Research Article (Araştırma Makalesi)

An Evaluation of Thrombocyte Functions in Patients with Idiopathic Subjective Tinnitus

İdiyopatik Subjektif Tinnituslu Hastaların Trombosit Fonksiyonlarının Değerlendirilmesi

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Özet

Amaç: Bu çalışmada, idiyopatik subjektif tinnituslu hastalarda, trombosit fonksiyonları değerlendirildi. Bu amaçla trombositlerin agonistlere cevap olarak yaptıkları agregasyon eğrisini inceleyen bir test olarak kullanılan adenozin difosfat (ADP) ve kollajen ile tetiklenen trombosit agregasyon düzeyleri araştırıldı.

Gereç ve Yöntemler: Çalışmaya, kliniğe en az 1 yıldır devam eden idiyopatik subjektif tinnitus şikâyeti olan hastalar alındı. İdioyopatik subjektif tinnitusu olan otuz üç hasta ve tinnitus şikayeti olmayan 33 kontrol grubu olmak üzere toplamda 66 kişi dâhil edildi. Tinnitus Handikap İndeksi (THI), tinnitus şiddetini ölçmek için tüm hastalar tarafından doldurduldu. Hastalardan alınan kanda, trombosit sayısı, protrombin zamanı (PT), parsiyel tromboplastin zamanı (aPTT), çalışıldı. Kanda adenozin difosfat kollajenaz (ADP) ve epinefrin kollajenaz ölçüldü. ADP ve kollajen, bir Chrono-log trombosit agregometre cihazında bir Chrono-log kiti kullanılarak ölçüldü.

Bulgular: THI'ya göre evre 1'de 4 hasta (%12.1), evre 2'de 8 hasta (%24.2), evre 3'de 10 hasta (%30.3), evre 4'de 7 hasta (%21.2), ve evre 5'de 4 hasta (%12.1) mevcuttu. Tinnituslu hastalarda ADP ile tetiklenen agregasyonda ortalama değer 83.85±16.80 (%), kontrol grubunda ise 91.88±20.67 (%) bulundu ve istatistiksel olarak anlamlı bir fark saptanmadı ($p>0.05$). Kollajen ile tetiklenen trombosit agregasyonunda tinnituslu hastalarda ortalama değer %119.24±24.76, kontrol grubunda %130.79±38.73 idi ve istatistiksel olarak anlamlı bir fark saptanmadı ($p>0.05$).

Sonuç: Sonuç olarak, trombosit fonksiyonları idiopatik subjektif tinnituslu hastalarda normal olarak değerlendirildi. Ancak, ölçülen değerlerin kontrol grubuna göre daha düşük olmasından dolayı trombosit agregasyonuna eğilimli olduğu tespit edildi.

Anahtar kelimeler: Adenozin difosfat, Kollajen. Tinnitus, Trombosit agregasyonu

Abstract

Objective: The aim of this study was to investigate the thrombocyte agggregation levels which are triggered by adenosine diphosphate (ADP) and collagen in patients with idiopathic subjective tinnitus.

Material and Methods: The study included 33 patients who presented at the clinic with complaints of idiopathic subjective tinnitus that had been ongoing for at least 1 year and a control group of 33 subjects with no complaints of tinnitus. The Tinnitus Handicap Index (THI) was completed by all the patients to measure tinnitus severity. Thrombocyte count, prothrombin time (PT), and partial thromboplastin time (aPTT) were examined in blood samples taken from the patients. Adenosine diphosphate (ADP) collagenase and epinephrine collagenase were measured in the blood.

Results: According to the THI, 4 (12.1%) patients were grade 1, 8 (24.2%) patients were grade 2, 10 (30.3%) patients were grade 3, 7 (21.2%) were grade 4 and 4 (12.1%) patients were grade 5. Mean aggregation triggered by ADP was determined as 83.85±16.80%in the tinnitus patients and 91.88±20.67% in the control group, with no statistically significant difference determined ($p>0.05$). The mean thrombocyte aggregation triggered by collagen was determined as 119.24±24.76% in the tinnitus patients and 130.79±38.73% in the control group, with no statistically significant difference determined ($p>0.05$).

Conclusion: The thrombocyte functions were evaluated as normal in patients with idiopathic subjective tinnitus. However, as the measured values were lower than those of the control group, there was determined to be a tendency for thrombocyte aggregation.

Keywords: Adenosine diphosphate, Collagen, Tinnitus, Thrombocyte aggregation

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INTRODUCTION

Tinnitus is defined as the perception of sound in the ears or inside the head without any acoustic stimulus. While 17% of the general population are affected, this rate increases to 33% in the elderly population (1,2). Males and females are affected at equivalent rates (3). In the differentiation of tinnitus, the form should be stated defined as pulsatile (synchronised with heartbeat) or non-pulsatile, subjective or objective, intermittent or continuous and unilateral or bilateral (4,5).

Hearing loss is a risk factor for tinnitus and the two combined are often seen together (6,7). Although, tinnitus may not be seen in every case of hearing loss (8,9). Obesity, smoking, alcohol consumption, head trauma, a history of arthritis and hypertension are possible risk factors (1-3). It may develop associated with otological diseases and the use of various drugs. Tinnitus is seen especially together with anxiety, depression and tempo-ro-mandibular joint diseases (10,11).

Light transmission aggregometry is accepted as the gold standard in the evaluation of thrombocyte functions. This method was first described by Born and O'Brien in 1962 (12,13). In this method, thrombocyte activation and aggregation is measured in an in vitro environment using thrombocyte agonists. It is the optimal screening test which can determine platelet function disorders and von Willebrand disease. Widely-used agonists are adenosine diphosphate (ADP), arachidonic acid, collagen, epinephrine, thrombin and ristocetin. In light transmission aggregrometry measurement, these agonists are added to a plasma rich in citrate platelets and the light permeability of this fluid is measured. It is used in the differential diagnosis of hemostasis disorders and the monitorisation of anti-platelet treatment (14,15). A short duration obtained in those using anti-platelet drugs shows an increased risk of thrombosis in high-risk patients and a prolonged duration indicates that the treatment applied is not sufficient (16).

ADP amplification has a critical role in bleeding diathesis, the efficacy of anti-thrombotic treatment, hemostasis and thrombosis. The ADP measurement duration is recorded as Closure Time (CT) in seconds (secs), and is a marker of thrombocyte function in the full blood sample under analysis. The normal limits are 85-157 secs for collagen/epinephrine and 65-125 secs for collagen/ADP.

Previous studies have determined thrombosis in the etiology of obstructive sleep apnea and sudden hearing loss. The aim of this study was to investigate the effect of thrombocyte functions in the causes of tinnitus and to evaluate the thrombocyte aggregation levels triggered by ADP and collagen.

MATERIALS AND METHODS

The approval of the Local Ethic Committee was obtained about this study (2017/21). All procedures followed were in accordance with the Declaration of Helsinki.

The study included 33 patients who presented at the clinic with complaints of idiopathic subjective tinnitus that had been ongoing for at least 1 year and a control group of 33 subjects with no complaints of tinnitus. Following an otorhinolaryngological examination, full blood count, kidney and liver function tests, blood lipid profile, thyroid function tests, vitamin B12, folic acid, ferritin, audiometric measurement, magnetic resonance measurement and vertebrobasilar artery Doppler ultrasonography examination were applied to all patients. Blood samples were taken for the examination of thrombocyte count, prothrombin time (PT), partial prothrombin time (aPTT), collagen ADP and collagen epinephrine. To determine the severity of tinnitus, the Tinnitus Handicap Index (THI) was completed by all patients.

ADP and collagen were measured using a Chrono-log kit in a Chrono-log platelet aggregometer device. Collagen ADP and collagen epinephrine were examined on a Siemens PFA 100 model device (Germany). The audiometric tests were applied using an Interacoustics AC40 Pure Tone Audiometer device and pure tone average (PTA) was evaluated in the range of 125Hz–8000Hz. Patients with PTA<30dB were included in the study.

To discount intracranial and ear pathologies, patients with normal temporal MRI results were included in the study. Exclusion criteria were a history of ear surgery or patients with tinnitus associated with acoustic schwannoma, meningioma, hearing loss, osteosclerosis, acoustic trauma, Meniere disease or other causes.

Statistical Analysis

Data obtained in the study were analysed statistically using IBM SPSS versionn 22 software. Conformity of the data to normal distribution was assessed using the Shapiro-Wilk test. In the comparison of two independent groups of variables with normal distribution, the Independent Samples t-test was applied. Statistical para-

meters were stated as mean±standard deviation (SD). A value of p<0.05 was accepted as statistically significant.

RESULTS

The patient group comprised 23 females and 10 males with a mean age of 42.67±15.76 years and the control group comprised 17 females and 16 males with a mean age of 39.72±13.57 years. No statistically significant difference was determined between the groups in respect of age. The tinnitus in the patient group was in the right ear in 14 cases, the left ear in 7 and bilateral in 12. According to the THI, 4 (12.1%) patients were grade 1, 8 (24.2%) patients were grade 2, 10 (30.3%) patients were grade 3, 7 (21.2%) were grade 4 and 4 (12.1%) patients were grade 5.

Mean aggregation triggered by ADP was determined as 83.85±16.80% in the tinnitus patients and 91.88±20.67% in the control group (p>0.05) (**Figure 1**). The mean thrombocyte aggregation triggered by collagen was determined as 119.24±24.76% in the tinnitus patients and 130.79±38.73% in the control group (p>0.05) (**Figure 2**). The ADP collagenase and epinephrine collagenase levels were determined to be lower in the patient group than in the control group. No statistically significant difference was determined between the patient and control groups in respect of ADP collagenase, epinephrine collagenase, thrombocyte count anda PTT (p>0.05). A statistically significant result was only obtained in PT (p=0.043), but the mean PT values of both the patient and control groups were within normal limits (**Table 1**).

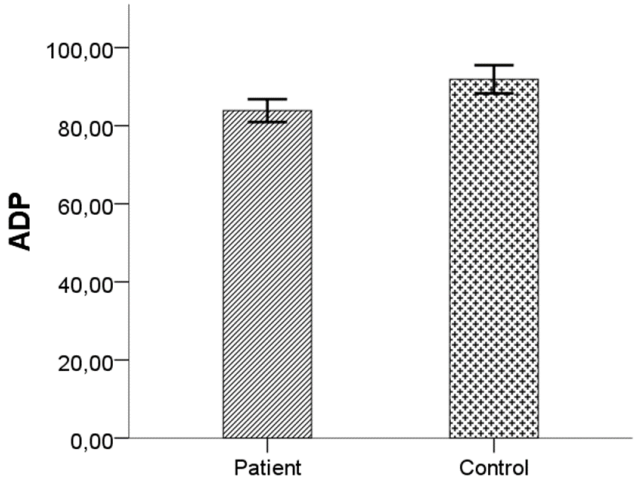

Figure 1. Serum collagen ADP levels in patient and control groups

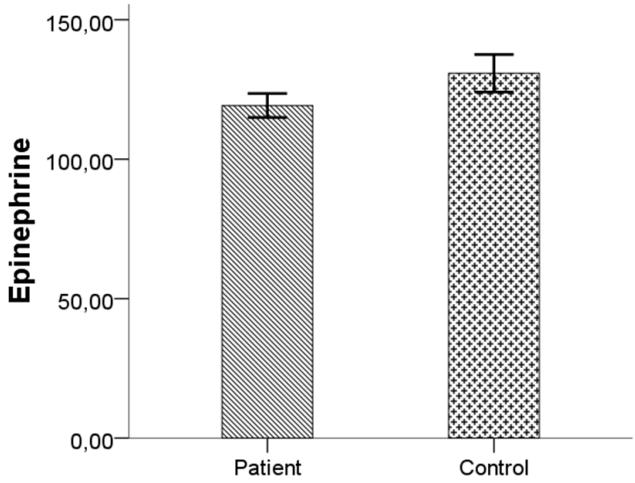

Figure 2. Serum collagen epinephrine levels in patient and control groups

Table 1. Comparison of blood parameter results of patient and control group

	Group		
	Patient (n=33)	**Control (n=33)**	
	Mean±SD	**Mean±SD**	p
Thrombocyte count	254.39±56.30	259.64±66.98	0.732
Collagen ADP	83.85±16.80	91.88±20.67	0.088
Collagen epinephrine	119.24±24.76	130.79±38.73	0.154
PT	95.44±11.47	104.29±21.74	0.043*
aPTT	26.48±1.88	27.28±3.28	0.230

ADP: Adenosin Diphosphate, PT: Prothrombin Time, aPTT: Activated Partial Thromboplastin Time *:p<0.05

DISCUSSION

The results of this study showed that although the ADP collagenase and epinephrine collagenase levels in the patient group were within normal limits, they were determined to be at a lower level than those of the control group. These findings suggested a tendency to aggregation in tinnitus patients compared to the control group. The results of the study support the view that tinnitus may develop associated with the possibility of ischaemia that emerges as a result of the increase in the tendency to thrombus.

Tinnitus is not a disease, but a symptom. There are no objective tests to determine this condition. The majority of previous studies conducted on this subject have been without evidence. Most of what is known about tinnitus has been acquired from animal experimental studies related to hearing loss (17,18). Tinnitus emerges associated with changes in the cochlea. However, reasons affecting the cochlea are multifactorial.

The cause of tinnitus in some patients may be inflammation or ischaemia (19). Some pro-inflammatory cytokines associated with ischaemia, such as TNF-α (Tumor NEcrosis Factor- α), start to be expressed in this area (20). Özbay et al. (21) reported that stress could cause inflammation and tinnitus could be related to stress. The neutrophil-lymphocyte ratio was also found to be higher in tinnitus patients and there was shown to be a relationship between tinnitus and inflammation (20). In a study by Kemal et al. (22), the mean thrombocyte volume was determined to be high in tinnitus patients.

Although a weak thrombocyte collecting agent itself, ADP is a key platelet agonist. At least 3 ADP receptors have been defined in platelet activation and aggregation; P2X1 ionotropic receptor, P2Y1 receptor, and P2Y2 receptor coupled to Gai2 (23,24). ADP triggers platelet activation. P2Y1 is accepted as the most important receptor for ADP. The defect seen in these receptors causes a congenital defect in thrombocyte reponse in patients. ADP receptor antagonists are inhibited by clopidrogel and the use of this drug inhibits ADP-induced aggregation and triggers collagen-induced aggregation. Collagen activates collagen receptors and causing the expression of arachidonic acid, converts to TxA2 and then activates thrombocytes.

ADP, which is found in intense amounts in thrombocytes, plays an important role in thrombus pathogenesis and the formation of hemostatic plaque. When stimulated with various agents, it is expressed from thrombocytes and strengthens thrombocyte aggregation. In the absence of ADP receptor, bleeding diathesis occurs when ADP is deficient in thrombocyte granules (25,26).

In another study, in which the blood of healthy newborns was compared with adult blood, it was determined that the bleeding time measured with primary hemostasis and PFA-100 closure time was shorter (27). The functional phenotype of neonatal thrombocytes was different from that of adults but there were reported to be limited tests that could determine this. In the current study, the closure time in the patient group was determined to be shorter than that of the healthy control group, suggesting that it is necessary to take into consideration that thrombocyte phenotype could be different in these patients.

In a study by Eistert B et al. (28), no correlation was determined between collagen and ADP-induced agglutination in patients with secondary bleeding following tonsilectomy. Palareti et al. (29) observed reduced thrombocyte reactivation with ristocetin, collagen and epinephrine in 21 hypothyroid patients. Myrup and Palareti (30,31), studied primary hemostasis in patients with thyroid dysfunction and determined an increased thrombocyte aggregation response to ADP. No relationship was determined between thrombosis and thrombocyte aggregation in ovarian cancers in a study by Feng S et al (32). In another study of Buerger disease seen with thrombosis, patients were not found to have a tendency to thrombocyte aggregation (33). Although there are many different results in literature related to induced thrombocyte aggregation, there have been very few clinical studies. The results of the current study showed normal laboratory values in the patient group, but they were at a lower level than those of the control group.

When measurement is to be made in a patient, whether or not there is thrombocytopenia or anaemia must certainly be checked, because this measurement is affected by a low thrombocyte count, Von Willebrand factor deficiency, a low hematocrit level, drug use, thrombocyte receptor defects and expressions from defeccts. However, it has been reported that there is no effect from deficiencies in hemophilia A, hemophilia B, fibrinogen and factor V, VII, XI and XII (34-35).

In conclusion, the results of this study demonstrated that thrombocyte functions were evaluated as normal. However, the values of the patient group were determined to be lower compared to the control group. It can be

recommended that conditions requiring anti-aggregant drug use in tinnitus are further researched and there is a need for more extensive studies in respect of conditions that provoke the formation of thrombus.

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Ethics Approval: The approval was obtained from Kahramanmaras Sutcu Imam University Local Ethics Comittee (2017/21).

Conflict of Interest: In this study, there is no conflict of interest among the authors on any subject.

Author Contribution: All authors contributed equally to the article.

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