INTRODUCTION
Hearing impairment is a crucial health problem in childhood. Hearing loss is detected in 1–6 of every 1000 live births [1]. In infants, early detection of hearing impairment is essential because of its detrimental effects on speech and language development [2]. Thereby, language, speech, and cognitive functions can be improved using hearing aids or cochlear or brainstem implants [3]. For children with hearing loss, the period around 6 months of age is critical for therapy using hearing aids in order to maximize speech and language development [4]. Today, various audiological test batteries are used for the detection of hearing impairment. Unfortunately, behavioral hearing testing, also called visual reinforcement audiometry, is not reliable before 6 months of age [5]. In infants, auditory brainstem responses (ABRs) and otoacoustic emissions (OAEs) are the main audiological tests for the detection of hearing impairments worldwide [6]. However, both audiological tests have some advantages and disadvantages. As a matter of fact, OAE is a quick test to determine whether hearing is normal or abnormal, whereas ABR provides an assessment of the level of hearing loss [6].

Auditory steady-state response (ASSR) is an alternative objective audiometric test for the detection of hearing loss in neonates, infants, and adults. It has several advantages: (i) the results at each frequency of stimulation can be automatically detected, (ii) there is no need for audiologists to identify the peaks, and (iii) it is easy to apply [7]. Moreover, unlike screening OAE, ASSR can evaluate the frequency-specific responses [8].

Recently, several clinical studies have evaluated the role of ASSR in for the detection of hearing impairment among infants. The aim of this study was to assess the effectiveness of ASSR, determine the cut-off values for each frequency, and detect the best correlated frequencies when compared with the ABR thresholds.
MATERIALS and METHODS

Participants
In total, 88 healthy term babies under the age of 12 months (43 females and 45 males) with a mean age of 2.98 (1–11) months participated in this study and successfully completed the ABR and ASSR tests. The exclusion criteria were as follows: (i) any abnormal otological finding at physical examination; (ii) presence of a systemic disease, intrauterine problems, craniofacial anomalies, and trauma; and (iii) preterm infants. The parents of all the subjects provided a written informed consent, and this study was approved by the Ethics Committee of Celal Bayar University (20478486-119/24.03.2016).

Audiological Test Batteries
The click-auditory brainstem response and auditory steady-state response tests, which were recorded using ICS Charter (GN Otometrics, Taastrup, Denmark), were performed on the children in a silent room while they were asleep. The click-ABR test was typically performed at 90 dB nHL, and the stimulus intensity was stepwise reduced by 10 dB nHL until the wave V peaks disappeared with a 10-ms duration in a repetition rate of 21.1/s. The signal was recorded using Ag/AgCl disc electrodes in Cz with mastoids M1 and M2 as reference, and electrode impedance was kept below 5 kOhm. Amplifier gain was 100k with a bandpass filter of 100–3000 Hz. Each ABR record was measured twice, and the reproducibility of waves was checked by measuring them twice. For the ABR results, a cut-off value of 30 dB nHL was selected for normal hearing level depending on the literature data [9].

Auditory steady-state responses were evoked using a dichotic multiple-frequency technique stimulating both ears with carrier frequencies of 500, 1000, 2000, and 4000 Hz. The stimuli were modulated at modulation frequencies of 90, 82, 88, and 84 Hz for the right ear and at 94, 86, 85, and 83 Hz for the left ear for the 0.5-, 1-, 2-, and 4-kHz tones, respectively. Ag/AgCl disc electrodes were placed in Cz, midline posterior neck, and Fpz. Impedances of the electrodes were kept below 5 kOhm and interelectrode impedances below 3 kOhm. The bioelectric activity was amplified and filtered using a high-pass filter of 65-Hz and low-pass filter of 300-Hz. Each recording sweep was composed of 16 epochs of 1024 data points. Sweeps were averaged in the time domain and subsequently analyzed in the frequency domain using a fast Fourier Transform. The presence of a response was calculated using the F-ratio when the response was significantly different (p<0.05) from background noise at the stimulation modulation frequencies. When the signal-to-noise ratio reached significance (p<0.05), an ASSR is considered to be present. The ASSR thresholds were determined for each of the frequencies at 500, 1000, 2000, and 4000 Hz by stepwise increasing or decreasing the stimulus presentation level by 10 dB. The thresholds of ASSR were considered as the minimum intensity of the detected responses. Stimulation was monaurally performed separately to the right and left ears.

Statistical Analysis
All data are presented as mean ± standard deviation. The statistical analysis was performed using Statistical Package for the Social Sciences v2.0.0 for Mac (SPSS Inc.; Chicago, IL, USA). Independent samples t-tests were used to compare the results of ASSR between the normal hearing and abnormal hearing groups. Pearson’s correlation test was used to analyze the correlations between the ABR and ASSR (for each frequency) thresholds, individually. The strength of correlation was classified as previously described [10]. A receiver operating characteristic (ROC) curve analysis was also performed. Differences with p<0.05 were considered to be statistically significant.

RESULTS
In total, 174 ears of 88 (43 females and 45 males) infants (two ears of two babies were excluded as they started to cry during assessment of the second ear) were evaluated. Of them, 135 ears had normal hearing values and 39 ears had hearing loss according to the ABR thresholds. The mean ASSR thresholds for each frequency are shown in Table 1. For all frequencies, statistically significant differences were determined when the ASSR values of normal hearing ears were compared with those of hearing-impaired ears (p<0.05).

In both normal and hearing-impaired ears, statistically significant correlations were detected between the ABR and ASSR thresholds at 500, 1000, 2000, and 4000 Hz and the mean values of 1000–4000 Hz and 2000–4000 Hz as presented in Table 2 (r=0.233, p=0.002; r=0.270, p=0.000; r=0.290, p=0.001; r=0.324, p=0.000; and r=0.346, p=0.000, respectively). The results showed that there was a moderate positive correlation between the results of the ABR and ASSR responses at 1000 Hz and the mean values of 1000–4000 Hz and 2000–4000 Hz in the hearing-impaired group (r=0.419, p=0.008; r=0.370, p=0.02; and r=0.408, p=0.01, respectively). In addition, a strong correlation was found between 4000 Hz ASSR thresholds and ABR thresholds in the hearing-impaired subjects (r=0.506, p=0.001).

The receiver operating characteristic curves and the cut-off values for each ASSR frequency for the detection of hearing-impairment thresholds are shown in Figure 1. The best cut-off values for each group were: 45 dB for 500 Hz (sensitivity=0.821, specificity=0.430), 35 dB for 1000 Hz (sensitivity=0.821, specificity=0.467), 35 dB for 2000 Hz (sensitivity=0.846, specificity=0.548), 35 dB for 4000 Hz (sensitivity=0.769, specificity=0.481), 37.5 dB for mean value of 1–4 kHz (sensitivity=0.846, specificity=0.496), and 37.5 dB for mean value of 2–4 kHz (sensitivity=0.846, specificity=0.474).
DISCUSSION

Early detection and identification of hearing impairment are very important for speech, language, hearing, social, psychological, and educational developments. ABR and OAE are the auditory tests commonly used for hearing screening among newborns. Although the ABR test is a gold standard test for newborn auditory screening, it has some limitations, such as a long testing time and precise interpretation required for wave V analysis. On the other hand, OAE provides rapid detection and is easier to evaluate for hearing screening; however, it does not provide any information about the severity of hearing impairment and is affected by ear wax or effusion. ASSR is an objective rapid auditory test that has the advantages of the ABR and OAE tests. It can be easily performed automatically without the need for a professional. Moreover, ASSR can be evoked by stimuli at different frequencies and shows the level of hearing at each frequency. This feature could be used for choosing the treatment using hearing aids or cochlear implants for congenital hearing loss and for medicolegal auditory assessment.

The auditory steady-state response thresholds for the different frequencies showed that the results at 500-Hz stimulus had the highest thresholds for all frequencies (Table 1). In addition, previous studies have shown that thresholds for 500-Hz stimulus might be at higher levels than those for the other frequencies. Various hypotheses had been suggested to explain the underlying mechanism of this finding. Lins et al. stated that stimulation at lower frequencies affects an extensive area of the basilar membrane because the time required for waves to travel to the apical region of the cochlea is longer and there might be a delayed jitter between receptors and stimulations. The other hypothesis is the immature neural synchronization in low-frequency responses. In addition, problems with earphones such as unsuitable placement, causing collapse or distortion of the external auditory canal may have an effect, particularly on lower frequencies.

In the present study, moderate correlations were determined between the ABR and ASSR thresholds at 4000 Hz and at the mean values of 1000–4000 and 2000–4000 Hz in all subjects (Table 2). Some investigators reported better correlations than those reported by us. Vander Werff et al. found strong correlations between the 2-kHz threshold and the mean values of the 2–4 kHz ASSR responses and click-ABR results (r=0.96 and r=0.97, respectively). Similarly, Swanepoel and Ebrahim reported that the mean values of 1–4 and 2–4 kHz had the best correlations between ASSR and ABR; however, strong correlations were found among other frequencies as well (r_{1kHz}=0.82, r_{2kHz}=0.86, r_{3kHz}=0.85, r_{4kHz}=0.85, r_{average1–4kHz}=0.92, and r_{average2–4kHz}=0.92). The possible reason for the difference could be the mean age of our subjects (2.98 ±2.58 months), which was much lower than that in previous clinical studies. As a matter of fact, the myelinization and development of the auditory system is a continuous process during infancy; therefore, both the ABR and ASSR responses keep changing during maturation.

In the hearing-impaired group, there were moderate correlations between the results of the two tests, particularly for 1 kHz, and mean values of 1–4 and 2–4 kHz. The values of correlation coefficients in babies with hearing loss showed that the best and strongest correlation was between the ABR and ASSR thresholds at 4 kHz. On the other hand, Swanepoel and Ebrahim found the poorest correlation at 4 kHz in patients with sensorineural hearing loss. This situation could be explained as follows: In that study, the subjects were subdivided into two groups, those with conductive hearing loss and those with sensorineural hearing loss. The correlation levels were different for the two groups. Infants with conductive hearing loss had higher correlation coefficients for 4-kHz responses than those for 1 and 2 kHz, despite the fact that lower levels were found in patients with sensorineural hearing loss. In that study, the patients were diagnosed with conductive hearing loss using tympanometry; however, this method was not used in our subjects, excluding those with abnormal ear findings in physical examinations. In addition, tympanometry may not be useful for assessing conductive hearing loss in infants, especially when accomplished using a standard probe tone frequency of 220 or 226 Hz. Otitis media with effusion and membrane retractions could be easily diagnosed with physical examination. On the other hand, consistent with our data, Firszt et al. found significant correlations between high frequencies of the ASSR and ABR thresholds in subjects with hearing loss. One of the possible reasons might be that click-ABR stimulated mainly the higher frequencies, such as 2 and 4 kHz; therefore, these frequencies could provide more information about the hearing status of the patient, and a stronger correlation could be seen.

<table>
<thead>
<tr>
<th>Table 1. Mean values of ASSR results for each frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients Normal-hearing patients Hearing-impaired patients</td>
</tr>
<tr>
<td>n=174</td>
</tr>
<tr>
<td>ABR thresholds 30.1 (±13.49)</td>
</tr>
<tr>
<td>ASSR 1000 Hz (dB) 45.6 (±18.94)</td>
</tr>
<tr>
<td>ASSR 4000 Hz (dB) 44.5 (±18.73)</td>
</tr>
<tr>
<td>Mean values for 1–4 kHz (dB) 44.2 (±15.96)</td>
</tr>
<tr>
<td>ABR: Auditory brainstem response; ASSR: auditory steady-state response</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Correlation coefficients between the ABR and ASSR thresholds for all subjects and hearing-impaired subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects Hearing-impaired subjects</td>
</tr>
<tr>
<td>n=174</td>
</tr>
<tr>
<td>Correlation coefficient p</td>
</tr>
<tr>
<td>ASSR 500 Hz 0.233 0.002</td>
</tr>
<tr>
<td>ASSR 1000 Hz 0.270 0.000</td>
</tr>
<tr>
<td>ASSR 2000 Hz 0.290 0.001</td>
</tr>
<tr>
<td>ASSR 4000 Hz 0.310 0.000</td>
</tr>
<tr>
<td>ASSR 1000–4000 Hz 0.324 0.000</td>
</tr>
<tr>
<td>ASSR 2000–4000 Hz 0.346 0.000</td>
</tr>
<tr>
<td>ABR: Auditory brainstem response; ASSR: auditory steady-state response</td>
</tr>
</tbody>
</table>
In the literature, there are a few studies comparing the ABR and ASSR thresholds in the same subjects. A strong correlation between the ABR and ASSR thresholds has been reported [16,17,21]. In contrast, our data demonstrated that the correlations between the two auditory tests were not as strong as reported in previous studies. It is likely that because the subjects in our study were asleep, they had reduced myogenic activity, which is elevated when awake, and this changed the ABR and ASSR thresholds [24, 25]. In addition, no sedative drugs were administered during the study; therefore, some of the subjects might not have been fully asleep, especially during the detection of ASSR thresholds. The evaluation of the ABR test was started just after the baby had fallen asleep. However, the ASSR responses might have elevated in some infants who woke up during ASSR testing due to the length of testing time; the ASSR responses would be more exaggerated in these infants.

In conclusion, ASSR may not be a beneficial and/or reliable screening test for hearing impairment in infants. However, it might detect the frequencies affected in patients with hearing loss and may confirm the results of other tests. Thus, ASSR may be considered to be a complementary test rather than an alternative to ABR.

In this study, the cut-off values for each ASSR frequency that showed hearing impairment were evaluated. The cut-off values for ASSR levels were 45, 35, 35, 35, 37.5, and 37.5 dB for 500 Hz, 1000 Hz, 2000 Hz, 4000 Hz, means of 1000–4000 Hz, and means of 2000–4000 Hz, respectively. Van Maanen and Stapells [24] recommended 50, 45, 40, and 40 dB could be considered as cutoff values between normal hearing and hearing loss for 500, 1000, 2000, and 4000 Hz, respectively. We believe that using tone-ABR and a higher mean age of subjects (the mean age of the patients was 18.3 months in that study) might be responsible for this difference. In addition, another study reported a cut-off value of 30 dB for all frequencies in adults [25]. The differences between studies may be related to the poorer detection of the ASSR responses in patients with mild hearing loss [26].

In conclusion, ASSR may not be a beneficial and/or reliable screening test for hearing impairment in infants. However, it might detect the frequencies affected in patients with hearing loss and may confirm the results of other tests. Thus, ASSR may be considered to be a complementary test rather than an alternative to ABR.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Celal Bayar University.

Informed Consent: Written informed consent was obtained from the parents of the patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - OÇ; Design - OÇ, UU; Supervision - OÇ, GE; Resources - OÇ, GE; Materials - OÇ, GE; Data Collection and/or Processing - OÇ, GE; Analysis and/or Interpretation - OÇ, UU; Literature Search - UU; Writing Manuscript - UU; Critical Review - OÇ; Other - GE.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES


2. Pimperton H, Kennedy CR. The impact of early identification of permanent childhood hearing impairment on speech and language outcomes. Arch Dis Child 2012; 97: 648-53. [Crossref]


5. Casey KA, Small SA. Comparisons of auditory steady state response and behavioral air conduction and bone conduction thresholds for infants and adults with normal hearing. Ear Hear 2014; 35: 423-39. [Crossref]


9. Stapells DR, Gravel JS, Martin BA. Thresholds for auditory brainstem responses to tones in notched noise from infants and young children with normal hearing or sensorineural hearing loss. Ear Hear 1995; 16: 361-71. [Crossref]


23. Scherf F, Brox J, Wuyts FL, Van de Heyning PH. The ASSR: clinical application in normal-hearing and hearing-impaired infants and adults,
comparison with the click-evoked ABR and pure-tone audiometry. Int J Audiol 2006; 45: 281-6. [Crossref]


