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Evaluation of the Relationship Between the SP/AP Ratio at Electrocochleography and Vitamin B_{12} Levels in Tinnitus Patients with Normal Hearing

Normal İşitmeye Sahip Tinnitus Hastalarında Elektrokokleografideki SP/AP Oranı ile B_{12} Vitamini Düzeyleri Arasındaki İlişkinin Değerlendirilmesi

Murat YAŞAR[a], Fatma ATALAY[a]

[a]Kastamonu University Faculty of Medicine, Department of Otorhinolaryngology, Kastamonu, Türkiye

ABSTRACT Objective: This study aimed to investigate the association between vitamin B_{12} levels and the summation potential/action potential (SP/AP) ratio, a parameter employed in the diagnosis of cochlear synaptopathy (CS), in tinnitus patients with normal hearing thresholds. **Material and Methods:** Fifty-five patients who presented due to tinnitus with normal hearing thresholds, who underwent electrocochleography (ECochG), and whose vitamin B_{12} levels had been measured within the previous 3 months were included in the study. All patients were administered the Tinnitus Handicap Inventory (THI) . Otoacoustic emission measurements were performed on all participants. All the participants' ECochG results recorded using tiptrode electrodes were compared. The SP/AP amplitude ratios were analyzed. Patients with an SP/AP amplitude ratio greater than 50% in at least one ear at the ECochG test were classified as Group 1, and those with values lower than 50% in both ears were classified as Group 2. **Results:** No significant gender and age difference was determined between the groups (p=0.826, p=0.803, respectively). The SP/AP amplitude ratio in Group 1 was significantly higher than that in Group 2, while vitamin B_{12} levels were significantly lower (p<0.001 and p=0.012, respectively). THI levels differed significantly between the groups. A significant positive correlation was observed between the total THI scores and subdimensions, and the subdimensions also exhibited significant positive correlation among themselves. **Conclusion:** Vitamin B_{12} deficiency may cause a rise in the SP/AP ratio employed in the identification of CS. Serum vitamin B_{12} levels should not be overlooked in the evaluation of auditory functions in patients with tinnitus.

Keywords: Tinnitus; vitamin B_{12}; electrocochleography; cochlear synaptopathy

ÖZET Amaç: Bu çalışmada, normal işitme eşiklerine sahip tinnituslu hastalarda koklear sinaptopati (KS) tanısında kullanılan bir parametre olan sumasyon potansiyeli/aksiyon potansiyeli (SP/AP) oranı ile B_{12} vitamini düzeyleri arasındaki ilişkinin araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Çalışmaya, tinnitus şikâyeti ile başvuran, işitme eşikleri normal olan, elektrokokleografi (ECochG) yapılan ve son 3 ay içinde B_{12} vitamini düzeyleri ölçülen 55 hasta dâhil edildi. Tüm hastalara Tinnitus Handikap Envanteri [Tinnitus Handicap Inventory (THI)] uygulandı. Tüm katılımcıların otoakustik emisyon ölçümleri yapıldı. Tüm katılımcıların tiptrode elektrotlar kullanılarak kaydedilen ECochG sonuçları karşılaştırıldı. SP/AP genlik oranları analiz edildi. ECochG testinde en az bir kulakta SP/AP genlik oranı %50'nin üzerinde olan hastalar Grup 1, her iki kulakta %50'nin altında olan hastalar Grup 2 olarak sınıflandırıldı. **Bulgular:** Gruplar arasında cinsiyet ve yaş açısından anlamlı fark saptanmadı (sırasıyla p=0,826, p=0,803). Grup 1'de SP/AP genlik oranı Grup 2'ye göre anlamlı derecede yüksek, vitamin B12 düzeyleri ise anlamlı derecede düşük bulundu (sırasıyla p<0,001 ve p=0,012). THI düzeyleri gruplar arasında önemli ölçüde farklılık gösterdi. Toplam THI puanları ve alt boyutlar arasında önemli bir pozitif korelasyon izlendi ve alt boyutlar kendi aralarında da önemli bir pozitif korelasyon gösterdi. **Sonuç:** B_{12} vitamini eksikliği KS'nin tanımlanmasında kullanılan SP/AP oranında artışa neden olabilir. Serum B_{12} vitamini düzeyleri tinnituslu hastalarda işitsel fonksiyonların değerlendirilmesinde göz ardı edilmemelidir.

Anahtar Kelimeler: Tinnitus; B_{12} vitamini; elektrokokleografi; koklear sinaptopati

Correspondence: Fatma ATALAY
Kastamonu University Faculty of Medicine, Department of Otorhinolaryngology, Kastamonu, Türkiye
E-mail: fatmatalay_88@hotmail.com

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Tinnitus is defined as the perception of a sound occurring with no acoustic stimulus.[1] The reported prevalence is between 2-32%.[2] The pathological mechanisms of tinnitus are still not fully understood. Tinnitus is classified as subjective and objective, which is the most common classification. Lesions in the acoustic nerve and inner ear are frequently implicated among the causes leading to subjective tinnitus.[3] Tinnitus is frequently accompanied by hearing loss, and loss of hearing also increases the probability of tinnitus.[2] However, tinnitus can also emerge in individuals with normal hearing as the result of cochlear damage and loss of outer hair cells.[4,5]

Cochlear synaptopathy (CS) and/or cochlear dead regions are thought to be implicated in the development of tinnitus in individuals with normal hearing.[6] Defined as hidden hearing loss, CS occurs as a result of damage to the synapses between the cochlear nerve fibers and the inner hair cells in the spiral ganglion. CS can cause clinical symptoms such as tinnitus and hyperacusis and difficulty understanding speech in noisy environments.[7,8] An increased summation potential/action potential (SP/AP) ratio at electrocochleography (ECochG) in tinnitus patients with normal hearing can be useful in the evaluation of "hidden hearing loss".[6]

Vitamin B_{12} plays an important role in the synthesis of myelin protein. Vitamin B_{12} deficiency can result in axonal degeneration and demyelination. Deficiency in vitamin B_{12} and folate leads to an increase in homocysteine, a neurotoxin.[2,9] Some studies have reported that vitamin B_{12} deficiency can lead to tinnitus and hearing loss by affecting the myelination of the neurons in the cochlea.[10,11] However, the pathological and neurophysiological mechanisms associated with tinnitus have yet to be fully elucidated. We encountered no previous study investigating the relationship between the SP/AP ratio at ECochG and vitamin B_{12} levels. Therefore, the present study aimed to investigate the association between vitamin B_{12} levels and the SP/AP ratio, a parameter employed in the diagnosis of CS, in tinnitus patients with normal hearing thresholds.

MATERIAL AND METHODS

This study was conducted retrospectively, in accordance with the Declaration of Helsinki and with the approval of the Kastamonu University Clinical Research Ethics Committee (date: November 1, 2023; no: 2023-KAEK-130). Fifty-five patients who presented to the Kastamonu Training and Research Hospital ear nose and throat clinic due to tinnitus persisting for at least 3 months between October 2022-October 2023, with normal hearing thresholds, who underwent ECochG, and whose vitamin B_{12} levels had been measured within the previous 3 months were included in the study. All the participants' otoscopic examinations and pure sound audiometry and tympanometry results were within normal limits. All patients were administered the 25-question Tinnitus Handicap Inventory (THI) during the examinations. Three response options are available for each question on this inventory, "Yes" (4 points), "Sometimes" (2 points), and "No" (0 points). Depending on their scores, the cases were classified as very mild (0-16), mild (18-36), moderate (38-56), or severe (58-100). The THI also evaluates patients with functional, catastrophic, and emotional subscales. Otoacoustic emission (OAE) measurements were performed on all participants, and individuals who passed the test were included in the study. Patients with middle ear pathologies, histories of otological surgery, or with psychiatric and neurological diseases were also excluded.

All the participants' ECochG results recorded using tiptrode electrodes were compared. The SP/AP amplitude ratios determined using ECochG were analyzed. Patients with an SP/AP amplitude ratio greater than 50% in at least one ear at the ECochG test were classified as Group 1, and those with values lower than 50% in both ears were classified as Group 2. In case of SP/AP amplitude ratios above 50% in both ears, the higher of the 2 values was employed.

OTOACOUSTIC EMISSION ASSESSMENT

OAE measurements in both ears were performed using a Madsen OAE device (Otometrics, Denmark, 2011) and OTOsuite software (Otometrics, Denmark). Transient evoked OAE (TEOAE) tests were conducted using standard techniques at a 1-4 kHz frequency range. For the measurement, an alternating polarity click stimulator at the 85 dB sound pressure level (SPL) in a nonlinear module was used. All

TEOAE tests were conducted using the same device.

AUDIOMETRY AND IMMITANCEMETRY TEST PARAMETERS AND APPLICATION

The tympanometry tests were performed using an Interacoustics AD226 (Denmark) immitancemetry device, with Type A (pressure -50 and +50 daPa) being regarded as normal. Audiometric thresholds were obtained using an Interacoustics AD629 audiometer (Interacoustics, Middelfart, Denmark) and Telephonics TDH-39 earphones (Telephonics Corp., Farmingdale, NY, USA). Pure tone audiometric thresholds were measured at frequencies of 0.25-8 kHz. Pure- tone audiometric thresholds between 1-20 decibel (dB) at all frequencies were considered normal. Patients who demonstrated an inter-frequency difference exceeding 5 dB were excluded from the study.

ELECTROCOCHLEOGRAPHY TEST PARAMETERS AND APPLICATION

ECochG recordings were taken with stimuli created using a Hedera Biomedics Socrates auditory evoked potentials device (Hedera Biomedics, Ancona, Italy), Sanibel electrode cables (Sanibel Supply, Roskilde, Denmark), Ambu® Neuroline™ 720 surface electrodes (Ambu A/S, Ballerup, Denmark), and E-A-RLINK™ gold-foil tiptrode electrodes (3M, St. Paul, MN, USA). A velocity transducer at a rate of 11.1/s, a click stimulus with an intensity of 95 dB nHL, and an alternating polarity click stimulus were employed for the ECoghG tests. One thousand scans were performed for each trace. The active tiptrode electrode was placed in the tested ear, the ground electrode in the center of the forehead, and the reference electrode over the contralateral mastoid. Before electrode insertion, the outer ear was filled with isotonic serum at body temperature, which was left in situ for 1 min. Once the fluid had been removed from the outer ear canal, the electrode was attached as close as possible to the tympanic membrane. It was ensured that the impedance of the electrodes was below 3,000 ohms and the impedance difference between the electrodes was 1,000 ohms or less. The device calculated the SP/AP amplitude ratios automatically once the waves appeared on it. SP/AP amplitude ratios above 50% were regarded as abnormal.[12]

STATISTICAL ANALYSES

Statistical analyses were performed using the SPSS (IBM, Inc., Chicago, IL, USA) version 22 software. Descriptive statistics were expressed as number and percentage values for categorical data and as mean±standard deviation for continuous data. Chi-square analysis (Pearson's chi-square) was applied in the comparison of categorical variables between the groups. The normality of the distribution of the continuous variables was evaluated using the Kolmogorov-Smirnov test. The Student's t-test was applied for paired group comparisons. Pearson's correlation test was used to examine the relationships between the continuous variables. A p values <0.05 was statistically significant.

RESULTS

A total of 55 patients were included in the study, with 27 patients in Group 1 and 28 patients in Group 2. In Group 1, 25.9% were male and 74.1% were female, while in Group 2, 28.6% were male and 71.4% were female. There was no statistically significant difference in the gender distribution between the groups (p=0.826). The mean age was 37.7±14.6 years in Group 1 and 36.8±12.5 years in Group 2, with no significant difference observed (p=0.803). However, the SP/AP amplitude level was significantly higher in Group 1 than in Group 2 (64.0±16.4 vs. 29.0±10.8, p<0.001), whereas the vitamin B_{12} levels were significantly lower in Group 1 than in Group 2 (167.1±102.7 vs. 245.5±120.8, p=0.012). No significant differences were found between the groups in terms of the total THI scores (41.6±21.5 vs. 39.6±20.6, p=0.774) or its functional (21.6±11.1 vs. 20.2±11.2, p=0.657), emotional (18.7±11.8 vs. 18.1±11.3, p=0.831), and catastrophic subscale scores (1.3±3.2 vs. 1.3±3.1, p=0.728) (Table 1).

Table 2 compares the THI scores of patients in Group 1 and Group 2. Similar distributions were observed between the very mild, mild, moderate, and severe levels in both groups. In Group 1, 22.2% of patients were classified as very mild, 14.8% as mild, 40.7% as moderate, and 22.2% as severe; in Group 2, the corresponding percentages were 25.0%, 17.9%, 42.9%, and 14.3%, respectively. According to the

TABLE 1: A comparison of the group characteristics						
		Group 1		Group 2		
		n	%	n	%	p value
Gender	Male	7	25.9	8	28.6	0.826*
	Female	20	74.1	20	71.4	
		$\bar{X}$±SD		$\bar{X}$±SD		
Age		37.7±14.6		36.8±12.5		0.803**
SP/AP amplitude		64.0±16.4		29.0±10.8		**<0.001****
Vitamin B_{12}		167.1±102.7		245.5±120.8		**0.012****
THI (total scores)		41.6±21.5		39.6±20.6		0.774**
THI functional		21.6±11.1		20.2±11.2		0.657**
THI emotional		18.7±11.8		18.1±11.3		0.831**
THI catastrophic		1.3±3.2		1.3±3.1		0.728**

*Chi-square analysis; **Student's t test was applied; SP/AP: Summation potential/action potential; THI: Tinnitus Handicap Inventory

TABLE 2: A comparison of the groups' THI levels					
	Group 1		Group 2		
	n	%	n	%	p value*
Very mild (0-16)	6	22.2	7	25.0	0.926
Mild (18-36)	4	14.8	5	17.9	
Moderate (38-56)	11	40.7	12	42.9	
Severe (58-100)	6	22.2	4	14.3	

*Chi-square analysis was applied.

chi-square test results, there was no statistically significant difference between the groups in terms of THI levels (p=0.926). This finding supports the notion that both groups showed similar distributions in terms of tinnitus severity.

No statistically significant correlation was found between the SP/AP amplitude and the THI total score (r=0.142, p=0.300), THI functional (r=0.169, p=0.216), THI emotional (r=0.072, p=0.602), and THI catastrophic subscales (r=0.087, p=0.526). A strong and significant positive correlation was found between the THI total score and the THI functional (r=0.830, p<0.001), THI emotional (r=0.840, p<0.001), and THI catastrophic (r=0.660, p<0.001) subscales. The subdimensions also exhibited a significant positive correlation among themselves. No significant relationship was found between age and SP/AP amplitude, THI total score, and subscales (p>0.05). Vitamin B_{12} levels exhibited a weak negative correlation with the SP/AP amplitude that approached statistical significance (r=-0.265, p=0.050), but no significant correlations with the THI scores or age were observed (Table 3).

TABLE 3: Correlation between SP/AP amplitude ratios and THI levels							
		SP/AP amplitude	THI (total scores)	THI functional	THI emotional	THI catastrophic	Age (years)
THI (total scores)	r value	0.142					
	p value	0.300					
THI functional	r value	0.169	**0.830**				
	p value	0.216	p<0.001				
THI emotional	r value	0.072	**0.840**	**0.415**			
	p value	0.602	p<0.001	**0.002**			
THI catastrophic	r value	0.087	**0.660**	**0.477**	**0.469**		
	p value	0.526	p<0.001	**0.000**	**0.000**		
Age (years)	r value	0.192	-0.133	-0.158	-0.072	-0.065	
	p value	0.159	0.333	0.250	0.600	0.638	
Vitamin B_{12}	r value	-0.265	-0.160	-0.072	-0.250	0.106	0.147
	p value	0.050	0.243	0.600	0.066	0.441	0.284

SP/AP: Summation potential/action potential; THI: Tinnitus Handicap Inventory

DISCUSSION

Determining the number of cochlear synapses is the gold standard for defining CS. However, it is impossible to determine this in living individuals. ECochG is an objective, non-invasive, electrophysiological measurement used to evaluate the status of the cochlea and cochlear nerve. It can also be used to detect CS. The SP/AP amplitude ratio measured using ECochG shows that CS is probably involved in the development of tinnitus in patients with normal hearing.[13-15] In the present study, the vitamin B_{12} levels of patients with normal audiometric thresholds were significantly lower in the group with a high SP/AP amplitude ratio. This suggests that vitamin B_{12} deficiency may cause CS and to tinnitus in association with this.

Vitamin B_{12} deficiencies are a widespread cause of significant morbidity in all age groups, and since they affect more than one system in the body, they can also have an adverse impact on the quality of life. Because of the many systemic or local effects involved, it is relatively difficult to determine the relationship between the auditory pathway and vitamin B_{12} deficiency or the effect of the duration of vitamin B_{12} deficiency on hearing sensitivity.[16] Because no definite information concerning the duration of vitamin B_{12} deficiency could be obtained in the present study, the effect of that duration on the results is uncertain.

Shemesh et al. examined 113 military personnel exposed to noise and detected chronic tinnitus and sensorineural hearing loss in 57, sensorineural hearing loss alone in 29, and normal audiogram findings in 27. The authors reported that vitamin B_{12} deficiency was more severe and widespread in the group with tinnitus than in the other groups. They also reported some degree of improvement following vitamin B_{12} replacement therapy. Those authors concluded that vitamin B_{12} deficiency was associated with functional impairment in the auditory pathway.[9] Electrophysiological studies have revealed the presence of sensory motor axonal neuropathy in patients with vitamin B_{12} deficiency.[17] A number of animal studies have also revealed the neuropathic effects of vitamin B_1 deficiency.[18] Such a deficiency is capable of leading to hearing loss and tinnitus because of its adverse effect on the myelination of neurons in the cochlea.[10,11] In addition, vitamin B_{12} deficiency is considered capable of causing loss of hearing and tinnitus by leading to the destruction of the microvascular system in the stria vascularis and a decrease in endocochlear potential.[19]

Few studies have investigated the cochlear functions in vitamin B_{12} deficiency. Abnormal auditory brainstem response (ABR) measurements have been detected in tinnitus patients with vitamin B_1 deficiency, and this has been attributed to that deficiency.[10] In their study of individuals with normal hearing, Karli et al. observed a significant association between vitamin B_{12} deficiency and cochlear dysfunction. Those authors recommended the routine measurement of serum vitamin B_{12} levels when evaluating patients with symptomatic hearing loss.[16] However, there are also studies reporting that although vitamin B_{12} deficiency affects the central nervous system in individuals with normal hearing, normal latencies and amplitudes are obtained on the ABR test.[20]

Vitamin B_{12} deficiency exhibits adverse effects on hearing by affecting myelination at any level of the retrocochlear region of the auditory system.[16] We encountered no previous studies investigating the relationship between the ECochG test and B_{12} vitamin levels and cochlear function. This study is the first to compare vitamin B_{12} levels and ECochG measurements in tinnitus patients with normal hearing. The SP/AP amplitude ratio at ECochG was significantly higher in individuals with tinnitus with low vitamin B_{12} levels than in tinnitus patients with normal B_{12} levels. This also suggests that vitamin B_{12} deficiency may cause CS, and tinnitus as a result, by causing an adverse effect on the cochlea.

The limitations of this study include the fact that not all known causes of tinnitus could be excluded and the absence of any data concerning the duration of vitamin B_{12} deficiency. The main obstacle to the development of successful methods of treatment for tinnitus is that the disease's pathological and neurophysiological mechanisms are not fully understood. Cochlear functions in patients with tinnitus can be evaluated using the ECochG test.

CONCLUSION

Vitamin B_{12} deficiency may cause a rise in the SP/AP amplitude ratio employed in the identification of CS. More detailed studies are now needed to fully elucidate the effects of vitamin B_{12} deficiency on cochlear functions. Serum vitamin B_{12} levels should not be overlooked in the evaluation of auditory functions in patients with tinnitus.

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Murat Yaşar;* ***Design:*** *Murat Yaşar;* ***Control/Supervision:*** *Fatma Atalay;* ***Data Collection and/or Processing:*** *Murat Yaşar;* ***Analysis and/or Interpretation:*** *Murat Yaşar;* ***Literature Review:*** *Fatma Atalay;* ***Writing the Article:*** *Murat Yaşar;* ***Critical Review:*** *Fatma Atalay;* ***References and Fundings:*** *Fatma Atalay;* ***Materials:*** *Murat Yaşar.*

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