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The possible relationship between epistaxis and protein Z plasma levels

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Abstract

Epistaxis represents a very common emergency in any ear, nose, and throat (ENT) department around the world. Frequently seen in the systemic vascular patients, nasal anatomy the lack of vessels muscular structure, the absence of vasoconstriction ability, the clot formed in the bleeding area further increases the amount of bleeding supports the importance of vascular causes in the etiology. The purpose of our research is to evaluate the relationship between epistaxis and plasma protein Z levels. 18 patients with epistaxis and 30 healthy subjects were investigated. 8 of patients group (44.4%) participating the research were women, 10 of them (55.6%) were men, in total 18 people. Control group consisted of 16 women (53.3%), 14 men (46.7%), 30 people in total. Both groups were measured about protein C (PC), protein S (PS), and protein Z (PZ). PC and PS concentrations were determined in plasma by using colorimetric and the formation clotting methods, respectively. Sandwich enzyme immunoassay method was used for the determination of PZ plasma concentrations. While statistically there were no significant differences detected comparing Protein S and Protein C levels data of control group, ($p=0.27$ ve $p=0.29$ respectively). Protein Z levels of patient group was found significantly lower than Protein Z levels of control group and this was found to be statistically significant ($p<0.001$). Vascular causes are known to take part in the etiology of epistaxis. Defects of Protein Z function and/or secretion, are associated also with bleeding, venous thrombosis and arterial thromboembolism as well as including especially epistaxis.

Keywords: Epistaxis, protein C, protein S, protein Z

Introduction

Epistaxis is one of the most common emergencies in ENT surgery and is seen in 60% of the people at anytime. Epistaxis is important for the terms of frequency and urgency. Epistaxis is classified clinically into anterior and posterior, and 90-95% of those are on the anterior region and most of them are on the little region, reveals in Kiesselbach plexus [1,2]. Sphenopalatine artery is responsible for most of the posterior epistaxis [3]. Bleeding caused by local traumas rather than anterior, whereas bleeding caused by systemic diseases usually generate from the posterior [3,4].

The most common causes are nose picking, trauma, infection, hypertension, medication are at increased risk for epistaxis [5]. Protein C, S and Z are vitamin K dependent glycoproteins. These proteins had an important role in the regulating of coagulation system. Deficiencies or changes in these proteins lead to an increased risk of heamorrhage or tromboembolism.

Most commonly investigated markers (PT and aPTT etc.) providing evaluation of intrinsic and extrinsic

sections coagulation pathway at patients with coagulation pathology. These markers may be used at lack of protein C (PC), protein S (PS) and protein Z (PZ). In 1977 one of these proteins defined coagulation factor depending on vitamin K in glycoprotein structure at bovine plasma, it was named as PZ [6].

However it was isolated in human plazma in 1984 [7]. PZ, is the coagulation factor synthesized at liver, in single chain glycoprotein structure at 62 kD weight. It is similar to aminoterminal end FII, FVII, FIX, FX, PC and PS like other proteins depending on vitamin K. PZ provides thrombin formation at phospholipids surfaces depending on Ca^{++} [8]. PZ at cell surfaces of phospholipid structure, may be taking substrate role for fresh protease binding protein or proteolytic reactions. Miletich and Broze in 1987, looked at PZ levels of 455 healthy adults serum and found an average value like $2.9 \pm 1.0 \mu\text{g/ml}$. They determined there were no connection between PZ values and age or gender [9].

However, coagulation markers for epistaxis are widely investigated in different studies, the possible effect of a for mentioned parameters is still unkown. They defend it tends to be bleeding in the lack of PZ because of the PZ interaction necessity for thrombin at phospholipid endothelial level becoming active. Thus; the aim of this study was to investigate the possible relationship between epistaxis and protein Z plasma levels.

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Material and Method

The study was a collaboration between Ankara Numune Training and Research Hospital and Ear, Nose, Throat and Medical Biochemical Clinics. It was performed prospectively during May 2013 and February 2014, by planning medication of hospitalized patient group that consisted on 18 people in total, 10 men and 8 women, age to 39-72; and control group consisting on 30 people in total 14 men and 16 women, age to 30-64. The patients were informed about their disease and applicable treatment, blood taking and they gave consent about using the blood in this research. The patients were asked about hemogram, routine biochemical, hemostasis parameters (aPT, aPTT, INR), PC, PS, PZ levels during treatment and was made. None of the patients used anticoagulation medicine before the therapy and all of the patients who had liver functional tests in reference range and did not have trauma history and before epistaxis none of the patients have had thromboembolic events. Control group who had not epistaxis, venous thrombosis, arterial thrombo embolism or pregnancy stories.

Blood samples were collected from all participants. Two types of blood collection tubes [red top tube and citrate containing tube] were used. To avoid the effect of coagulation activation, the first one ml blood were discarded. All samples centrifugated at 1000g for 15 minutes and then samples were stored at -80 C until analysis. The measurements of PZ concentrations were determined in plasma by using enzyme-linked immunosorbent assay Kit For Protein Z (PROZ) (Houston/USA, USCN). Measurement performed by using ELISA microplate strip washer (ELX50; BioTek Instruments, USA) and ELISA microplate reader (ELX808; BioTek Instruments, USA). The reportable range was 0,156-10 ng/mL. Assay had a precision of < 10%.

PC and PS concentrations were determined in plasma by using colorimetric and the formation clotting methods, respectively. Measurements performed by using STA analyser (Diagnostics Stago, Parsippany, NJ, USA).

This study was conducted at Ankara Numune Teaching and Research Hospital with approval of the local Ethics Committee and suitable with the Declaration of Helsinki (576-2013).

Statistical analysis

Data analyses were performed using SPSS 21.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL). The normal distribution of the considered variables was first evaluated 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 0,05.

Results

The participants of this research were hospitalised patients group consisting of (44.4%) 8 women and (55.6%) 10 men, 18 people in total, who had bleeding of the nose but not trauma history; and control group consisted of (53.3%) 16 women and (46.7%) 14 men, 30 people in total who had not have nose bleeding before. Total number of participants was 48. The median age of patient and control group was 59.50(39-72), 48.50(30-64) years old

respectively. There were not found any differs in terms of gender for both groups. (p=0,24).

There were no significant differences to be detected while comparing the PS and PC levels data of patient group, (p=0.27 and p=0.29 respectively). PZ levels of patient group were found lower than PZ levels of control group and this was found to be statistically significant (p<0.001) (Table 1).

Table 1. Comparison of Protein Z, Protein S and Protein C levels of patient group and control group

	Epistaxis group	Control group	P value**
Protein Z	2068 ± 1249	2273 ± 162	<0.001
Protein S	83.9 ± 28.5	88.3 ± 19.1	0.27
Protein C	99.4 ± 26.5	108.3 ± 14.4	0.29

** Mann Whitney U test

Discussion

Epistaxis etiology focuses on chemical irritant from environmental causes cold and dry air, sudden changes in atmospheric pressure, vascular abnormalities of local causes, infectious diseases, trauma, iatrogenic conditions, neoplasms, foreign bodies, hypertension from systemic causes, atherosclerosis, bleeding diathesis, low plateletes or disfunction, coagulopathy, hepatic and renal disorders [1,5,10].

Epistaxis is frequently seen at people with systemic vascular disease, the lack of vasoconstriction ability as the absence of nose vessels muscular structure, coagulum made in bleeding area further increasing supports the importance of vascular causes [1,5,10,11].

PZ, depending on vitamin K, synthesized in liver, is cofactor [12] of protease inhibitors (ZPI) depending on weight 72 kD and PZ-ZPI association FXa and inhibits FXIa, decreases thrombin structure [13,14]. ZPI in plasma, is at very high levels according to PZ and it is connected to PZ with 1:1 proportion. For this reason when there is no free PZ in plasma, it circulated like stable PZ/ZPI complex [15,16]. Average plazma PZ levels, changes between 1.16 and 2.71 µg/ml with chronic liver diseases together, is affected by age, gender, vitamin K levels, anticoagulant usage, genetic factors like pregnancy and race and non genetic factors [17-19]. PZ levels grows fast at first months of life but later this increase will be reduced at adults levels during puberty.

Plazma PZ levels in men is found to be higher than women [19]. The lack of PZ, as the reason for FXa secretion increase, and pro coagulant events reasons like thrombin production and arterial thromboembolism and venous thrombosis (VT), was founded to cause also different bleeding [18-20].

Eventhough it is well known that plasma proteins depending on Vitamin-K play an important part for coagulation and regulation, the function of PZ is not clear. Another research found that lack of PZ may cause different bleedings. According to this research made by Matthes and Kemkes-Matthes formed by 36 people PZ levels at 21 patients with unkonown bleeding disorders [21].

Different researches are made to find relations between protein Z and bleeding and coagulation evaluating tests (prothrombin

time, INR, PT, aPTT and platelet count) in patients coming with bleeding. They found an inverse relationship between Protein Z and INR [21,22].

We found few articles in the literature about the relation between PZ and epistaxis. On the other hand, there are a lot of researches about the relation between and habitual abortion with PZ/ZPI complex levels and venous thrombosis occurring at hematological diseases like A hemophilia (HA) and Idiopathic Thrombocytopenic Purpura (ITP), colon cancer, small cell lung cancer (NSCLC), breast, stomach, pancreas, liver cancer and multiple myelom (MM).

Thromboembolic incidents in cancer patients are the most frequent complications and the main reasons for death [23,24]. Different researches specified that proteins belonging to coagulation system act important part to parenchyma growth and spread [25]. At first coagulation cascade, activation of FX, thrombin and at last fibrin formation are important steps for cancer tissue [25]. According to Sierko E. research releasing inadequate and ineffective PZ/ZPI released by cancer cells forming column parenchyma [26,27]. In another research made by Sierko E. et al, plasma PZ levels were lower inpatients with small cell liver cancer, compared to those who didn't have it, yet patients with cancer proved this decrease as tumor area increases. Hereby, they argued that hemostatic system proteins like PZ, ZPI and similar to them may take part at NSCLC pathogenesis [28].

Bolkun L et al., made a research about patients who had been diagnosed with MM. There were no statistical difference between serum PZ levels of MM patients and healthy people. However it was found that serum PZ levels of patients at third stage of disease were significant lower than serum PZ levels of patients at first stage of disease. Additionally they diagnosed that further the stage of disease the further thrombose and thromboembolism danger goes.

Consequently, in additon to PZ impairment, procoagulant and anticoagulant activities are increasing in patient group with additional risk factors like hematologic malignancy and applied chemotherapy for these malignancies treatment. Otherwise, in MM treatment, while the danger of using alone thalidomide with thrombosis and thromboembolism is <5%, by adding dexamethasone treatment it is 14%, this risk is further increased by adding antineoplastic like doxorubicin, cyclophosphamide [29].

In Bolkun L. et al., research, they stated that patients with heavy HA (F8 <1%) diagnose have statistically significant low PZ/ZPI complex levels, other patients do not have any relation, at the end HA patients with lack of F8 together with lack of PZ/ZPI cause to bleeding [29].

In 1991 Hogg and Steflo, stated that thrombin PZ presence in a relationship with phospolipid levels ensures coagulation, but in the lack of PZ it does not occur [8]. This observation prompted the suggestion that this phenomenon may provide a mechanism whereby thrombin is kept from diffusing into the vascular lumen and away from the site of injury. According to this they stated that low PZ levels may cause different bleedings. To determine the accuracy of this they worked with 36 patients with bleedings, not receiving anticoagulant therapy and having normal liver functions and control group consisting of 36 people too matching to patient group about age and gender. 22 patients from 36 had hematoma, 14

patients from 28 had post operative bleeding, 14 patients from 35 had bleeding after cut and bruises, 11 women patients from 32 had abnormal menstrual bleeding, 10 patients from 32 had bleeding after tooth extraction, 6 patients from 35 epistaxis and 5 of 36 petechial bleedings. They found significantly lower comparing the average PZ levels of bleeding group to control group. At the end they defined the lack of PZ as a new coagulopathy type. They stated that can not be detected by routine coagulation tests and causing capillary fragility syndrome will cause bleeding. In contrast to our study they stated that average PZ levels of women patients are lower than the average of men [21].

According to Ravi S. et al., research the PZ levels of healthy group was found in normal range in men and women. But serum PZ levels of women in working group eventhough had no relation to coagulation factors depending on vitamin K and age, was found significant lower than men serum PZ levels. None of the 48 patients with bleeding had low PZ levels. Their data shown that low-normal protein Z levels were not associated with a bleeding tendency [9].

Gamba G. research evaluated 15 patients with bleeding. 8 of 15 patients had epistaxis. Even in this research protein Z values were lower according to control group. Especially patients undertaking warfarin treatment detected evident drop [30].

An important part of epistaxis pathology consitutes of hematologic causes. Outside the hematological pathology that may cause frequent etiologic well known in this patient group, a part of it takes part at the patient releasing inadequate an/or ineffective PZ/ZPI complex. We compared in our research the PZ, PS and PC levels of patient group consisting of 18 people who were diagnosed by epistaxis caused by non traumatic causes, hospitalised in our clinic, their treatment was planned, they did not have venous thrombosis, arterial thrombo embolism incidents; and control group consisting of 30 people who had not epistaxis, venous thrombosis, arterial thrombo embolism or pregnancy stories.

There were no significant differences between PS and PC levels between groups ($p=0.27$, $p=0.29$), but PZ levels of patient group was lower than PZ levels of control group and this result was significant ($p<0.001$). As a result, falling of PZ levels increases bleeding, being effective on coagulation and as a cause of capillary fragilite syndrome, it prevents coagulation while bleeding and makes it difficult to control epitaxis.

Conclusion

A part of frequent epistaxis causes consist on hematological pathologies. Also PZ as one of the coagulation factors depending on vitamin K, its releasing may cause bleeding and different thrombotic incidents. Researches have highlighted that low PZ levels causes venous thrombosis and/or thromboembolism and bleedings. PZ levels of patient group is found to be significantly lower than PZ levels of control group in our research and by causing negative effects to coagulation mechanism, by causing a decrease in thrombin activity and shifting the balance in the direction of antithrombotic, eventually causing preventing of coagulation bleeding in nasal passages makes it difficult to control epistaxis. However we have to do more extensive and multicenter studies are needed to investigate the relationship between epistaxis and PZ.

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