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Tissue and serum prolidase activity in patients with nasal polyposis

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ÖZET

Nazal polipozisli hastalarda doku ve serum prolidaz aktivitesi

Biz nazal polip (NP) li ve NP siz hastalarda serum ve doku prolidaz aktivitelerini (PA) karşılaştırarak NP patogenezinde prolidazın rolünü aydınlatmayı amaçladık. Çalışma grubuna kabul edilmiş hastalarda NP yaygınlığı ameliyat öncesi bilgisayarlı tomoğrafi kayıtlarında Lund-Mackay skorlama sistemi (LMS) ile değerlendirildi. Bu çalışmada kontrol grubunu alt konka küçültme ameliyatı uygulanan polipsiz hastalar oluşturdu. Tüm hastaların serum ve doku PA leri ölçüldü. Polip grubunda doku PA seviyeleri kontrol grubundan önemli derecede yüksek olmasına rağmen kontrol grubunda serum PA seviyeleri polip grubundan önemli derecede yüksek bulundu. Serum PA ve LMS skorları arasında önemli derecede negatif ilişki vardı. Çalışma grubunda doku PA ile serum PA ve LMS skorları arasında anlamlı ilişki yoktu.
Biz doku ve serum PA lerinin NP gelişiminde önemli rol oynadığını düşünüyoruz.

Anahtar Kelimeler: *Nazal polipozis, Patogenez, Prolidaz enzimi, Prolidaz aktivitesi şeklinde olacak*

SUMMARY

We aimed to elucidate the role of prolidase in NP pathogenesis by comparing serum and tissue prolidase activity (PA) in patients with and without NP. Patients with NP enrolled to the study group and widespread of NP evaluated using the scoring system of Lund-Mackay (LMS) according to the computer tomography records preoperatively. Patients without NP and who underwent inferior turbinate reduction operation were included to the control group in this study. All patients serum and tissue PA were analyzed. The levels of serum PA were significantly higher in the control group compared with the polyp group, whereas the levels of tissue PA were significantly higher in the polyp group than the controls. There was a significant negative correlation between serum PA and LMS. There was no significant correlation between tissue PA and serum PA, neither tissue PA and LMS in study group. We think that tissue and serum PA play an important role NP development.

Key words: *Nazal polipozis, Patogenez, Prolidaz enzimi, Prolidaz aktivitesi*

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Introduction

Nasal polyps (NP) are inflammatory outgrowths that originate from the mucous membranes of the nasal cavity or paranasal sinuses. NP pathogenesis has not been fully elucidated and no single cause has been found, but many authors assert NP to be an inflammatory pathology. Histologically, NP are characterized by the proliferation and the thickening of the mucosal epithelium, myofibroblast differentiation and extracellular matrix (ECM) accumulation (1,2). Eosinophilic NP display edema, rare glandularity, and minimal collagen deposition except within the basement membrane. Non-eosinophilic NP are present glandular hypertrophy, dense collagen deposition, and mononuclear cellular infiltrate (3). Accumulation of ECM is an important process in the development of NP. Myofibroblasts are the activated cell phenotype of fibroblast and have a high capacity for ECM protein secretion (2). In NP, growth factors, such as transforming growth factors (TGF) stimulate fibroblast proliferation, myofibroblast differentiation, collagen synthesis and ECM deposition. Fibroblasts that play important role in connective tissue development and deposition also have an important role in NP pathogenesis (1,4-6).

Prolidase is the main regulatory enzyme in the metabolism of proline and hydroxyproline, which constitute 20% of all collagen in the human body. Prolidase is in the latest stage of protein catabolism and it is a step-limiting factor in regulation of collagen biosynthesis, thus being involved in matrix remodeling and cell growth. As an important marker of collagen turnover, serum prolidase activity (PA) has been evaluated in various diseases (7-9). Accumulation of ECM is important process in the characteristic structural modification of NP.

Thus, we aimed to elucidate role of prolidase in NP pathogenesis by determining serum and tissue PA in NP patients and comparing the results with patients who had normal nasal mucosa that obtained during inferior turbinate reduction operation. We also analyzed relationship between extent of NP that assessed using Lund-Mackay scoring system and tissue and serum PA in patients with NP.

Materials and methods

Patients

This prospective study was performed on total of 82 subjects who admitted to Otolaryngology Department of GATA Haydarpasa Training Hospital between February 2012 and February 2013. The study was approved by the local ethical committee (23.02.2012/2012-32), and all participants gave informed consent. Each patient underwent endoscopic examination.

Patients with NP enrolled to study group and CT scans were performed on patients in study group for the differential diagnosis and to assess the extent of pathology. Patients who underwent inferior turbinate reduction operation and had no evidence of NP and sinusitis in endoscopic examination were enrolled to control group in this study.

The Lund-Mackay scoring system was used for the evaluation of CT scans of NP patients (10). It is a measure of the degree of opacification in maxillary, ethmoid, sphenoid sinuses, ostiomeatal complex, and frontal sinus regions bilaterally (score range: 0-24). The severity of sinus opacification was scored as 0 (complete lucency), 1 (partial lucency) or 2 (complete opacity). The ostiomeatal complex was scored as either 0 (not obstructed) or 2 (obstructed). In addition, mild mucosal thickening without fluid collecting was scored as 0; mild mucosal thickening with fluid collecting causing partial lucency scored as 1; and, moderate or severe mucosal thickening without fluid collecting causing partial lucency, but not complete opacity, scored as 1.

The exclusion criteria for the participants were as follows: being under 18 years old, steroid usage within the last month, previously sinonasal surgery, recent acute upper respiratory tract infection (within the last month), systemic diseases such as hypertension, diabetes, connective tissue disorders, malignancy, autoimmune disorders, allergic rhinitis, and other any chronic diseases.

Samples

Polyp tissue samples of the patients in study group were taken endoscopically during surgery. Tissue samples of patients in control group were taken during inferior turbinate reduction operation. Punch biopsy specimens taken from NP and inferior turbinates by using 5 mm tru-cut biopsy forceps (Smith and Nephew, Memphis, TN, USA). Tissue samples were directly drawn into eppendorf tubes. Blood samples were drawn into regular bottles and the serum were separated from the blood by centrifugation at 5000 rpm for 5 minutes and then drawn into eppendorf tubes. The serum and tissue samples stored at -80°C until analyzed. The tests were carried out in our hospital laboratory.

Prolidase enzyme activity

We measured the tissue and serum concentrations of prolidase by the previously reported (11) and slightly modified method (12) based on the spectrophotometric determination of proline levels liberated from glycyl-L-proline by prolidase enzyme. Serum and tissue samples were removed from the freezer (-80°C) and allowed to stand at room temperature to dissolve. Tissue samples were homogenized by ultrasonic homogenizer (Lab Line, CV 26, USA). 25 µL of serum or homogenized tissue fluid samples (pre-diluted with serum physiologic, 1:1, v:v), 75 µL of incubation buffer containing 50 mM MnCl2H2O, and 30.7 mg/dL reduced glutathione (GSH) in 50mM TrisHCl buffer (pH 7.0) were mixed and incubated at 37 °C for 30 min. Then 100 µL of substrate solution (144 mM glycyl-L-proline in 50 mM TrisHCl buffer containing 50mMMnCl2H2O) was added and waited at 37 °C for 5 min. After the end of incubation 2.3 mL of modified Chinard reagent (1 mL of glacial acetic acid +300 µL of 50 mM Tris HCl buffer (pH 7.0)+1 mL of ninhydrin solution (20 g/L ninhydrin in 0.5 Morthophosphoric acid) was added into the reaction mixture. The mixture was then further incubated at 90°C for 30 min. After 1 mL of concentrated glacial acetic acid was added into the reaction mixture, absorbances of prolin standards and samples were read at 515 nm wavelength against sample blank in a spectrophotometer (Beckman Coulter DU 530 UV/VIS Spectrophotometer, USA) using reagent blank as zero. The unit of prolidase enzyme activity was determined as a capable enzyme amount to produce proline in 1 min.

Statistical analysis

IBM SPSS (Statistical Package Social Sciences) version 20.0 was used for the statistical analysis. Differences between the groups were determined using Chi-square test. The significance of differences in prolidase activity among groups was compared using Mann-Whitney U test. Pearson's correlation test was used for comparison of serum PA and tissue PA with each other and with Lund-Mackay scores (LMS) in NP patients. Within the 95% confidence interval, P ≤ 0.05 was considered statistically significant.

Results

This study was completed with 44 patients (34 males, 10 females, mean age 26.3 ± 5.7 old years) in NP group and 38 patients (31 males, 7 females, mean age 25.1 ± 5 old years) in the control group. There were no statistically significant differences in age (p = 0.5) and gender (p = 0.6) between the groups. The average LMS was 16.6 ± 3.4 (min=10,max=24) in NP group. The levels of serum prolidase activity were significantly higher in control group when compared with NP group, whereas the levels of tissue prolidase activity were significantly higher in NP group (p<0.001) (Table 1).

Table 1. Serum and tissue prolidase activity in control and nasal polyp groups.

	Control group (n=38)	Nwasal polyp group (n=44)	p
Serum prolidase activity (U/L ±SD)	1262.3 ± 278	738 ± 247	<0,001
Tissue prolidase activity (U/L ±SD)	37.2 ± 51	200 ± 122	<0,001

There was no statistically significant correlation between serum and tissue PA levels in NP group (r = 0.05, p = 0.75) (Figure 1). There was also no statistically significant correlation between tissue PA levels and LMS in NP group (r = -0.22; p = 0.16) (Figure 2). Statistically significant negative correlation was detected between serum PA levels and LMS in NP group (r = -0.61, p<0.001) (Figure 3).

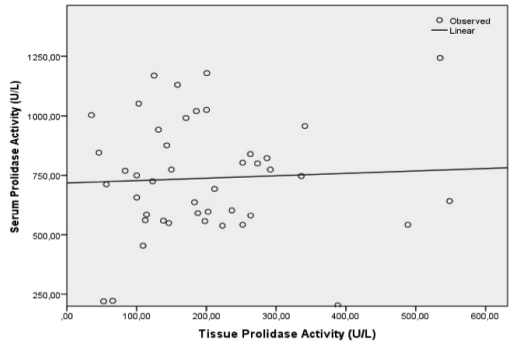

Figure 1. There was no correlation between serum prolidase activity levels and tissue prolidase activity levels in nasal polyp group (Pearson Correlation , r=0.05 p=0,75).

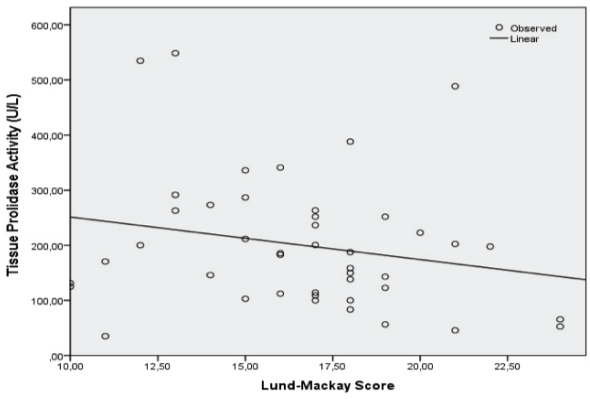

Figure 2. There was no correlation between tissue prolidase activity levels and Lund-Mackay scores in nasal polyp group (Pearson Correlation , r= -0.22 p=0,16).

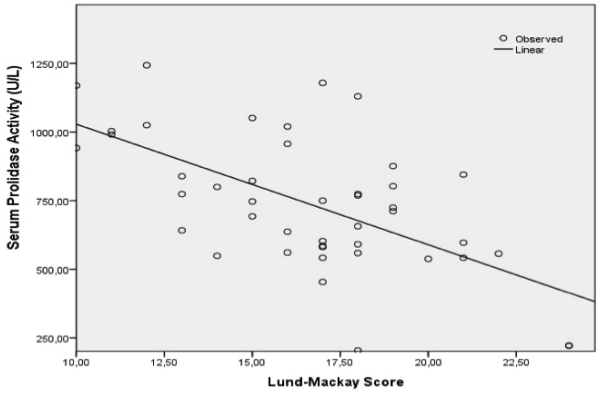

Figure 3. There were a significant negative correlation between serum prolidase activity levels and Lund-Mackay scores in nasal polyp group. (Pearson Correlation , r= -0.61 p< 0.001)

Discussion

NP's are a chronic inflammatory condition of paranasal sinuses and have increased numbers of activated eosinophils, mast cells, and IgE (3). The overall prevalence ranges from 1-4% in the general population (2). Although NP pathogenesis still remains unclear, accumulation of ECM is important process in the characteristic structural modification of NP. Collagen is an important structural component of ECM. The differentiation of fibroblasts into myofibroblasts and collagen production is significantly induced by TGF in nasal polyp derived fibroblasts. Finally, significant increase in subepitelial collagen deposition and fibrosis develop in nasal polyp tissue (2,3,6). Prolidase is a cytosolic exopeptidase, which plays an important role in the recycling of proline and hydroxyproline from imido-dipeptides and imido-tripeptides (mostly derived from degradation products of collagen) for re-synthesis of collagen. Prolidase is a rate-limiting factor in collagen turnover, in the final step of collagen degradation (13). Proline and hydroxyproline play important role in collagen stability. The hydroxyproline levels were found significantly higher in NP tissue compared with normal nasal mucosa tissue (14).

Serum PA has been evaluated in various diseases such as uremic bone disease (15), Leck-Calve-Perthes disease (16), coronary artery disease (17), chronic hypertension (9), chronic liver disease (18), type-2 diabetes mellitus (19), cardiac hypertrophy (9), psoriasis (20) and osteoarthritis (21) and serum PA have been found to be elevated in these diseases. Chronic inflammation and increment of collagen turnover and deposition were held responsible for increased serum PA in these studies. However, conflicting results also have been published. Cakmak et al (22), reported that they found lower serum PA levels in asthma patients compared with healthy subjects. Myara et al (23) found higher serum PA levels in only 5 of 27 cirrhosis patients; they also failed to identify any correlation between the degrees of fibrosis and PA levels. However, they did detect a significant association between serum PA and early stages of cirrhosis. They claimed that, serum PA levels were affected by the degree of collagen turnover, rather than by the extend fibrosis. In end-stage cirrhosis, there may be insufficient collagen turnover to increase serum PA, meaning that collagen turnover becomes lower than normal, possibly leading to lower serum PA. In a study on patients with diabetic foot ulcers, it has been proposed that collagen deposition is the possible reason of the high levels of PA in the wound fluid so the decrease in serum levels of PA may reflect an appropriate wound healing and end of collagen accumulation (24). We found lower serum PA levels and higher tissue PA levels in nasal polyposis patients compared with control subjects. From these results it can be considered serum prolidase enzyme deficiency play a role in nasal polyposis pathogenesis, but prolidase enzyme deficiency is rare, the reason is still unknown an autosomal recessive disease that presents with a variety of clinical manifestation and associated with several diseases. In prolidase deficiency, mainly effected system is connective tissue, particularly its main component collagen. Prolidase deficiency is usually presented as clinical condition associated with chronic ulcerative dermatitis, chronic skin lesions, delayed wound healing, splenomegaly, mental retardation, frequent infections, respiratory dysfunction, disturbed psychomotor development and massive urinary excretion of imino-dipeptides. The onset of clinical symptoms generally is before the age of 12 years (13,25). In our study, none of the patients presented any of these clinical manifestations and the disparity between the prevalence of the two diseases have also moved away us from the idea of prolidase deficiency plays a role in the pathogenesis of nasal polyposis. In our study, statistically significant negative correlation was detected between serum PA levels and LMS while there was no statistically significant correlation between tissue PA levels and LMS in NP group. There was no statistically significant correlation between tissue PA levels and serum PA levels in NP group. The average LMS was 16.61 and there was widespread disease in our patients in NP group. In accordance with the results of Cakmak (22) and Myara (23), we determined lower serum PA levels in NP patients compared to control group in our study. We think that a negative feedback mechanism develops in advanced stage of NP disease that causes the slowdown of collagen turner over and accumulation of ECM and we also believe that this is the reason of low serum PA levels.

As a result, we think that serum PA levels and tissue PA levels are effective in nasal polyp development. Serum PA levels may give us an idea about stage of the disease if sufficient

data generated by clinical trials. Because of this, there is need for clinical trials which contains more and in different stages of the disease patients and evaluate correlation between PA levels and widespread of nasal polyps.

Kaynaklar

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