Comparing the Results of Acoustically Evoked Short Latency Negative Responses Recorded by 500 and 1000 Hz Tone Burst

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In the auditory brainstem response of the patients with profound hearing loss with severe acoustic stimulation, a negative deflection with 3–4 ms latency has been reported, which is named N3 potential or acoustically evoked short latency negative response (ASNR). It is assumed to be a vestibular, and specifically saccular evoked potential. The purpose of the current study was to compare the results of two 500 and 1000 Hz frequencies in ASNR recording, which is a new test for assessing saccular and inferior vestibular nerve. The present cross-sectional study was performed on 20 congenital profoundly hearing-impaired volunteers (age range of 18-40 years old) who were selected by non-probability sampling method from the deaf community in Tehran. All the participants were examined by auditory brainstem response test with 500 and 1000 Hz tone burst and vestibular evoked myogenic potential test. The applied statistical tests included Kolmogorov-Smirnov and paired t tests. There was statistically significant difference between the threshold and N3 potential latency at the frequencies of 500 and 1000 Hz (p-value=0.037 and P-value=0.050, respectively). No statistically significant difference was found between the response amplitude at these two measured frequencies (P-value=0.901). 500 Hz ASNR was recorded in 33 ears (97.05%) from 34 ears with normal p13 and n23 while this value decreased by 79.41% (27 ears) for 1000 Hz. Despite N3 recording with the frequency of 500 Hz in 7 ears, no response was observed at 1000 Hz. Since ASNR was observed mostly in profoundly hearing impaired ears with normal p13 and n23, it can be concluded that ASNR can be used in cases in which there is no possibility of recording VEMP. Also, the frequency of 500 Hz is more capable in motivating ASNR when compared with 1000 Hz tone burst.

Key words: Vestibular Evoked Myogenic Potentials (VEMP), Acoustically Evoked Short Latency Negative Response (ASNR), N3 potential, Congenital Profound Hearing Loss, Tone burst stimulus.

Internal ear is composed of two main parts of hearing and balance. The balance performance is controlled by semicircular canals and otoliths (utricles and saccules). Cochlea is not the only organ in the internal ear which is activated by acoustic energy. Otolithic organs, especially saccules, also respond to intense sounds. Physiological studies on humans and neurophysiological studies on animals have shown that sounds can lead to the stimulation of vestibular system. Currently, vestibular evoked myogenic potentials (VEMP) are the only diagnostic instruments for the clinical evaluation of saccule and inferior part of vestibular nerve. The recorded

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response is in response to the short-term severe acoustic stimuli caused by the contraction elimination of sternocleidomastoid (SCM) muscle\(^3\). Afferent nerve, inferior vestibular nerve and its efferent pathway are the spinal-vestibular pathway\(^4\). VEMP is independent from the performance of cochlea since it can be also recorded in the patients with severe to profound sensorineural hearing loss\(^5,6\). Auditory brainstem response (ABR) has been widely used as a clinical test of hearing pathway. ABR includes fast and slow waves within 10 ms after the beginning of the stimulation. Five positive peaks from fast waves (I-V waves) have been identified\(^2\). Apart from these positive waves, Kato et al.,\(^7\) observed a large negative stimulation with the latency close to 3 ms during the ABR test with 1-5 kHz click stimulus in some profound hearing-impaired patients and called it N3 or acoustically evoked short latency negative response (ASNR). They expressed that this potential was not an electrical artifact but was a nervous physiological response which was evoked by a loud sound; its ASNR and latency increased and decreased with the rise in the stimulus intensity, respectively. They proposed that this 3 ms response can be a saccular-dependent acoustic response\(^7\).

Short latency, v-shaped wave and dependence on high intensity of stimuli are the three most important features of N3\(^7\). Due to the v shape, wave depends on the vestibule and is distinct from the waves aroused from the hearing pathway (such as ABR)\(^2,7\). Nong et al.,\(^2\) studied the patients with sensorineural hearing loss using the ABR click and stated that ASNR can have a vestibular origin which is specifically dependent on the VEMP-similar saccule.

The investigation on 23 deaf children by Emara\(^8\) revealed that ASNR could be recorded in 30.43% of children, all of whom were normal VEMP people. He found no relationship between the presence of ASNR and residual hearing.

Moreover, Versino et al.,\(^9\) compared ASNR and VEMP (acoustic and galvanic) and introduced N3 potential as a far-field brainstem potential which was most probably generated from vestibular nuclei and recorded independent from SCM muscle contraction and pathway integration from secondary nuclei to SCM muscle.

When ASNR has a vestibular origin, it can be considered a new clinical test for the investigation of vestibular system which facilitates the implementation of the VEMP test because SCM muscle should be contracted for VEMP recording. However, ASNR can be used for the evaluation of people who cannot contract their cervical muscle or those who do not understand the instruction of muscle contraction. Since different neural pathways are assumed for these two responses, they can be considered as two supplementary methods or the evaluation of the saccular performance\(^9\). Therefore, the aim of the present study was to examine ASNR more detailed (latency, amplitude and threshold) at two experimental frequencies of 500 and 1000 Hz and compare the results of these two experimental frequencies for ASNR recording.

**MATERIALS AND METHODS**

The present cross-sectional study was performed on 20 congenital profoundly hearing-impaired volunteers (8 women and 12 men) in the age range of 18-40 years old who were selected from the statistical community by non-probability sampling method. All the volunteers received VEMP and ABR tests in the Audiology Clinic of Faculty of Rehabilitation, Shahid Beheshti University of Medical Sciences. The inclusion criteria of the study included having airway hearing thresholds of 90 DB nHL at the octave frequency of 250-8000 Hz (profound hearing loss), no history of balance disorders, having normal tympanogram, no history of taking ototoxic medications, or suffering from cervical problems like arthritis and no history of traumatic brain injury (TBI). After determining the required samples and obtaining written consent, the history of all the participants was taken using a questionnaire. Those who had the inclusion criteria were entered into the research project. Then, vestibular-hearing tests including pure tone audiometry, tympanometry, ABR and VEMP tests were performed.

This study was approved by the Ethical Committee of Tehran University of Medical Sciences and all the people participating in this study presented their written testimonial.

Airway hearing thresholds at the octave frequencies of 250 to 8000 Hz were measured using the two-canal audiometry system of Interacoustic, Model AC30 (made in Denmark). To investigate
the health and normal performance of middle ear and tympanic membrane, tympanometry was done using the acoustic immittance system of Interacoustic, Model AT229 (made in Denmark).

VEMP test was done on all the participants of the study by an ICS Charter EP system (made in America). SCM muscle was considered as a target muscle. To activate SCM muscle, the participant was asked to sit on a chair and tilt his/her head 30 degrees forward and 30 degrees toward the opposite side of the desired muscle. To control and maintain muscular contraction during the test, the feedback method [10] was applied. In this method, the cuff of a sphygmomanometer was inflated up to 20 mm Hg and the participant placed the cuff between his/her chin and shoulder and pressed the bag in order to keep the pressure constant at 40 mm Hg until the end of the experiment. The invert and noninvert electrodes were placed in the upper end of sternum and upper one-third of SCM muscle, respectively. The impedance between the electrodes was less than 5 ohm and the responses were intensified by 5000 times. To determine the amounts of latency of p13 and n23, the 500 Hz tone burst acoustic stimulus with 2-0-2 cycle at the intensity level of 97 DB nHL was used. The number of stimuli per second (rate), band-pass filter, time window and the number of stimulation repetition (sweep) were 5.1 Hz, 10-2000 Hz, 50 ms and 150 cases, respectively. The reproducibility or lack of reproducibility of response was interpreted as the presence or lack of VEMP. Moreover, in order to avoid confounded responses as a result of cervical fatigue, one min resting was considered after recording each wave.

To record N3, all the participants were evaluated by ABR using the ICS Charter EP system (made in U.S.A). Invert and noninvert electrodes were placed on the mastoid of the experimented ear and the middle region of the forehead on the hair line, respectively. Since saccular afferents have the best response to the 500 and 1000 Hz frequencies compared with other frequencies[11, 500 Hz tone burst (rise and fall time of 1 ms and plateau time of 2 ms)]12 and 1000 Hz tone burst (rise and fall times of 1 ms and plateau time of 0 ms)13 were used in ASNR recording. Tone burst stimulus at the intensity levels of 70-100 DB nHL was presented via an insert phone to the experimental ear. For the 1000 and 500 Hz stimulation, rarefaction and alternating polarities were used, respectively. Number of stimuli per second, investigation period, band-pass filter and number of stimulation repetition were considered as 10.1 Hz, 10ms, 100-3000 Hz and 1000-1800 times, respectively. The responses were intensified by 105 times.

In case of having three following features, the recorded wave was N3 potential: 1) emergence of response peak of 3-5 ms after starting the

### Table 1. Comparing threshold, latency and amplitude of N3 potential at two experimental frequencies of 500 and 1000 Hz in the studied people

<table>
<thead>
<tr>
<th>Variable of N3 potential</th>
<th>500 Hz tone burst</th>
<th>1000 Hz tone burst</th>
<th>*Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold dBNHL</td>
<td>89.43±1.03</td>
<td>91.96±0.91</td>
<td>0.037</td>
</tr>
<tr>
<td>Latency ms</td>
<td>3.37±0.07</td>
<td>3.52±0.10</td>
<td>0.050</td>
</tr>
<tr>
<td>µVAmplitude</td>
<td>0.24±0.01</td>
<td>0.25±0.02</td>
<td>0.901</td>
</tr>
</tbody>
</table>

* In all cases, the significance level is 0.05.

### Table 2. Investigating the relationship between the results of VEMP and ASNR tests with the frequencies of 500 and 1000 Hz in the studied people

<table>
<thead>
<tr>
<th>Presence of ASNR</th>
<th>Lack of ASNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Hz</td>
<td>1000 Hz</td>
</tr>
<tr>
<td>VEMP</td>
<td>Response presence</td>
</tr>
<tr>
<td>Lack of response presence</td>
<td>4(80%)</td>
</tr>
</tbody>
</table>
stimulation, 2) reproducible response, and 3) the amplitude of more than 0.05 µv. The responses at each intensity level were tracked several times. Increase in latency was tried to be obtained by tracking at lower intensity levels; otherwise, the wave was not considered a response. If there were two or several samples for the N3 response, the highest peak was selected as the peak. In each ear, latency, amplitude and threshold of N3 potential were measured at two experimental frequencies of 500 and 1000 Hz.

To examine the normal distribution of data, Kolmogorov-Smirnov test was used. To compare the variables, the paired t test was applied. It seemed that the best method for examining the relationship between VEMP and ASNR was the comparison of response percents. Data analyses were done in SPSS17 software at the significance level of 0.05.

Findings

The volunteers were 20 deaf people (39 ears) with the age range of 18 to 40 years old (mean of 27.69±0.84 years old), none of whom had the history of balance disorder and dizziness. Due to the lack of cooperation, the tests were not done on one of the ears. From among 39 tested ears, normal VEMP was recorded in 34 ears and no response was observed in 5 ears. The mean latency of p13 and n23 was 15.73±0.25 and 24.35±0.26 ms, respectively, and n23-p13 response amplitude was obtained as 125.30±13.16 µv. In Fig. 1, the VEMP waves obtained from the ears of a young man are demonstrated.

Fig. 2 shows a sample of N3 recorded using 1000 Hz tone burst from the left ear of a young man.

The mean amount of latency, threshold and N3 amplitude obtained for the frequency of 1000 Hz in 30 ears was 3.52 ± 0.10 ms, 91.96 ±0.91 DB nHL and 0.25±0.02 µv, respectively; they were 3.27±0.07 ms, 89.43±1.03 DB nHL and 0.01±0.24 µv, respectively, for the frequency of 500 Hz in 37 ears. Considering the significance levels mentioned in Table 1, there was a significant difference between threshold and latency of N3 potential at two frequencies of 500 and 1000 Hz while there was no significant difference between the amplitude of N3 at these two frequencies.

In 34 ears in which normal VEMP was observed, 33 ears (97.05%) had N3 potential for the frequency of 500 Hz while this amount decreased to 27 ears (79.41%) for the frequency of 1000 Hz. In the five ears which did not have any

*Number of ears

Fig. 1. A sample of VEMP waves obtained from the ears of a young man
VEMP, N3 was not observed in 4 and 3 ears with the frequencies of 500 and 1000 Hz, respectively. In one ear, no response (N3 potential and VEMP) was recorded. In 7 ears, although N3 was recorded for the 500 Hz frequency, it did not appear for the 1000 Hz frequency. In table 2, the results of the presence of VEMP and N3 potential at two experimental frequencies are given.

**DISCUSSION**

In this study, it was assumed that ASNR had most probably a saccular-dependent origin. Vestibular nerve fiber is activated via acoustic stimulation of saccule and has the spontaneous discharge rate in the frequency range of 200-1000 Hz\(^{14}\). Thus, the fiber of vestibular afferent of hair cells of a saccule has the highest response to the frequencies of 500 and 1000 Hz and the least sensitivity to the stimuli higher than 3000 Hz\(^{14, 15}\). This selective response of the sensory system of saccule to the special frequency range denotes the importance of tone burst stimulus in the test recording protocol. Additionally, the number of stimuli per second (rate) is also an important parameter; therefore, this study used the 500 and 1000 Hz tone burst as the stimulus for recording ANSR. Moreover, 10.1 Hz was used as the number of stimuli per second; these two cases led to the contradiction in the results of the present research with those of most other studied, which are explained below.

Few studies have compared experimental frequencies in ASNR recording. Thus far, only two studies have examined the stimulus type and experimental frequency. In the first one which was done by Nong et al.,\(^{16}\), the results of ABR click (with the highest energy concentration on 3000-4000 Hz frequencies) obtained from 2384 8 month-old to 70 year-old patients (hearing or hearing-impaired) recorded from 1980 to 1998 were evaluated; it was declared that, from among 653 patients with profound hearing loss, only 12.3% (80 people) showed ASNR response. In 56 patients, ABR evaluation was done with 500 and 1000 Hz tone pip stimuli (rise and fall time of 2 ms and plateau time of 0 ms); 26 patients of whom had profound hearing loss and 10 patients (38.5%) or 43 ears demonstrated ASNR with tone pip stimuli. These reports are not in line with the 97.05% and 76.92% 500 and 1000 Hz tone burst amounts of the present study since the study of Nong et al.,\(^{16}\) was a retrospective study which included all people with and without vestibular disorders and there was no limitation on the studied group. Furthermore, application of the click stimuli and 20 Hz as the number of stimuli per second were the
cases which decreased the occurrence rate of ASNR response.

Also, Nong et al.,16 compared the results of click and tone pip stimuli and stated that the occurrence percentage of ASNR response with 1000 Hz tone pip was more than that of 500 Hz tone pip and click stimuli; however, in the present study, 500 Hz frequency could evoke ASNR in 7 ears which had no response to the 1000 Hz frequency. In other words, in 97.05% of ears with normal saccular performance, N3 was recorded with 500 Hz tone burst stimulus while 1000 Hz frequency of ASNR response was emerged in 79.41% of these ears. In fact, these results were expected because the final organ for the ASNR generation was assumed to be saccule and studies have shown that the main vestibular end organ which responds to sound is saccule and it has the highest sensitivity to the low frequencies12,15.

The second study was related to the ASNR investigation in 17 3-month-old profound hearing-impaired children (15 children in the risk of internal ear problems due to different factors) which was done by Zagolski17. In that study, he used click stimuli and 500 Hz tone burst and reported no significant difference between the distribution of click amplitude and 500 Hz tone burst in ASNR.

Although no click stimulus was used in this study, no statistically significant difference was observed between the response amplitude at two experimental frequencies of 500 and 1000 Hz. Since ASNR is a far-field brainstem potential which is recorded by ABR equipment, therefore, its amplitude is usually small and does not have much clinical variation due to the fact that it is influenced by various factors including personal, stimulus type, recording parameters and, to most extent, by physiological noise levels and impedance of electrodes18,19. Thus, response amplitude is not a proper criterion for the investigation and comparison of stimuli and different frequencies in ASNR recording. Zagolski17 reported the mean N3 response for click and 500 Hz tone burst stimuli as 698.2 and 670.3 nV, which are different from the amounts of 0.24 iv for 500 Hz tone burst and 0.25 iv for the 1000 Hz obtained in this study. This difference can be attributed to the inherent variability of N3 response amplitude, difference in the studied population (children in the risk of internal ear problems versus adults) and differences in the features of the 500 Hz stimulus itself; however, he did not refer to the features of 500 Hz tone burst in his study.

By presenting 1000 Hz short tone burst stimuli with high intensity in the presence of white noise to the experimented ear, Murofushi et al.,11 reported that they could record ASNR response in people with normal hearing for the first time. White noise was probably used for decreasing the possibility of any response with cochlea origin. ASNR was observed with high reproducibility in 23 out of 24 ears (95.8%).

According to the results of Murofushi et al.,’s study, the possibility of recording vestibular response using low tone burst frequency and high intensity was reinforced11. Therefore, tone burst stimulus was used in the present study. 95.7% of their study was close to the occurrence rate of 97.05% of 500 Hz tone burst and 76.92% of 1000 Hz obtained in the present study.

Although their studied population was normal hearing people, the results obtained from this study about the patients with profound hearing loss can be compared with their findings to some extent since the possibility of cochlea intervention in response generation was removed by the presentation of white noise.

Versino et al.,9 evaluated 15 patients with MS along with 31 normal people by galvanic VEMP, acoustic VEMP and ABR. They used the technique of Murofushi et al., (i.e. simultaneous presentation of 1000 Hz short tone burst and white noise to the experimented ear) for recording ASNR. The occurrence rate of ASNR by Versino et al.,9 in normal people (100% with 1000 Hz tone burst) was close to the amount obtained in this study; this was mostly due to the application of 10.1 Hz as the tone burst stimulus and number of stimulus for evoking ASNR response in people with no history of vestibular disorders. This issue re-confirms the importance of stimulus type, low number of stimulus per second and health of vestibular system in recording ASNR response.

CONCLUSION

Considering the findings of the present study, it seems that the occurrence possibility of ASNR response with 500 Hz frequency of tone
burst is higher than 1000 Hz. Also, 500 Hz tone burst provides lower amounts in terms of threshold and latency of ASNR compared with 1000 Hz tone burst. Similar studies with the application of tone burst stimulus in the recording protocol can help in reinforcing the results of this study to a large extent.

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