Original Article

Transforming growth factor beta 1 (TGF-β1) in the pathophysiology of nasal polyp

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ABSTRACT

Objectives: This study aims to use immunohistochemistry to compare histopathological findings and transforming growth factor beta 1 (TGF-β1) levels in polyp tissue in cases of nasal polyp with histopathological findings and TGF-β1 levels in the nasal mucosa in patients with obstructive nasal deformity.

Patients and Methods: The study group consisted of 20 patients. The control group consisted to 20 patients scheduled for nasal surgery due to septum deviation. Edema, mixed infection cell infiltration, eosinophil leukocyte infiltration and squamous metaplasia development were studied from hematoxylin-eosin stained slides containing polyp and conchal mucosa tissue specimens collected from the study and control groups. These were then assessed semi-quantitatively. The presence or absence of TGF-β1 antibody and positive reaction in the mucosal epithelium, submucosal glands, fibroblasts and inflammatory cells were evaluated immunohistochemically.

Results: A positive association was determined between eosinophilia and edema on tissue staining with hematoxylin and eosin in cases of nasal polyp, while no edema was observed in any cases in the control group. Comparison of the control and study groups on immunohistochemical (TGF-β1 positive reaction) staining revealed no significant difference between epithelium, gland and fibroblast staining. Immunohistochemical staining of inflammatory cells was more pronounced in the study group.

Conclusion: We conclude that TGF-β1 may play a role together with other mediators, although not alone, in the pathophysiology of nasal polyp.

Keywords: Nasal polyposis; tissue eosinophilia; transforming growth factor beta 1.

Polyps are benign mucosal formations that develop for various reasons. Nasal polyps (NP) are edematous masses associated with chronic inflammation and characterized by squamous metaplasia of the nasal mucosa, secretory hyperplasia, inflammatory cell infiltration, extracellular matrix accumulation and fibrosis.[1] They are also characterized by chronic mucosal inflammation of the nasal and paranasal sinuses.[2]

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The causes leading to polyp formation are still unclear, but damage to the mucosal epithelium mediated by mesenchymal tissue or inflammatory mediators have been proved to play a key role. Inflammatory cell inflammation and thickening of the basal membrane together with epithelial proliferation and extracellular matrix accumulation occur with recurring injury. Fibroblasts are found in the stroma and stimulate extracellular matrix accumulation.[3] Broad variation may be observed among polyp structures. The essential features of the bilateral eosinophilic polyp include thickening in the basal membrane, pseudocyst formation, a decrease in vessel and gland numbers together with edematous fibrotic stromal tissue and an absence of neuronal structures.[4]

Recent studies have suggested that the release of immunological mediators is the most important factor in polyp development. Interleukin (IL)-3, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-8 have been reported as the most important of these mediators. These cytokines represent the essential cause of chronic inflammation, eosinophilia, superficial epithelial changes and edema.[5]

Eosinophils are the dominant inflammatory cells in NP tissue. The cytokines transforming growth factor alpha (TGF-α) and ILI-β regulate the extravasation of eosinophils toward the NP lamina propria. Eosinophils, mast cells, macrophages and T cells are the main source of TGF. The liposaccharide (LPS) in human blood is the most important potent stimulus for TGF-α production. This induces the release of adhesion molecules and several other cytokines and inflammatory proteins.[6,7]

Interleukin-3, IL-5 and GM-CSF are cytokines responsible for the activation and survival of eosinophils. Granulocyte-macrophage colony-stimulating factor increases in parallel to the active eosinophils in NP tissue. It contributes to chronic eosinophilic response by increasing eosinophil migration, survival and activation.[8]

The TGF-β1 family is one of the extracellular growth factors controlling development in a multi-directional manner. It is involved in the growth, repair and hemostasis of all tissues in the body.[9] Transforming growth factor-β1 is a cytokine with multi-directional effects that modulates proliferation, differentiation and other processes in cell survival.[10] Structurally, it contains a large number of polypeptide growth factors. These are involved in the regulation of such cellular processes as proliferation, differentiation, motility, adhesion and cell death.[9]

Transforming growth factor-β1's effect on the immune system takes the form of inhibition. It suppresses production of immunoglobulin by B lymphocytes. The effect of TGF-β1 on cell proliferation depends on the type of cell. It exhibits an inhibitory effect on epithelial cells, endothelial cells, fibroblasts and T and B lymphocytes. Its effect on human mesenchymal cells, in contrast, is biphasic. At high concentrations, TGF-β1 prevents cell growth, while at low concentrations it has a mitogenic effect.[10]

Transforming growth factor-β1 is released by various cells in the body, such as lymphocytes, eosinophils, macrophages and fibroblasts. Transforming growth factor-β1 leads to fibrosis in respiratory tissue and exhibits a pro-inflammatory regulatory effect in fibroblastic activity. Transforming growth factor-β1 is known to be a potent stimulator of fibroblast proliferation in the respiratory epithelium. Fibroblasts are present in NP tissue stroma and have been associated with active extracellular matrix accumulation. According to this thesis, they contribute to fibroblast proliferation and connective tissue deposition in chronic rhinosinusitis.[3]

The purpose of this study was to use immunohistochemistry to compare histopathological findings and TGF-β1 levels in polyp tissue in cases of NP with histopathological findings and TGF-β1 levels in the nasal mucosa in patients with obstructive nasal deformity.

PATIENTS AND METHODS

Patients presenting to the Gaziosmanpaşa University Ear, Nose and Throat clinic with nasal polyposis determined in their histories and on endoscopic examination were included

in the study. Cases of NP with non-specific and specific (granulomatous inflammation) upper respiratory tract inflammatory disease or with cystic fibrosis, primary ciliary dyskinesia or other systemic disease were excluded. Twenty patients were enrolled in the study group. Care was taken that patients in the study group had received no treatment for nasal polyposis in the one-month period before surgery. The control group of 20 subjects was selected from patients with no history of asthma or allergic, inflammatory or granulomatous disease and scheduled for surgery due to septum deviation or obstructive nasal deformity. Biopsy tissue specimens were collected from the inferior conchal mucosa following general anesthesia in these subjects.

Consent was obtained from patients in both study and control groups. Approval was granted by the Gaziosmanpaşa University Medical Faculty Ethical Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Following routine tissue procedures, the NP and normal conchal mucosa tissue specimens from the study and control groups were placed in paraffin blocks. Sections 4 micrometers in thickness were then taken from these. Polyp tissues in sections stained with hematoxylin-eosin (H-E) were assessed in terms of edema, mixed inflammatory cell infiltration, eosinophil leukocyte infiltration and development of squamous metaplasia. The same parameters were used in the assessment of conchal mucosa specimens from the control group.

Immunohistochemical analysis

Immunohistochemical analysis was performed on paraffin sections from both the study and control groups. Four-micrometer sections from the paraffin blocks were first kept overnight in a stove at 65°C and then deparaffinized in xylene. Deparaffinized sections were passed through decreasing alcohol series (90%, 80% and 70% ethyl alcohol). Sections were placed in citrate buffer solution for antigen retrieval, and boiled in a microwave for 2 5-min cycles, at high and low energy. They were then left to cool at room temperature for 20 min. After cooling, the slides were washed in distilled water. Once dry, the outside of the slides was marked with a slide marker and hydrogen peroxide was applied for 10 min in order to prevent nonspecific background staining. Following 20-min incubation of tissue sections with ultraviolet block, TGF-β1 antibody was diluted to 1:50 with antibody diluent and applied to the sections. One-hour incubation was then performed for the primary antibody. Next, sections were incubated for 30 min at each step with biotinylated goat serum and streptavidin peroxidase solution. Amino ethyl carbazole (AEC) chromogen was applied for 15 min in order to show the resulting reaction. Following application of Mayer's hematoxylin for background staining, the slides were covered with aqueous material.

Assessment of immunohistochemical staining was performed under a light microscope. Positive staining was assessed on the basis of the presence or absence of positive reaction in the mucosal epithelium, submucosal glands, fibroblasts and inflammatory cells. Semi-quantitative evaluation was performed in the form of intensity of staining and stained area percentages.

Parameters evaluated semi-quantitatively in sections stained with H-E and the presence or

Table 1. Severity of edema in tissues stained with hematoxylin-eosin

	Study group	
	n	%
Edema		
None	1	5
Mild	2	10
Moderate	3	15
Severe	14	70

Table 2. Intensities of eosinophilia in polyp tissue stained with hematoxylin-eosin in the study group

	Study group	
	n	%
Eosinophilia		
None	2	10
Mild	7	35
Moderate	8	40
Severe	3	15

Table 3. Densities of mixed inflammatory cells in the study and control groups

	Study group		Control group	
	n	%	n	%
Mixed inflammatory cells				
None	0	0	8	40
Mild	6	30	8	40
Moderate	8	40	2	10
Severe	6	30	2	10

absence of TGF-β1 positive reaction in the tissue components cited above were compared within and between groups using appropriate statistical analysis in order to determine potentially significant relations.

Statistical analysis

All the variables in this study consisted of qualitative data. The chi square test on 2x2 grids was therefore used for comparisons between variables. Under conditions in which this test was not applied (when the expected frequencies in any well were less than 5), the Fisher exact chi square test was used. Correlation analysis (Spearman Rho coefficient) was used to investigate relations between variables involving descriptive data.

RESULTS

Ages of the patients with NP ranged between 12 and 72, with a mean age of 37.4. Ages in the control group ranged from 25 to 58, with a mean value of 27.1.

Fifteen (75%) of the patients in the study group were male and five (25%) were female. Sixteen (80%) of the subjects in the control group were male and four (20%) were female. Six patients (30%) in the study group had allergies, and fourteen (70%) had none. Five patients (25%) in the study group and four (20%) in the control group were smokers.

Seven patients (35%) in the study group had previously undergone surgery for NP once, one (5%) had been operated on twice, and 12 (60%) had never been operated for NP. Fifteen patients (75%) used nasal steroids, while the other five (25%) had not.

No edema was determined in tissues stained with H-E in the control group (Figure 1). The study group was divided into four groups on the basis of severity of edema-none, mildly edematous tissue, moderate edematous tissue and severely edematous tissue (Table 1, Figure 2).

Analysis of the presence of eosinophils in tissues revealed no eosinophils in preparates stained with H-E in the control group (Figure 3).

Levels of eosinophilia using the same staining method in the study group are shown in Table 2. Mild eosinophilia was defined as a dispersed state, moderate eosinophilia as small groups and severe eosinophilia and eosinophilia masses (Figure 4).

Table 4. Epithelial staining using immunohistochemical (TGF-β1 positive reaction) staining

	Staining in the epithelium			
	Study group		Control group	
	n	%	n	%
No staining	6	30	4	20
Staining	14	70	16	80

Table 5. Staining in glands on immunohistochemical staining (TGF-β1 positive reaction) staining

	Staining in glands			
	Study group		Control group	
	n	%	n	%
No staining	8	40	6	30
Staining	12	60	14	70

Table 6. Staining in fibroblasts on immunohistochemical staining (TGF-β1 positive reaction) staining

	Staining in fibroblasts			
	Study group		Control group	
	n	%	n	%
No staining	14	70	18	90
Staining	6	30	2	10

Table 7. Staining in inflammatory cells on immunohistochemical staining (TGF-β1 positive reaction) staining

	Inflammatory cell staining			
	Study group		Control group	
	n	%	n	%
No staining	6	30	15	75
Staining	14	70	5	25

Varying levels of mixed inflammatory cells were observed in tissue on staining with H-E in both the study and control groups (Table 3, Figure 5). The difference in mixed inflammatory cell density distributions between the groups based on the Chi-square test was significant (χ^2=13.886, p=0.003) (Figure 6).

No squamous dysplasia or basal membrane thickening was observed in any of the preparates stained with H-E from the study or control groups.

Presence of staining in the epithelium in the study and control groups was compared using immunohistochemical (TGF-β1 positive reaction) (Table 4, Figure 7). A statistically insignificant difference was determined based on the chi-square test (χ^2=0.533, p=0.465) (Figure 8).

Staining was observed in glands in both the study and control groups on immunohistochemical staining (TGF-β1 positive reaction) (Table 5, Figure 9). The differences according to the chi-square test were statistically insignificant (χ^2=0.440, p=0.507) (Figure 10).

Staining was present in fibroblasts in both the study and control groups on

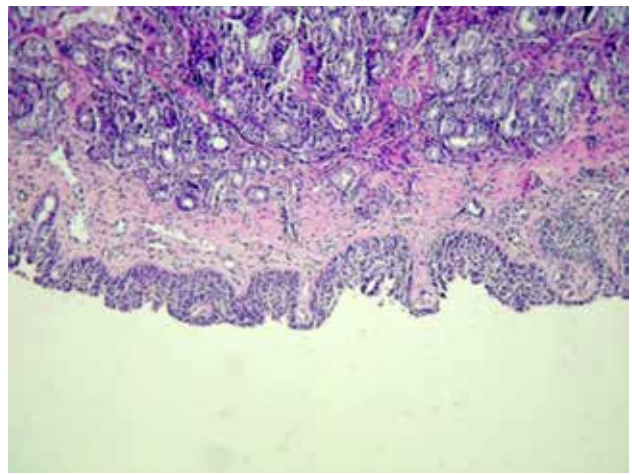

Figure 1. *Control group mucosal edema stained with H-E×10 magnification.*

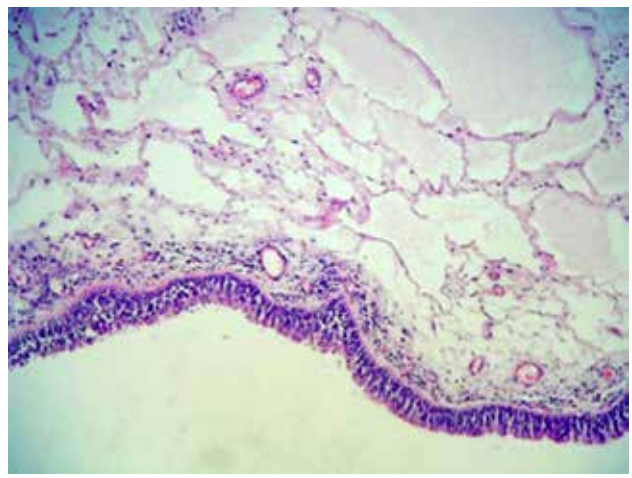

Figure 2. *Study group mucosal edema stained with H-E×10 magnification.*

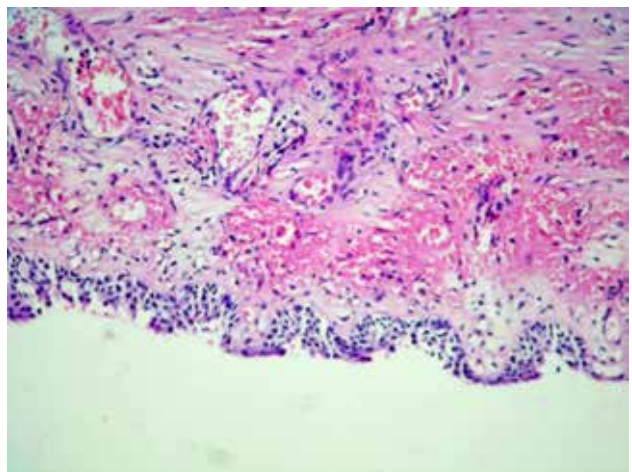

Figure 3. *Control group mucosal eosinophils stained with H-E×20 magnification.*

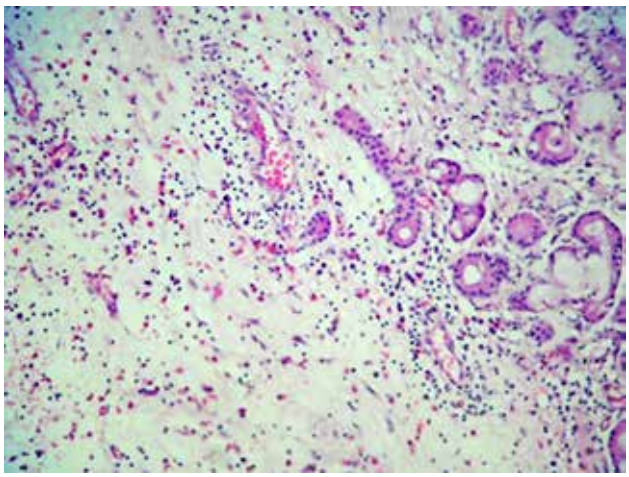

Figure 4. Study group mucosal eosinophils stained with H-E×20 magnification.

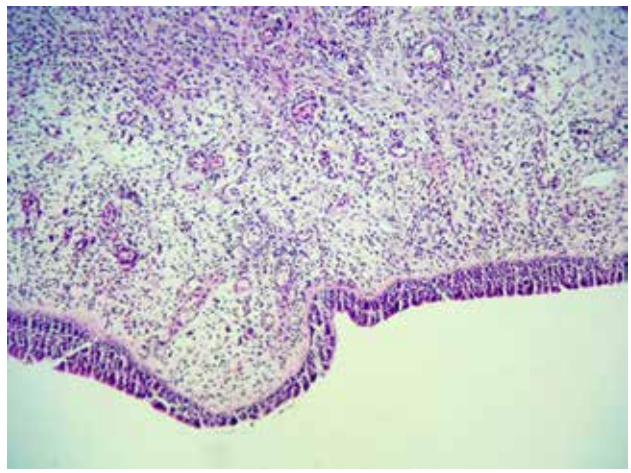

Figure 5. Control group mucosal mixed inflammatory stained with H-E×10 magnification.

immunohistochemical staining (TGF-β1 positive reaction) (Table 6). A statistically insignificant difference was determined between the groups on the basis of Fisher exact chi-square test (p=0.235).

Staining was present in inflammatory cells in both the study and control groups on immunohistochemical staining (TGF-β1 positive reaction) (Table 7). A statistically significant difference was determined on the basis of the chi square test (χ^2=8.120, p=0.004).

Simple correlation analysis (Spearman correlation coefficient method) revealed a positive, strong significant correlation between edema and eosinophilia in preparates stained with H-E in the study group (r=0.601, p=.005). A negative, weak but significant correlation was observed between mixed inflammation and eosinophilia (r=0.451, p=0.046).

There was no correlation in the study group on immunohistochemical (TGF-β1 positive reaction) staining between epithelial staining, gland staining and eosinophil density on staining with H-E (r=0.02, p=0.933).

A positive, weak and insignificant correlation was determined in the study group between

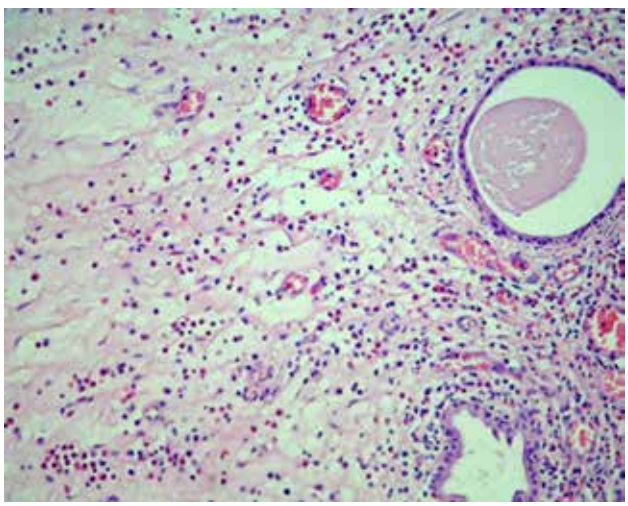

Figure 6. Study group mucosal mixed inflammatory stained with H-E×10 magnification.

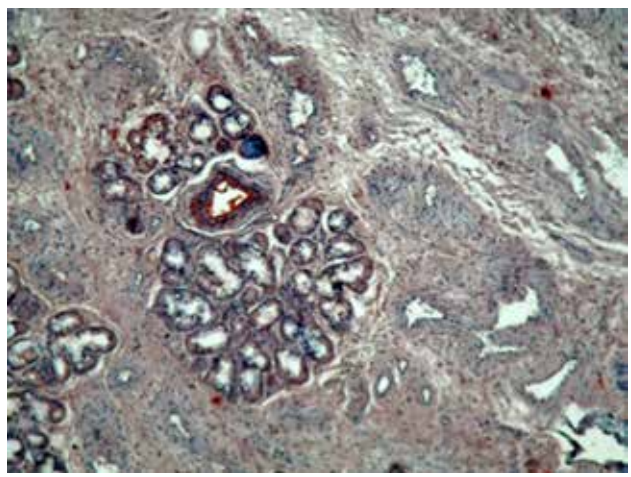

Figure 7. Control group mucosal edema stained with immunohistochemical (TGF-β1 positive reaction) ×20 magnification.

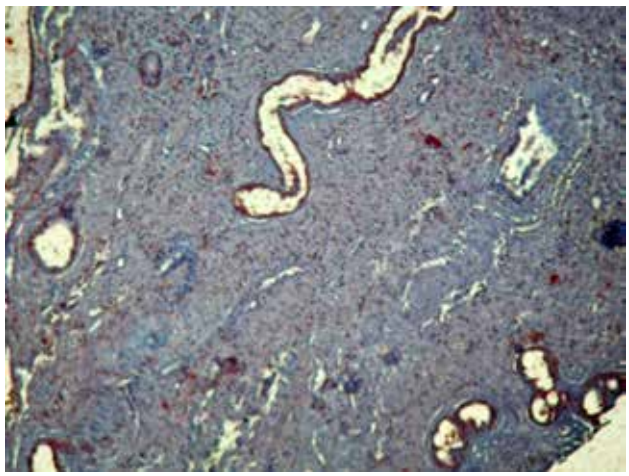

Figure 8. *Study group mucosal edema stained with immunohistochemical (TGF-β1 positive reaction) ×10 magnification.*

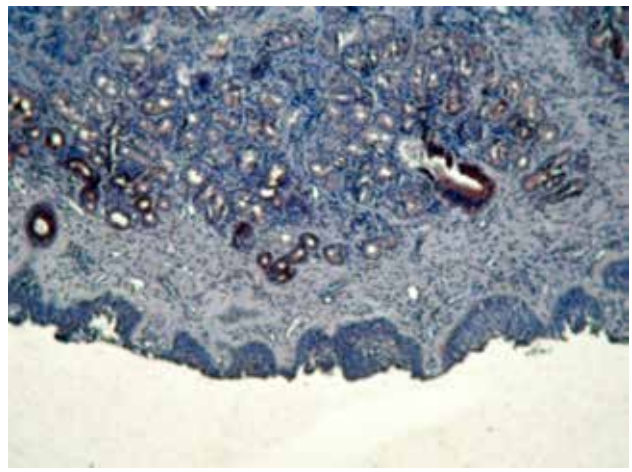

Figure 9. *Control group mucosal gland stained with immunohistochemical (TGF-β1 positive reaction) ×10 magnification.*

fibroblast staining on TGF-β1 positive reaction staining and eosinophil density on staining with H-E (r=0.170, p=0.473).

A positive, weak and insignificant correlation was also determined in the study group between inflammatory cell staining on TGF-β1 positive reaction staining and eosinophil density on staining with H-E (r=0.421, p=0.065).

A negative, strong and significant correlation was observed in the study group between gland staining with TGF-β1 positive reaction and edema density on staining with H-E (r=-0.525, p=0.017).

No correlation was determined between fibroblast staining on immunohistochemical staining (TGF-β1 positive reaction) and density of edema on H-E staining (r=0.023, p=0.922).

A positive, weak and insignificant correlation was observed between inflammatory staining on immunohistochemical staining (TGF-β1 positive reaction) and density of edema on H-E staining (r=0.386, p=0.93).

No correlation was determined between epithelial, gland or fibroblast staining on immunohistochemical staining (TGF-β1 positive reaction) and density of mixed inflammation on H-E staining (r=0.00, p=l.00).

A negative, weak and insignificant correlation was observed between inflammatory staining on immunohistochemical staining (TGF-β1 positive reaction) and density of mixed inflammation on H-E staining (r=-0.423, p=0.063).

A negative, weak but significant correlation was observed in the study group between eosinophilia and mixed inflammation in preparates stained with H-E (r=-0.451, p=-0.046).

A negative, weak and insignificant correlation was determined in the study group between mixed inflammation and edema in preparates stained with H-E (r=-0.325, p=0.162).

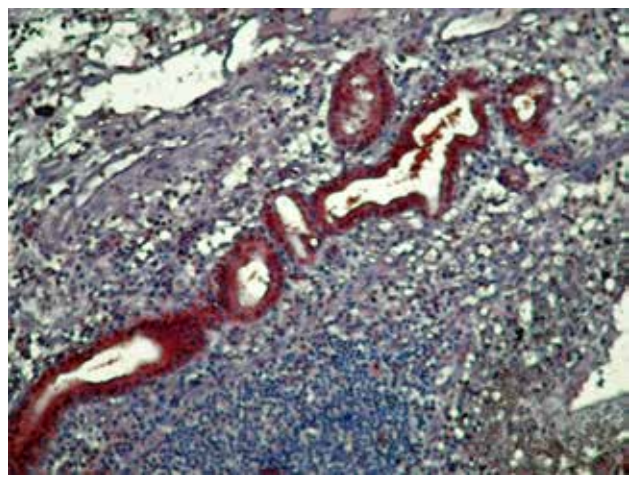

Figure 10. *Study group mucosal gland stained with immunohistochemical (TGF-β1 positive reaction) ×20 magnification.*

Positive, weak and insignificant correlations were determined on immunohistochemical staining (TGF-β1 positive reaction) in the study group between epithelial staining and gland staining (r=0.138, p=-0.574), epithelial staining and fibroblast staining (r=0.429, p=0.59), and fibroblast staining and gland staining (r=0312, p=0.181).

No statistically significant correlation was determined on immunohistochemical staining (TGF-β1 positive reaction) in the study group between epithelial and inflammatory cell staining (r=0.048, p=0.842).

No statistically significant correlation was determined on immunohistochemical staining (TGF-β1 positive reaction) in the study group between inflammatory cell and gland staining (r=0.89, p=0.709).

A positive, weak and insignificant correlation was observed on immunohistochemical staining (TGF-β1 positive reaction) in the study group between fibroblast and inflammatory cell staining (r=0.190, p=0.421).

In the control group, a negative, weak and insignificant correlation was observed between mixed inflammation on H-E staining and epithelial staining (r=-0.232, p=0.325), gland staining (r=-0.122, p=0.610) and fibroblast staining (r=-0.124, p=0.603) on immunohistochemical staining (TGF-β1 positive reaction).

A positive, weak but insignificant correlation was observed in the control group between mixed inflammation on H-E staining and inflammatory staining on immunohistochemical staining (TGF-β1 positive reaction) (r=0.472, p=0.036).

A positive, strong and significant correlation was observed in the control group between epithelial and gland staining on immunohistochemical staining (TGF-β1 positive reaction) (r=0.491, p=0.028).

A positive, weak and insignificant correlation was observed in the control group between epithelial and fibroblast staining on immunohistochemical staining (TGF-β1 positive reaction) (r=0.167, p=0.482).

No correlation was observed in the control group between epithelial and inflammatory cell staining on immunohistochemical staining (TGF-β1 positive reaction) (r=0.00, p=l.00).

Positive, weak and insignificant correlations were observed in the control group between fibroblast and gland staining (r=0.218, p=0.355), inflammatory cell and gland (r=0.126, p=0.597), and fibroblast and inflammatory cell staining (r=0.192, p=0.416) on immunohistochemical staining (TGF-β1 positive reaction).

DISCUSSION

Nasal polyposis is a chronic inflammatory disease of the upper airways histologically characterized by infiltration of inflammatory cells such as eosinophils and neutrophils. Pathophysiological changes such as proliferation in the epithelial layer, thickening in the basal membrane, glandular hyperplasia, edema, stromal fibrosis, angiogenesis and cellular infiltration in the stroma layer are also seen.[11]

The basic characteristic of inflammation in NP is the presence of tissue eosinophilia and the release of associated inflammatory mediators.[12] Eosinophils are granulocytic leukocytes involved in diseases developing in association with immune reactions to allergic inflammation, asthma and parasites. In inflammation, two important mediators, IL-16 and RANTES, are released and are responsible for their own and CD4+ lymphocyte migration and for increased immune response.[5] There is a known parallel between tissue eosinophilia and disease severity. Studies examining the diffusion of the disease using paranasal CT have reported that tissue eosinophilia increases with diffusion.[13] Tissue eosinophilia has generally been determined numerically, using the level of eosinophils or the percentage of activated eosinophils.[14] In our study, eosinophilia was assessed numerically. Tissue eosinophilia increased in the study group patients, but was not observed in the control group. This supports the idea of an increase in eosinophil activation in polyp tissue and also shows that no change occurs in healthy mucosa in the nasal cavity.

Soler et al.[15] examined the nasal mucosa of patients with chronic rhinosinusitis and studied 147 histopathological specimens. They determined NP in 44.9% of specimens,

asthma in 38.1%, ASA intolerance in 10.2% and comorbidity with allergic rhinitis in 27.2%. On histopathological investigation they determined mucosal eosinophilia at a level of 78.9%. Mast cell, plasma cell and macrophage levels were 1.2%, 99.3% and 4.1%, respectively. Mean goblet cell level as a percentage of all cells on the basis of epithelial markers was 19.9%. The level of basal membrane thickening exceeding >5 pm was 49.7%, and that of squamous metaplasia in all patients was 53.1%. In terms of major findings in all specimens, they observed subepithelial edema (41.5%) and mucosal fibrosis (40.8%). Lymphocytes were present in 100% of specimens, eosinophils in 49.7% and neutrophils in 0.7%, and mucosal eosinophilia was associated with severity of disease.[17] No edema or eosinophilia was observed in the control group in our study, while mild edema was observed in two (10%) of the 20 patients in the study group, moderate edema in three (15%) and severe edema in 14 (70%). Dispersed eosinophils were observed in seven specimens (35%), in the form of small clusters in eight (40%) and in the form of large clusters in three specimens (15%). We observed no squamous metaplasia in any study group specimens. In addition, we observed a strong, positive and significant correlation between eosinophils and edema in preparates stained with H-E in the study group. We also determined a negative, weak but significant correlation between eosinophils and mixed inflammation in preparates stained with H-E in the study group.

Another significant element in the pathogenesis is how eosinophils lead to tissue damage, inflammation and polyp formation. Eosinophil migration is triggered by various inflammatory cytokines. These cytokines particularly cause the release from eosinophils of toxic mediators such as eosinophilic cationic protein (ECP) and neutrophil elastase, and this leads to tissue injury. This complex relationship between inflammatory mediators has not yet been fully clarified.[17,18] Cytokines are mediators that transmit intracellular messages as signal-bearing molecules in the development of immune response. They are responsible for the activation, degranulation and differentiation of mediators appearing in the immune system. [19] This tissue eosinophilia in NP has been attributed to immunological mechanisms involving cytokines. Cytokines associated with NP and tissue eosinophilia include IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-11 and IL-16, GM-CSF, RANTES, eotaxin, TGF-β and TGF-α.[16] These cytokines make significant contributions to the development, activation and survival of eosinophils.[4]

Transforming growth factor-β is an important growth factor involved in tissue remodeling. It is involved in almost all stages of the process of chemotaxis in the activation of structural or inflammatory cells.[20] In addition to being a member of the TGF cytokine family, it also plays a key role in the development and induction of inflammation and neoplasm.[4] The major role played by TGF-β in chronic inflammation reaction, extracellular matrix deposition and epithelial growth and differentiation has recently been emphasized in various cell culture and animal models.[21] Transforming growth factor-β is known to have an immunoregulator role. It performs this by activating the release of some cytokines and inhibiting that of others. Transforming growth factor-β is thought to play a significant role in aggravating inflammation in NP tissue and stimulating fibrosis.[4]

Transforming growth factor-β is a potent fibrogenic cytokine that stimulates extracellular matrix formation. It is also a chemoattractant for fibroblasts. However, it generally inhibits the growth and activation of inflammatory cells.[22] Transforming growth factor-β leads to fibrosis in respiratory tissue and has a notable pro-inflammatory regulatory effect in fibroblastic activity. Fibroblasts are present in NP tissue stroma and are actively associated with production of the extracellular matrix. One study showed greater fibroblast proliferation in tissue obtained from NP in the presence of TGF-β1 compared to non-stimulated tissue. Sime et al.[25] showed that prolonged and severe pleural and interstitial fibrosis developed as a result of continual release of TGF- β1 from the rat lung. An increase in fibronectin, procollagen, TGF-β2 and VEGF accompanies TGF-β1. One common feature of NP and idiopathic pulmonary fibrosis is varying degrees of fibrosis in stroma and thickening of the basal membranes. Transforming growth factor-β may be directly involved in this

structural change. Transforming growth factor-β's ability to stimulate collagen deposition may occur in the presence of other cytokines.[23]

Eosinophils in NP tissue can release TGF-βs isoforms and their receptors.[4] Although eosinophils are more frequently observed than macrophages in tissue, TGF-β is more common than macrophages and eosinophils.[21] Nonetheless, eosinophils are thought to represent an important source of TGF-β in NP tissue. Other data suggest that a low concentration of TGF-β induces eosinophil concentration, and that a high concentration lowers eosinophil survival, and that this is mediated by IL-5, 1L-3 and GM-CSF. A balance has been shown between IL-5 production and TGF-β from eosinophils. Transforming growth factor-β1 exhibits its effect on the hematopoietic regulation mechanism by preventing IL-5 activity and programming cell death. One study comparing normal nasal mucosa and NP tissue reported a higher concentration of IL-5 and a lower concentration of TGF-β1 in NP tissue compared to normal nasal mucosa.[22] Another study reported that TGF-β1 exhibits its anti-inflammatory effect through IL-5 antagonism, that it may be instrumental in eosinophil hemostasis and supports eosinophil apoptosis.[24]

There are at least as many TGF-β receptors and locally manufactured TGF-β isoforms (TGF-β1-β1-β3) at various levels in NP tissues as there are receptors. Although TGF-β isoforms and receptors are stained in normal tissue sections, this is less intense than in NP tissue.[4] In our study, we determined no statistical relation between TGF-β1 staining in epithelial and gland tissues at immunohistochemical staining and eosinophil staining with H-E. However, we observed a positive, weak and insignificant correlation between TGF-β1 staining in fibroblast and inflammatory cells at immunohistochemical staining and eosinophil staining with H-E. The level of TGF-β1 staining in inflammatory cells with immunohistochemical staining was 70% in the cases with NP and 25% in the control group. This may indicate that TGF-β1 is involved in inflammation.

Go et al.[11] investigated syndecan-1 and TGF-β release from nasal mucosa and NP tissue. Blood vessels, and nasal gland and inflammatory cells were positive for TGF-β in that study, while fibroblasts were negative. The epithelium, ciliary cells, seromucous gland and blood vessel endothelial cells stain positive, while the basal membrane is negative. Inflammatory cells staining positive for TGF-β are in subepithelial regions. However, staining for TGF-β is positive in the presence of pseudocyst formation. In our study, comparison of the study and control groups revealed no significant differences in TGF-β1 staining in glands, the epithelium and fibroblasts on immunohistochemical staining, while there was a statistically significant difference in inflammatory cell staining with TGF-β1.

Transforming growth factor-β, a cytokine with immunosuppressive effects, leads to cell proliferation and is involved in myofibroblast differentiation and altering extracellular matrix deposition.[20] It also plays a role in such diverse phenomena as epithelial cell regeneration, inflammation and tissue repair.[24] As with the extracellular matrix, eosinophil granule protein has been shown to increase permeability in animal models. A higher extracellular matrix concentration together with a higher albumin concentration have been shown in NP tissue compared to control mucosa, and this also supports the hypothesis that eosinophils may induce plasma leakage.[22]

One study that compared normal tissue specimens with untreated polyp specimens observed no greater eotaxin, extracellular matrix or albumin in polyp tissue, but reported significantly lower TGF-β1. No difference was observed between fibronectin, hyaluronic acid and LTC4/D4/E4 levels. Interleukin-5 and eotaxin levels in untreated NP tissue specimens were correlated with extracellular matrix, and albumin levels were correlated with fibronectin. Increases are observed in IL-5, the extracellular matrix, albumin and fibronectin concentrations in polyp tissue with oral corticosteroid therapy. In addition to reducing eosinophil numbers and activation, oral corticosteroid therapy also causes IL-5 suppression, and this also leads to a decrease in eosinophil activation, and has been proved to significantly suppress extracellular matrix levels. In addition to this eosinophilic

activation, oral corticosteroid therapy has been shown to reduce albumin deposition, resulting in diminution in NP.[22]

The highest total TGF-β (TGF-β1+TGF-β2) level has been identified in patients undergoing more than one polypectomy. Histologically, more fibrotic polyp types have been shown in patients with the highest TGF-β2 levels.[4]

Ohno et al.[23] analyzed RNA extracts correlated with TGF-β. Although they identified RNA extracts correlated with TGF-β in tissue from patients with NP and allergic rhinitis, they determined none in normal mucosa. They attributed this to TGF-β RNA release not being at detectable levels in normal tissue. They also showed TGF-β1 gene release in approximately half of eosinophil infiltrate in tissue. *In vitro* TGF-β1 production has been shown from macrophages and endothelial cells.

Conclusion

Specimens from patients with NP were compared with specimens from the control group. Severity of edema in tissues stained with H-E was 70% in the NP group, while no edema was detected in any tissues from the control group. Eosinophilia was observed at 90% in NP tissue, while again none was determined in the control group. Mixed inflammatory cell clusters were 70% moderate or severe in the NP group and 20% moderate and severe in the control group. No difference was observed between the groups in terms of staining in the epithelium. No squamous dysplasia or basal membrane thickening was observed in preparates from either group.

Changes such as proliferation in the epithelial layer, glandular hyperplasia, edema, stromal fibrosis, angiogenesis and cellular infiltration in the stromal later were observed in nasal polyp histopathology.

Transforming growth factor-β1 staining levels in glands with immunohistochemical staining were 65% in the NP group and 70% in the control group. Transforming growth factor-β1 staining in fibroblasts was 30% in the NP group and 10% in the control group. Transforming growth factor-β1 staining in inflammatory cells was 70% in the NP group and 25% in the control group. Eosinophilia, fibroblast and inflammation were significantly high in the NP group.

We conclude that transforming growth factor-β1 may play a significant role in the pathophysiology of NP together with other mediators, albeit not alone.

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