pattern, though the absorbance for the clinical groups was significantly lower than the normal group. On the other hand, at and above 4000 Hz there was no noticeable difference in mean absorbance across the groups for either WBA_{PP} or WBA₀.

A mixed ANOVA model was used to analyse the WBA, with pressure conditions (WBA_{PP} and WBA₀) and frequency (250 to 8000 Hz) as within-subject factors, and the study groups (normal MEP, MEP_N, and MEP_P) as betweensubject factors. The main effects of pressure conditions $(F = 1.775, p = 0.00, \eta_p^2 = 0.634)$, groups (F = 49.850, p = 0.00, p = 0.00) $\eta_{\rm p}^2 = 0.507$), and frequency (F = 281.32, p = 0.00, $\eta_{\rm p}^2 = 0.744$) were significant. The interaction effect between groups and pressure conditions ($F = 112.42, p = 0.00, \eta_p^2 = 0.699$), groups and frequency (F = 15.60, p = 0.00, $\eta_p^2 = 0.243$), and pressure conditions and frequency (F = 38.16, p = 0.00, $\eta_{\rm p}^2 = 0.282$) were also significant. Further, MANOVA was performed to investigate the frequencies at which the group differences occurred under the two different pressure conditions (peak, ambient). The result revealed a significant difference between the groups at low and mid-frequencies until 3000 Hz (p<0.05) for both pressure conditions, whereas high frequencies (4000-8000 Hz) did not show any significant difference (p>0.05).

To further investigate the interaction between groups and frequency across the pressure conditions, a post hoc Tukey's HSD test was performed. In comparison to the normal MEP group, a significant decrease in absorbance at WBA_{PP} was observed from 250 to 3000 Hz for the MEP_P group, and from 600 to 3000 Hz for the MEP_N group. Whereas between the MEP_N and MEP_P groups, a significantly lower absorbance was seen between 1000 and 2000 Hz for MEP_P compared to MEP_N. Compared to the normal MEP group, MEP_P had the most significant reduction in absorbance up to 3000 Hz,



Figure 1. Comparison of mean absorbance and standard error (whiskers) measured at (a) peak pressure (WBA_{PP}) and (b) ambient pressure (WBA₀) for each of the three groups

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Figure 2. Comparison of WBA measured at peak pressure and ambient pressure in (a) Normal MEP group; (b) Negative MEP group; (c) Positive MEP group; and (d) Mean difference in WBA between pressure conditions (WBA_{pp} – WBA₀)

followed by MEP_N (as seen in Figure 1a). In examining WBA₀ (Figure 1b), it was noted that both MEP_N and MEP_P showed a significant difference (p<0.05) in comparison to the normal MEP group, indicating a decrease in absorbance for frequencies between 250 and 3000 Hz. However, no significant difference was found between the MEP_N and MEP_P group, showing similar absorbance values across frequencies.

In comparing WBA_{PP} and WBA₀ between the groups, the normal MEP group had similar mean absorbance values across the frequencies for both peak and ambient pressures (Figure 2a). In contrast, lower absorbance was observed for ambient pressure up to 1250 Hz for the MEP_P group (Figure 2c) and up to 2500 Hz for the MEP_N group (Figure 2b). The results of a paired *T*-test performed between peak and ambient pressure within groups showed a significant difference at low and mid-frequencies between any two groups. Though little difference in absorbance values was observed for the normal MEP group, there was a small but significant difference between 250 and 1000 Hz (Figure 2a). Also, there was a significant difference for frequencies between 500 and 1000 Hz for the MEP_P group, and from 250 to 2500 Hz for the MEP_N group.

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The most marked observation that emerged from the data comparison, as seen in Figure 2d, was the mean difference in absorbance between peak and ambient pressure. For the normal MEP group, the difference in WBA across frequencies are hardly distinguishable, ranging from 0.02 to 0.04 absorbance units between 300 and 1000 Hz. Whereas, for the MEP_P group there was a marginal increase in mean difference between 500 and 1000 Hz (0.07 to 0.11 units). Of most interest, the mean difference was most significant for the MEP_N group, which increased from 0.07 absorbance at 250 Hz to a maximum of 0.36 absorbance at 1000 Hz and decreased beyond. At frequencies above 2500 Hz, there was a negligible mean difference for all the study groups.

Table 1 summarises the mean difference of WBA_{PP}–WBA₀ for each of the groups and the significance level obtained between the groups. Overall, the findings show a significant mean absorbance difference across the groups [*F*(32,166) = 9.17, *p* <0.000; Wilk's Λ = 0.131, $\eta_{\rm P}^2$ = 0.639]. Further analysis across frequencies revealed a significant difference for all frequencies up to 2500 Hz (*p*<0.05), with the effect size ($\eta_{\rm P}^2$) increasing from 0.31 at 250 Hz to 0.64 up to 1000 Hz and reducing to <0.3 above 1500 Hz. Additionally, post hoc Tukey's HSD analysis revealed significant differences between the normal MEP group and MEP_N up to 2500 Hz, and MEP_N and MEP_P up to 2000 Hz, whereas a significant difference was obtained only at 1000 Hz between the normal MEP group and the MEP_P group. On the whole, WBA at 1000 Hz showed a significant difference in all the groups, pointing to it being a distinctive indicator that could be considered for differentiating the abnormal pressure group from the normal MEP group.

To summarise, WBA at peak pressure was higher than at ambient pressure, especially at low and mid frequencies, irrespective of the study group. However, the difference in magnitude was more significant for the MEP_N group, negligible for the normal MEP group, and intermediate for the MEP_P group. The most notable mean difference between WBA_{PP} and WBA₀ was seen between 600 and 1000 Hz, with a significant difference at 1000 Hz for all the groups and this could be a distinctive indicator for differentiation. Also, in the MEP_N group the difference in absorbance between the two pressure conditions was extended across a wider frequency range. In contrast, for the MEP_P group it is restricted to a smaller frequency range. The findings of the study confirm the usefulness of measuring WBA_{PP} and WBA₀. Further, the difference in absorbance values between pressure conditions seems to be most sensitive across the mid-frequency range.

Discussion

The purpose of this study was to compare WBA at peak pressure and ambient pressure in ears with an abnormal

positive and negative pressure with that of normal individuals having normal middle ear pressure. The WBA was least at 250 Hz, increased gradually as the frequency increased and reached a maximum at 2000 Hz in ears with normal hearing. At higher frequencies, the absorbance reduced gradually to a minimum at 6000 Hz and beyond. These findings are very similar to what has been found previously and have an identical absorbance pattern. However, the reported frequencies of maximum absorbance are not exactly consistent across studies and are reported to be anywhere between 2000 and 4000 Hz [16-18]. A few studies have shown other maxima in the region of 1000 Hz to 1500 Hz [16,19], whereas the present study found maximum absorbance at 2000 Hz. This variation in absorbance values might be attributed to racial differences, since studies have reported differences in absorbance in different ethnic populations [17,20].

The outcomes of the present study indicate that, especially in the negative MEP group, there is a significant difference in WBA at low and mid-frequencies, with lower absorbance at ambient pressure compared to peak pressure. In contrast, a negligible difference in WBA occurs for adults with normal MEP. Limited studies have reported a change in WBA measured at abnormal MEP, and these have been performed mostly in young children [9–11,21]. Few efforts have been made to examine the effect of negative MEP on WBA in adults. Earlier studies on negative MEP indicated a decrease in absorbance for frequencies below 3 to 4 kHz and a small increase in absorbance at higher frequencies [4,7,12]. Our findings appear to well support the earlier studies. The present study showed decreased absorbance at low and mid

Table 1. Mean difference of WBApp-WBA0 for three groups and its significance level

Frequency (Hz)	Mean difference of WBApp-WBA ₀			Significance level (<i>p</i> -value)			Ffferet size
	CG	MEPP	MEPN	CG vs. MEP _P	CG vs. MEP _N	MEP _N vs. MEP _P	enect size (ηp²)
250	0.01	0.00	0.07	0.66	0.00*	0.00*	0.31
300	0.02	0.00	0.09	0.68	0.00*	0.00*	0.34
400	0.02	0.03	0.14	0.94	0.00*	0.00*	0.37
500	0.03	0.07	0.19	0.43	0.00*	0.00*	0.38
600	0.04	0.09	0.24	0.18	0.00*	0.00*	0.41
800	0.03	0.11	0.32	0.09	0.00*	0.00*	0.53
1000	0.02	0.09	0.36	0.05*	0.00*	0.00*	0.64
1250	0.00	0.03	0.28	0.65	0.00*	0.00*	0.52
1500	0.00	-0.01	0.20	0.99	0.00*	0.00*	0.33
2000	0.00	0.01	0.09	0.91	0.00*	0.04*	0.14
2500	0.00	0.02	0.06	0.50	0.00*	0.14	0.15
3000	0.00	0.01	0.02	0.48	0.09	0.89	0.05
4000	-0.01	-0.01	0.01	0.88	0.08	0.07	0.07
5000	0.00	-0.03	0.02	0.95	0.12	0.06	0.32
6000	0.00	-0.02	0.01	0.74	0.31	0.56	0.28
8000	-0.02	-0.02	-0.05	0.95	0.69	0.66	0.01

*Significance level *p*<0.05

CG - Control group (Normal MEP); MEP_N - Negative middle ear pressure group; MEP_P - Positive middle ear pressure group

frequencies up to 2500 Hz, with increased absorbance at high frequencies and a noticeable change of WBA between 1.0 to 1.5 kHz. Also, at ambient pressure, studies have reported a decrease in absorbance up to 4000 Hz for children with mild negative MEP of < -100 daPa [10]. Similar findings of reduced WBA from 0.5 to 1.5 kHz with self-induced negative MEP varying from -40 to -125 daPa were reported in adults, compared to baseline ambient pressure measurements [22].

In ears with positive MEP, the present study shows a decrease in WBA up to 3000 Hz, with the most significant reduction seen around 1000 to 2000 Hz for both the peak and ambient pressure. These results agree well with another study that has reported WBA in ears with positive MEP compared in normal MEP, a decrease in WBA of around 0.2 absorbance units at 1000 Hz, with a decrease to 0.11 absorbance units at 2850 Hz [23]. However, studies of the effect of positive MEP on WBA have rarely been reported.

Similarly, studies in the literature have reported a significant reduction in WBA at ambient pressure compared to the peak pressure, irrespective of the middle ear pressure conditions [7,11,13]. In the present study, WBA at peak pressure was higher than the ambient pressure conditions for both the normal MEP group and the clinical group. In the normal MEP group, the mean difference calculated between peak and ambient pressure was small, having a maximum mean difference of about 0.03 at 800 Hz. In the clinical group, the mean difference was most considerable for the negative MEP group, which had a mean difference ranging from 0.90 to 0.36 between 300 and 2000 Hz and reaching a maximum at 1000 Hz. On the other hand, positive MEP elicited a maximum difference of 0.11 at 800 Hz. Similar findings have also been reported in a recent study that showed the highest mean difference of 0.12 to 0.42 between 250 and 2000 Hz in an ETD group, i.e., individuals with negative MEP and a minimum difference for the normal MEP group of 0.06-0.09 from 600 to 1500 Hz [11].

The amount of reduction in absorbance at low and mid frequencies could be due to increased stiffness of the middle ear due to the presence of negative or positive MEP [13]. This generates a larger impedance at the level of the TM by increasing its stiffness, thereby reflecting more energy back to the ear canal [6,13,24]. Further, at higher frequencies the study indicated no significant difference in WBA regardless of a change in MEP, and the WBA values were similar across the study groups. This could be because the transmission of sound energy at high frequencies is not stiffness controlled [25]. Hence, the WBA values at those frequencies are not affected and have a similar pattern despite pressure variations.

However, for those with negative MEP, the WBA was significantly higher at peak pressure compared to ambient pressure, and reached absorbance values similar to those with normal MEP. This is because the TPP plays a significant role in equalising the pressure between the ear canal and middle ear, thereby allowing maximum energy to enter the middle ear [13]. Thus, applying ear canal pressure to compensate for the negative MEP helps restore the WBA values nearer to baseline values [7,11,13]. By way of contrast, in the positive MEP group the WBA did not improve

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much with compensated pressure, i.e. at TPP. Though there is no research focused on positive MEP, similar findings of reduced WBA at low and mid-frequencies with a minimum difference between peak and ambient pressures were reported in the early stages of otitis media [11].

In an apparent contradiction, there are studies which report similar WBA findings irrespective of the MEP, i.e. positive or negative. This might be attributed to a situation in which the position of TM and the direction of umbo displacement are generally similar, generating stiffness at the level of the TM for both conditions [12,23]. As indicated earlier, the stiffness of the TM significantly alters the transmission of low-frequency sounds more than the transmission of highfrequency sounds [6,13,24]. This is probably the reason why significantly lower absorbance values are measured at low frequencies. However, the reason for having different absorbance values at TPP between negative pressure and positive pressure groups is not clear. This difference suggests that, in addition to altered TM stiffness which is the major contributing factor, there may be different mechanisms that affect the transmission of sound. To the best of the authors' knowledge there have so far been no explanations that can support these differences.

However, there are two different mechanisms that could be postulated to explain the difference, both of which consider physiological changes due to MEP.

First, despite maintaining the ear canal pressure which is equal to MEP, the fact of the matter is that the MEP remains either positive or negative or might make it more positive or negative due to a change in position of the TM. Generally, positive pressure tends to push the mucous layer against the bony wall of the middle ear, leading to a more rigid surface that tends to reflect more sound. Whereas negative pressure tends to push the mucous layer against the wall making it more flaccid, and it tends to absorb sound [26]. The TM moves to and fro with the sound wave, which results in movement of the air particles present in the middle ear in a similar fashion having the same frequency. In the case of positive pressure in the middle ear, which increases the rigidity of the middle ear wall, would reflect the sound waves [26]. Thus, it might restrict the movement of TM, leading to a reduction in transmission of sound, which is more likely to affect the lower frequencies compared to high frequencies because of its wavelength properties.

Another possible reason could be that positive pressure in the middle ear would push the round window inside the scala tympani, leading to a reduction in volume. This would probably increase the pressure and restrict the movement of the stapes footplate, leading to reduced transmission of sounds [27]. However, an opposite action can be expected in case of negative MEP.

These two reasons could have altered the WBA; however, it has to be experimentally verified in vivo or in vitro.

As shown in our current results and also in support of the earlier literature [4,7,9,13], the mid-frequency region between 600 and 1000 Hz can be considered as a way to identify individuals with abnormal positive or negative pressure. Any difference in mean WBA between peak and ambient pressure of more than 0.19, observed from 600 to 1000 Hz, indicates negative pressure; a difference of around 0.03 to 0.11 indicates positive pressure; and no change or negligible change of less than 0.03 can be considered as an indicator of a healthy middle ear with normal MEP. Also, concerning the WBA pattern, one can expect a lesser change in absorbance between the peak and ambient pressure in individuals with normal MEP and normal hearing. Similarly, a more significant change in WBA observed only at ambient pressure is an indication of abnormal negative pressure, whereas individuals with abnormal positive pressure will show a more considerable change in WBA observed at both peak and ambient pressure.

Clinical implications

An important implication of these findings is the importance of the difference in WBA value obtained between peak and ambient pressure in ears with positive and negative MEP. The difference in WBA patterns obtained at TPP and ambient pressure could indicate the type of pressure within the middle ear. The deterioration of WBA at peak pressure to the level of ambient pressure without any difference between the two, suggests the presence of positive MEP, and thus the possibility of having an early stage of acute otitis media without effusion. Therefore, understanding the effects of MEP on WBA can aid in improving the diagnostic accuracy and differential diagnoses of middle ear pathologies. Although the study showed interesting results on the effect of MEP on WBA, it suffers from some limitations due to small sample size and the participants

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being selected based on the outcome of the conventional 226 Hz probe tone tympanometry.

Conclusion

The present study showed a difference in WBA measured between the peak and ambient pressure at low and mid-frequencies with a noticeable change seen between 600 and 1000 Hz. The study suggests that the differential criteria - the mean difference between WBAPP and WBA₀ - along with the WBA pattern, could be used to differentiate abnormal MEP from the MEP of healthy ears. Also, one can expect to see a larger difference in absorbance values between the two pressure conditions in the limited mid-frequency range for MEP_P individuals, with a wider frequency range for MEP_N individuals. Thus, the inclusion of WBA measured at peak and ambient pressure could be a supplementary tool for early identification of middle ear pathologies, in particular abnormal MEP due to middle ear effusion or ETD, and thereby could promote effective treatment.

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