Research Article

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The effects of oral steroid therapy on prolidase enzyme activity in patients with nasal polyps

Nazal polipli hastalarda oral steroid tedavisinin prolidaz enzim aktivitesi üzerine etkisi

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Abstract

Objective: To compare prolidase enzyme activity (PEA) in serum and polyp specimens of patients with nasal polyps obtained before and after the oral steroid therapy.
Materials and methods: Thirty three patients with nasal polyps (39 ± 13 years) received 1 mg/kg of oral steroids. Serum samples were collected from each patient, but nasal polyp specimens could be obtained only from 23 patients (38 ± 13 years) before and after the oral steroid therapy. PEA was measured by ELISA method.
Results: Serum PEA values were 210 (176–242) U/L and 184 (147–217) U/L before and after the oral steroid therapy, respectively (p = 0.015). Polyp tissue PEA was 1337 (738–2130) U/g and 871 (590–1663) U/g before and after the oral steroid therapy, respectively (p = 0.429).
Conclusion: In patients with nasal polyps, significantly lower serum PEA after the oral steroid therapy may be a consequence of the role of prolidase enzyme in inflammatory processes which are important for the development of nasal polyps. More comprehensive studies with larger sample sizes are needed to elucidate the role of PEA in the pathogenesis of nasal polyps.

Keywords: Nasal polyp; Prolidase; Steroid therapy; Inflammation; Fibrosis.

Öz

Amaç: Nazal polip hastalarının serum ve burun polip dokusu örneklerinde steroid tedavisinden önce ve sonra prolidaz enzim aktivitesini karşılaştırmaktır.
Gereç ve Yöntem: Nazal polipli 33 hasta (39 ± 13 yıl) 1 mg/kg oral steroid tedavisi aldı. Oral steroid tedavisi öncesi ve sonrasında her bir hastadan serum örnekleri alınabilirken, sadece 23 hastadan (38 ± 13 yıl) nazal polip dokusu örnekleri alınabildi. Prolidaz enzim aktivitesi ELISA yöntemiyle ölçüldü.
Bulgular: Serum prolidaz enzim aktivitesi tedavi öncesi 210 (176–242) U/L iken tedavi sonrası 184 (147–217) U/L idi (p = 0.015). Nazal polip dokusundaki prolidaz enzim aktivitesi ise tedavi öncesi 1.337 (738–2.130) U/g iken tedavi sonrası 871 (590–1.663) U/g idi (p = 0.429).
Sonuç: Nazal polipli hastalarda oral steroid tedavisinden sonra görülen anlamlı derecede daha düşük serum prolidaz enzim aktivitesi nazal polip gelişimi için önemli olan enflamatuar süreçlerde prolidaz enzim aktivitesinin bir rolünün olmasından dolayı olabilir. Nazal poliplerin patogenezinde prolidaz enzim aktivitesinin rolünü açıklığa kavuşturmak için daha fazla hastayla daha detaylı çalışmalar yapılması gerekir.

Anahtar kelimeler: Nazal polip; prolidaz; steroid tedavisi; enflamasyon; fibrozis.

Introduction

Nasal polyps (NPs) that occur as a result of the chronic inflammation in nasal cavity and paranasal sinuses are

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the inflammatory outgrowths of upper respiratory tract [1]. Although allergy, bronchial asthma, chronic local infections, aspirin intolerance, anatomical disorders, genetic factors, epithelial rupture, mucosal contact, Bernoulli's phenomenon, connective tissue disorders, immunological and biochemical factors have been proposed in the etiopathogenesis of NPs, itstill remains unclear [2]. Various cytokines, growth factors, chemical mediators and inflammatory cells cause the development of nasal mucosal inflammation and edema which are the leading cause of NPs [3, 4]. In the development of NPs, chronic inflammation is followed by mucosal epithelial proliferation, mucosal thickening, myofibroblast differentiation, extracellular matrix (ECM) deposition and fibrosis [5, 6].

Prolidase, a manganese-dependent cytosolic exopeptidase, plays an important role in ECM remodelling, collagen degradation and turnoverby cleaving imidodipeptides containing C-terminal proline or hydroxyproline [7, 8]. Increased plasma prolidase activities have been observed elevated in situations that are characterized by chronic inflammation of the tissue and/or increased collagen turnover. Therefore, alterations in PEA may contribute to the development of NPs [9].

Different kinds of agents are used for the medical therapy of NPs, but steroids are still the most widely used ones. Up to now, there are few studies about to the relationship between serum PEA and NPs in the literature. However, there is no published study for PEA both in serum and nasal polyp tissue before and after the therapy with steroids in patients with NPs. Therefore, we aimed to determine the levels of PEA in serum and polyp specimens of these patients before and after the oral steroid therapy.

Materials and methods

This prospective cohort study was performed between 15 May 2016 and 15 May 2017 at Otorhinolaryngology Clinic of Hitit University Erol Olcok Education and Research Hospital. Ethical approval was taken by Hitit University Clinical Research Ethics Committee with a number of 2016/30. Patients with cystic fibrosis, ciliary dyskinesia, chronic lung disease, atherosclerotic heart disease, malignancy, hypertension, severe systemic diseases, diabetes mellitus, any nasal pathology such as antrochoanal polyp, inverted papilloma, using nasal steroids, antibiotics or any other medication that could affect the results were excluded from this study. NPs were diagnosed by anterior rhinoscopy and endoscopy. Prevalence of the disease was evaluated by paranasal computed tomography. Each patient was informed about the possible side effects of methylprednisolone therapy, complications of the procedure, andthen a written informed consent form was obtained.

Patients received oral methylprednisolone therapy (prednol 16 mg, Mustafa Nevzat Pharmaceutical Industry, Istanbul, Turkey) at a daily dose of 1 mg per kg. Methylprednisolone was reduced by 10 mg in each following three days, and terminated approximately in 3 weeks. Preoperative polyp tissues and serum samples were taken 1 day before starting the steroid therapy. (In our clinic, blood tests and histoptological samples are routinely performed at the time of policlinic examination in patients with nasal polyps). Serum and nasal polyp samples were taken from the patients who were decided to have surgery within 5 days after the end of oral steroid treatment. Serum samples were taken during routine blood examination before anesthesia. Nasal polyp samples were taken during the operation and in the operating room among the routine tissues taken for pathological examination.

Measurements of the PEA in serum and nasal polyp tissues

After 12-h of fasting, 8 mL of venous blood sample was drawn into the clot activator containing tubes (Isotherm, Hongyu Medical, Weihai, China) from each patient between 08:00 AM and 10:00 AM. After the formation of the blood clot, serum was separated by centrifuging for 10 min at 3000 *g*, and then stored at −80°C in an eppendorf tube until being analyzed. Nasal polyp samples were taken from all patients before and after steroid treatment. These tissue samples were placed into the empty plastic tubes and then stored at −80°C until the PEA measurement.

Each nasal polyp specimen was weighed and placed into empty tubes. One milliliter of 0.9% saline solutionwas added per 100 mg of nasal polyp sample, and then each tissue sample was homogenized with a motor-driven homogenizer. After that, the specimens were sonicated in cold water ten times for 10 s, and were centrifuged at 3000 *g* for 10 min at 4°C. The obtained supernatant and serum were used for the measurement of PEA by human prolidase ELISA kit (Catolog number CSB-E16196h, Cusabio Biotech Co. Ltd.). Intra-assay and inter-assay coefficient of variations were <8% and <10%, respectively. Detection range was from 93.75 U/L to 6000 U/L, with a sensitivity less than 23.4 U/L. Measurements were read on automated ELISA microplate reader: ALISEI from Radim Diagnostics.

Statistical analysis

SPSS (Version 23.0, Chicago, IL, USA; license Hitit University) was used for all statistical analyses in this study. Normality of the distribution was assessed using the Shapiro-Wilk test. Descriptive statistics for continuous variables were summarized as mean ± standard deviation or median (25^{th}–75^{th} Inter Quartile Range) based on the distribution, and categorical data were presented as numbers and percentages. For the comparison of continuous variables, Wilcoxon Signed Rank test was used. The level of statistical significance was a p value less than 0.05.

Results

19 (57.6%) males and 14 (42.4%) females were included in this study (39 ± 13 years for all patients). Serum PEA levels were compared before and after the oral steroid therapy in all 33 patients, but polyp tissue PEA levels could be examined only in 23 patients, since polyps for 10 patients could not be obtained due to the localization of them. Therefore, PEA could not be measured in the nasal polyp tissues of these 10 patients. Serum PEA was 210 (176–242) U/L and 184 (147–217) U/L before and after the oral steroid therapy, respectively (p = 0.015) (Table 1) (Figure 1). Polyp tissue PEA was 1337 (738–2130) U/g before the oral steroid therapy, and was 871 (590–1663) U/g after the therapy (p = 0.429) (Table 1) (Figure 2).

Discussion

To the best of our knowledge this is the first study to investigate PEA both in serum and nasal polyp tissue specimens before and after the oral steroid therapy. The main findings of the present study showed a statistically significant difference between serum PEA levels before and after the oral steroid therapy. Although there was a decrease in polyp PEA levels after treatment with oral steroids, this decrease was not statistically significant due to the limited number of nasal polyp tissues analyzed. It is believed that NPs should be primarily treated with surgical removal. However, detection of the essential role of chronic inflammation in the pathophysiology of NPs took medical therapys to fore front. Steroids, especially the methylprednisolone and dexamethasone, are the medical therapy of choice in NPs [10]. They exert their effects mainly on lymphocytes, monocytes, macrophages, eosinophils, neutrophils, mast cells and basophils by reducing the circulating lymphocyte count, inhibiting the neutrophil migration towards inflammed areas and alleviating the local inflammatory reaction [11].

Table 1: Wilcoxon signed rank test results for prolidase activity in the serum and polyp tissue.

	Group	n	Median (min-max) U/L	p-Value
Serum	Pre-treatment	33	210.19 (133.9–441.1)	**0.015**[a]
	Post-treatment	33	183.67 (31.25–311.7)	
Tissue	Pre-treatment	23	1337.32 (737.9–2130.3)	0.429
	Post-treatment	23	871.27 (590.1–1663.3)	

[a]Statistically significant (p < 0.05); Min, minimum; Max, maximum.

ECM deposition is an important process in the structural modification of polyp formation [6, 12, 13]. Myofibroblasts represent an activated fibroblast phenotype with high capacity to release ECM proteins. Therefore, they have an important role in the development of NPs, in addition to their functions such as connective tissue formation and deposition [14, 15]. An increased plasma PEA has been considered to be an indicator of fibrosis in a number of studies [9, 15, 16]. Higher rates of fibrosis in NPs have been considered to be possibly associated with the increased serum PEA. We therefore considered that measurements of PEA both in serum and polyp specimens in patients with NPs may have positive contributions to the understanding of the pathogenesis as well as the therapy of disease.

Collagen is the main constituent of bones, tendons and membranous connective tissues. Twenty-five percent of the amino acids in the collagen structure are proline and hydroxyproline. Prolidase plays an important role in the degradation of collagen and regulation of collagen turnover. It is particularly important in the last stage of protein catabolism, in the degradation of procollagen which contains a higher amount of proline, and the reentry of proline into the collagen cycle [6]. Prolidase is the unique enzyme able to cleave the peptide bond between proline and glycyl in the structure of collagen, which suggests that Serum PEA may directly be associated with the rate of collagen turnover [17].

In a study conducted by Hissariaet al. in 2006, a group of patients with sinonasal polyps were treated with oral methylprednisolone at a dose of 50 mg daily for 14 days, and compared to the placebo group. As a result, significant improvements were observed in nasal symptoms and polyp sizes in patients treated with systemic steroids [18]. When our patients were re-evaluated by the nasal endoscopic examination, it was seen that the polyp sizes

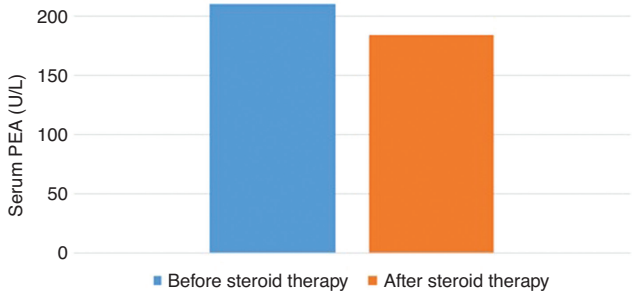

Figure 1: Serum prolidase enzyme activity levels before and after steroid treatment.

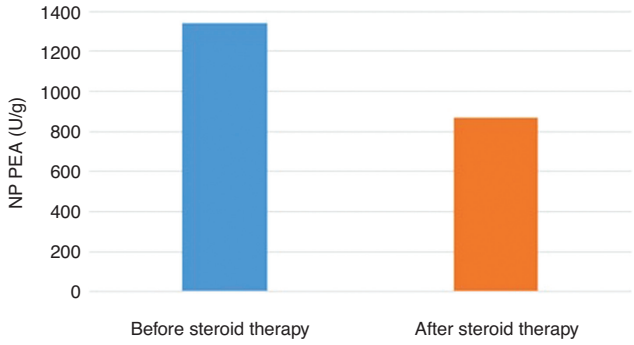

Figure 2: Tissue prolidase enzyme activity levels before and after steroid treatment.

after steroid therapy were relatively smaller than before the therapy.

San et al. compared PEA in serum and NPs of 30 patients to that in 30 patients with turbinate hypertrophy or septal deviation [16]. In patients with NPs, PEA was found to be higher than controls, both in the serum and polyp tissues. They suggested that the increased PEA might be a consequence of chronic inflammation, and concluded that higher levels of PEA might be an indicator of fibrotic processes in the development of NPs. In our study, decreased PEA both in serum and nasal polyp specimens as well as apparent reduction in polyp sizes following steroid therapy may indicate the potential importance of PEA in the pathogenesis of NPs.

Salihoglu et al. compared PEA in serum and nasal polyp specimens from patients with NPs to that in the control group [9]. The control group included patients without NPs who underwent inferior turbinate reduction surgery. PEA was measured in serum and polyp tissues of these patients. PEA in nasal polyp tissue and serum was significantly higher than the control group. In our study, we detected a significant reduction in serum PEA following the oral steroid therapy.

Cincik et al. compared hydroxyproline levels in polyp tissue specimens of patients with NPs to the levels in normal mucosa specimens from patients with a turbinate pathology [19]. In patients with NPs, hydroxyproline levels were found to be significantly higher than those in the control group. They also reported a significant reduction in hydroxyproline levels in patients with NPs, following intranasal or oral steroid therapy. Although serum PEA was not measured in this study, higher hydroxyproline levels in patients with NPs may indicate higher levels of PEA. In our study, we found significant reductions in serum PEA after oral steroid treatment. However, unlike Cincik et al. we did not detect statistically significant reduction in PEA in polyp tissues following oral steroid therapy. Therefore, we consider that measurements of the PEA in combination with hydroxyproline may be more valuable than the measurements of SPEA alone in patients with NPs.

Eosinophilic polyps are characterized by prominent edema in association with rarer glandularity and collagen deposition while significant glandular hypertrophy, dense collagen deposition and mononuclear cell infiltration are the features of non-eosinophilic polyps [20]. Dense collagen deposition in non-eosinophilic polyps suggests that higher PEA may result in the development of such polyps. However, we don't know whether the polyps obtained in our study are eosinophilic or not.

One of the limitations of our study is that nasal polyps included in this study were not stratified as eosinophilic polyps and non-eosinophilic polyps. We assume that it would be more useful to compare serum and nasal polyp PEA after separating the patients into eosinophilic, non-eosinophilic and control groups. The second limitation was the small sample size of study patients with NPs.

Conclusion

Significant reductions were observed in serum PEA of patients with NPs after the oral steroid therapy. This finding may suggest that PEA may have an important role in the inflammatrory processess of NPs. More comprehensive prospective cohort studies with larger samples are needed to elucidate the role of PEA in the pathogenesis of NPs.

Conflict of interest: The authors decleared that there was no conflict of interest for this article.
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