



Is there any relationship between sudden sensorineural hearing loss and protein Z plasma levels?

Protein Z plazma düzeyleri ile ani işitme kaybı arasında ilişki var mıdır?

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ABSTRACT

Objectives: This study aims to evaluate the relationship between sudden sensorineural hearing loss and protein Z plasma levels.

Patients and Methods: Twenty patients with sudden sensorineural hearing loss (14 males; 6 females; mean age 51.6±14.6 years; range 23 to 75 years) and 30 healthy subjects without any hearing problems (14 males, 16 females; mean age 46.8±8.9 years; range 30 to 64 years) were investigated. Protein C, protein S, and protein Z plasma levels were measured.

Results: When protein S and protein C levels were compared between the study groups, we did not find a statistically significant difference (p=0.78 and p=0.44), whereas, protein Z plasma levels were found to be considerably lower in the sudden sensorineural hearing loss group than in the control group, and the difference was statistically significant (p<0.001).

Conclusion: Vascular causes may be involved in the etiology of sudden hearing loss. Changes in protein Z plasma levels might be associated with arterial thromboembolism or venous thrombosis in patients with sudden sensorineural hearing loss.

Keywords: Etiology; protein Z; sudden sensorineural hearing loss; thromboembolism.

ÖZ

Amaç: Bu çalışmada protein Z plazma düzeyleri ile ani işitme kaybı arasında ilişki değerlendirildi.

Hastalar ve Yöntemler: Ani işitme kaybı olan 20 hasta (14 erkek, 6 kadın; ort. yaş 51.6±14.6 yıl; dağılım 23-75 yıl) ve herhangi bir işitme sorunu olmayan 30 sağlıklı birey (14 erkek, 16 kadın; ort. yaş 46.8±8.9 yıl; yaş aralığı 30-64 yıl) araştırmaya dahil edildi. Protein C, protein S ve protein Z plazma düzeyleri ölçüldü.

Bulgular: Çalışma grupları arasında protein S ve protein C düzeyleri karşılaştırıldığında istatistiksel olarak anlamlı bir fark bulunmazken (p=0.78 ve p=0.44), kontrol grubuna göre ani işitme kaybı olan grupta protein Z plazma düzeylerinin önemli ölçüde düşük olduğu bulundu ve fark istatistiksel olarak anlamlıydı (p<0.001).

Sonuç: Damarsal nedenler ani işitme kaybı etyolojisinde yer alabilir. Ani işitme kaybı olan hastalarda protein Z plazma düzeylerindeki değişim arteriyel tromboembolizm veya venöz tromboz ile ilişkili olabilir.

Anahtar sözcükler: Etiyoloji; protein Z; ani işitme kaybı; tromboembolizm.

Sudden sensorineural hearing loss (SSNHL), first defined by De Kleyn in 1944, is one of the most challenging issues in otolaryngology.^[1] Idiopathic SSNHL, the acute onset of hearing loss of at least 30 dB in three connected

frequencies occurring instantaneously or over a period of up to three days has an estimated incidence between 10 and 20 per 100.000 persons per year.^[2,3] Etiological factors can be found in only 10-15% of SSNHL

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patients, while other patients remain idiopathic. Others define SSNHL as a rapid-onset sensorineural hearing loss that develops within 24 hours.^[4,5] In a recent systematic analysis of 23 research papers, Chau et al.^[6] demonstrated that 71% of SSNHL cases were classified as idiopathic SSNHL because no underlying pathology could be found. In 13% of cases, infection was shown to be the culprit, whereas 5% of cases were attributed to otologic etiology, 4% to trauma, 3% to hematologic causes, 2% to neoplasia, and another 2% to other causes.^[6] Idiopathic SSNHL occurs mainly in patients between the ages of 30 and 60 years, with equal sex distribution, and can be devastating for the patient. It is almost always unilateral and is commonly associated with tinnitus and aural fullness. Therefore, SSNHL should be treated as a medical emergency. Multiple treatment protocols and agents have been proposed to treat SSNHL. Treatment options include steroids, antivirals, vasodilators, diuretics, hyperbaric oxygen therapy, and vitamins.^[7,8] A maximum of 32 to 65% of cases of SSNHL may recover spontaneously.

The vitamin K-dependent anticoagulant plasma glycoprotein protein Z (PZ) was first isolated from bovine plasma in 1977 and in 1984 from human plasma and was shown to exert an important role in the regulation of the coagulation cascade.^[9,10] While *in vitro* studies showed that bovine PZ could promote the assembly of thrombin with phospholipid surfaces, thereby enhancing coagulation, the human PZ form binds to thrombin poorly, with very little impact on the association of thrombin binding with phospholipids. More recent studies showed that PZ forms a calcium ion-dependent complex with Factor Xa on phospholipid surfaces, thereby serving as a cofactor for the inhibition of Factor Xa by a PZ-dependent protease inhibitor (ZPI).^[11] Deficiency in PZ secretion and/or function was linked with a procoagulant state and several thrombotic disorders, including arterial and venous thrombosis.

Proteins C, S, and Z are vitamin K-dependent glycoproteins. These proteins had an important role in regulating the coagulation system. Deficiencies in these proteins lead to an increased risk of venous thromboembolism. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) are commonly investigated markers in patients with coagulation disorders. Prothrombin time and aPTT measure the extrinsic and intrinsic pathways of coagulation, respectively. These markers are useful in the case of proteins C, S, and Z deficiency.

However, coagulation markers for SSNHL are widely investigated in different studies, and the possible effect of the mentioned parameters is still unknown.

Thus, the aim of this study was to investigate the possible relationship between sudden hearing loss and protein Z plasma levels.

PATIENTS AND METHODS

The study was carried out in collaboration with the Otorhinolaryngology and Biochemistry Clinics of Ankara Numune Training and Research Hospital. The study was performed prospectively by including 20 patients with SSNHL (14 males; 6 females; mean age 51.6±14.6 years; range ages 23 to 75 years), and 30 in the control group, (14 males, 16 females; mean age 46.8±8.9 years; range 30 to 64 years). Patients were informed about their disease and the treatment they would receive; they consented to give blood and have the blood used in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the ethical committee of Ankara Numune Training and Research Hospital (573/2013). All the patients were studied with hemogram, routine biochemistry, hemostasis parameters PT, aPTT, international normalized ration (INR), protein C (PC), protein S (PS), PZ, thyroid function (sT₃, sT₄, TSH), and an audiogram with high-resolution temporal bone computed tomography or magnetic resonance imaging. None of the patients used anticoagulant medicine before treatment, and all the patients' liver function tests were within the reference range. None of the SSNHL patients had developed thromboembolism. Blood samples were collected from all participants. Two types of blood collection tubes [red-top tube (standard empty vacuum container) and citrate-containing tube] were used. To avoid the coagulation activation effect, the first 1 mL of blood was discarded. All samples were centrifuged at 1000 g for 15 minutes and then samples were stored at -80 °C until analysis. The measurements of PZ concentrations were determined in plasma by using the enzyme-linked immunosorbent assay Kit For Protein Z (PROZ; Houston/USA, USCN). An ELISA microplate strip washer (EXL50; BioTek Instruments, USA) and an ELISA microplate reader (ELX808; BioTek Instruments, USA) were used to perform measurements. The reportable range was 0.156-10 ng/mL. The assay had a precision of less than 10%.

Protein C and PS concentrations were determined in plasma by using colorimetric and formation clotting methods, respectively. Measurements were performed with a Simultaneous Thermal Analyzer (STA; Diagnostic Stago, Parsippany, NJ, USA).

Statistical analysis

Data analyses were performed using the IBM SPSS version 21.0 software (IBM Corporation,

Armonk, NY, USA). The normal distribution of the considered variables was evaluated first using the Shapiro-Wilk test. The subjects' demographic data were compared using the Mann-Whitney U test or chi-square test. The correlation analysis was done with Spearman's Rho test. The level of significance was set at $p < 0.05$.

RESULTS

There was no significant difference between the two groups in terms of gender ($p = 0.24$). While there were no significant differences between levels of PS and PC in the patient group and the control group (respectively $p = 0.78$ and $p = 0.44$), the PZ levels in the patient group were considerably lower than the PZ levels in the control group; the difference was found to be statistically significant ($p < 0.001$) (Table 1).

DISCUSSION

The major finding of the current investigation was the significant difference between study groups in terms of protein Z plasma levels. As mentioned in the result section, the protein Z plasma levels were significantly lower in patients with SSNHL when compared with the control group.

Etiology can be determined in only 10-15% of SSNHL patients, and most (71%) of the cases were classified as idiopathic SSNHL, because no underlying pathology could be found.^[4] In 13% of cases, infection was shown to be the culprit, whereas 5% of the cases were attributed to an otologic etiology, 4% to trauma, 3% to hematologic causes, 2% to neoplasia, and another 2% to other causes.^[6]

Protein Z is a 62 kDa vitamin K-dependent single-chain glycoprotein consisting of 360 amino acids containing the N-terminal γ -carboxyglutamic acid domain necessary for its effective secretion, followed by two epidermal growth factor-like domains, and a C-terminal pseudocatalytic domain.^[10] Protein Z acts

as a cofactor for the 72 kDa serpin ZPI, which rapidly inhibits Factors Xa and XIa, hence reducing thrombin generation.^[10-12] Relative to PZ, ZPI is present in plasma at higher levels relative to PZ, where it binds all PZ in a 1:1 ratio, hence circulating as a stable PZ/ZPI complex, with virtually no free PZ detected.^[13,14]

Mean protein Z plasma levels ranged from 1.16 to 2.71 $\mu\text{g/mL}$ and were influenced by genetic and nongenetic factors, including chronic liver disease, age, gender, vitamin K levels, concurrent use of anticoagulants, pregnancy, and ethnic origin.^[9,15] Protein Z plasma levels rapidly increase during the first months of childhood, then slowly taper off, with adult levels reached during puberty, and higher PZ levels were reported in men than in women.^[16,17] Protein Z plasma levels are reduced during inflammatory episodes. Contradictory findings were also reported, with both increased and decreased PZ levels described in obese individuals; this was attributed to altered inflammatory cytokine expression associated with obesity, the presence of anti-PZ antibodies, the ethnic background of study subjects, and possibly increased excretion of PZ.^[18-20] Compared to studies on PZ levels, only four studies addressed plasma ZPI levels but had inconclusive findings. An association of reduced ZPI levels, peripheral arterial disease, and venous thrombosis was documented, while no differences in ZPI levels were reported between normal controls and antiphospholipid antibody-positive cases.^[7,21]

Protein Z deficiency ($< 1 \mu\text{g/mL}$) was linked with a procoagulant state, associated with excessive Factor Xa secretion and thrombin production.^[13,17] A recent meta analysis demonstrated that reduced PZ levels were linked with an increased risk of thrombotic events, such as arterial vascular and venous thromboembolic diseases.^[21] Whether PZ deficiency constitutes an independent risk factor for thrombosis is inconclusive, since the association of PZ deficiency with increased risk of thrombosis was reported in the presence of other prothrombotic risk factors such as factor V Leiden prothrombin mutation and hyperhomocysteinemia.^[17,22] It was further suggested that PZ deficiency did not constitute an independent risk factor for venous thrombosis (VT), but only increased the VT risk with factor V Leiden. Furthermore, a rare inherited coagulation disorder, hereditary combined vitamin K-dependent clotting factor deficiency, was reported.^[23]

We could not find articles on the subject of connections between PZ and SSNHL in the literature. However, there are many studies of relationships with PZ/ZPI complex levels between subject areas in the colon, and small lung cell, breast, stomach, pancreas, and liver cancers, as well as multiple myeloma (MM), idiopathic

Table 1

Protein Z, protein S, and protein C levels comparison of patient and control groups

	Patient group	Control group	p^*
	(ng/mL)	(ng/mL)	
	Mean \pm SD	Mean \pm SD	
Protein Z	1946 \pm 263	2273 \pm 162	<0.001
Protein S	88.2 \pm 25.4	88.3 \pm 19.1	0.78
Protein C	113 \pm 40.2	108.3 \pm 14.4	0.44

* Mann Whitney U test.

thrombocytopenic purpura (ITP), and in hematological diseases such as hemophilia A (HA) which are formed venous thrombosis and habitual abortion.

Thromboembolic episodes are the most common complications and the second-leading cause of death in cancer patients.^[24] It is widely documented that coagulation system proteins play a role in cancer growth and metastatic dissemination.^[25-27]

Protein Z plasma levels are used for the diagnosis of MM in patients with bleeding or thrombosis and also used for following the efficiency of MM treatment. According to the study, conducted by the Bolkun et al.,^[28] it was found that there was no significant difference between the serum PZ levels of healthy people and patients with MM, whereas it was found to be a significant difference between patients with stage 3 disease and stage 1 disease in terms of PZ levels. Also while the treatment of MM thrombosis by thalidomide is used alone, the risk of thromboses and thromboembolism is less than 5%; by adding dexamethasone to the treatment, this risk rises to 14%, and doxorubicin, cyclophosphamide, and melfen antineoplastics further increase this risk.

Martinelli et al.,^[29] said that if a very low PZ level could serve as an isolated venous thrombosis risk factor, moderate PZ deficiency certainly would increase the venous thromboembolic risk of other well-known prothrombotic risk factors. Additionally, Cobankara et al.^[30] showed a significantly higher PZ concentration in patients with ITP. This clinical observation, suggested by the author, might be attributed to more hemostatically active residual platelets. Bolkun et al.,^[28] have found in their study that in patients with severe HA (F8<1%) and in patients diagnosed with PZ/ZPI complex, levels are significantly lower, and determined that there is no relationship in the remaining patients.^[28]

Pregnancy is associated with a state of hypercoagulation linked to excessive thrombin generation, which is crucial for controlling bleeding at delivery.^[31,32] Growing evidence implicates coagulation abnormalities with adverse pregnancy outcomes, including recurrent and nonrecurrent pregnancy losses, intrauterine growth retardation, placental abruption, intrauterine fetal death, preeclampsia, and maternal or neonatal thrombosis.^[32,33] In addition, a decreased quantity of natural anticoagulants, such as PS and PC, and reduction in overall fibrinolytic activity accompany most pregnancy complications.^[34] Progressively higher PZ levels were seen with gestational age in normal pregnancies, especially among obese pregnant women, and were attributed to pregnancy-associated imbalance of fibrinolytic and hemostatic mechanisms.^[35,36] Protein

Z plasma levels correlated with gestational age, evidenced by the progressive increases in PZ levels (20%) during the three trimesters of pregnancy, which were attributed to compensatory mechanisms induced by increased Factor Xa concentrations, and decline thereafter by as much as 30% to levels below those of the first trimester. The increase in PZ levels becomes attenuated in patients with abnormal pregnancy outcomes, including low birth-weight babies or gestational hypertension or diabetes mellitus.^[37,38] Reduced PZ levels (<1 µg/mL) were linked with several pregnancy complications including fetal demise, preeclampsia, small-for-gestational age, intrauterine growth restriction, HELLP syndrome, and idiopathic recurrent miscarriage.^[37-41]

As is known, approximately 3% of the etiology of SSNHL is from the return of venous thromboembolism, which is obstructing the circulation in the labyrinthine artery. Inadequate and/or ineffective release of ZP/ZPI complex may also participate the pathophysiology of SSNHL, particularly in patients with idiopathic SSNHL. In our study, 20 people who was diagnosed as SSNHL and had not a history of pre-owned venous thrombosis, arterial thromboembolism and problematic pregnancy were compared with 30 participants who had not a problem with their hearing and who not a history pre-owned venous thrombosis, arterial thromboembolism and problematic pregnancy in terms of PZ, PS and PC levels. Statistically significant difference was not found in PS and PC levels between the groups (p=0.78, p=0.44), whereas, PZ levels of the patients with SSNHL were significantly lower than the PZ levels of participants in the control group (p<0.001). As a result of low PZ levels, particularly in the cochlear apical segments, including nutrition and arterial thrombosis in the venous anastomosis network and in the inner ear limited/or may cause micro-emboli, causing SSNHL.

In conclusion, according to our study decreased PZ levels might be one of the possible causes of SSNHL. When the current literature was evaluated among the known causes, 3% of SSNHL cases were due to hematologic pathologies. Protein Z and a low vitamin K-dependent anticoagulant can cause various thrombotic and thromboembolic events. Protein Z plasma levels of the patients in our study group were significantly lower than in the control group. However, further studies are needed to investigate the relationship between thromboembolic events and SSNHL.

Declaration of conflicting interests

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