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Original Article

The Role of Transforming Growth Factor Beta 1 (TGFβ1) in Nasal and Paranasal Sinuses Polyposis

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ABSTRACT

Background and Objectives: **Nasal polyposis is a diseases resulting from complex pathogenetic mechanisms. Some studies showed that TGFβ1 had significant role in this pathogenesis. In this study, we investigated the roe of cytokines and mediators in polyp development.**

Material and Methods: **In this case- control study, healthy nasal mucosal samples were obtained from 24 people undergoing septoplasty or rhinoplasty and polyp samples were obtained from 15 patients with nasal and paranasal sinuses polyposis undergoing endoscopic sinus surgery. TGFβ1 concentration was measured with ELISA in homogenized polyp and control samples. The difference of the mean concentrations was analyzed with Mann-Whitney test.**

Results: **We detected TGFβ1 in 11 patients' samples and in 22 control samples. There was not significant differentiation between the mean of TGFβ1 levels in two groups.**

Conclusion: **Measuring level of TGFβ1 with ELISA technique in homogenized polyp and control samples have not significant differentiation.**

Key words: Nasal, Paranasal, TGFβ1, ELISA

Introduction

Nasal polyposis is thought to develop as a manifestation of a chronic inflammatory process involving the upper airway (1).

Nasal polyps commonly arise from the paranasal sinuses (2). According to the European position paper on rhinosinusits and nasal polyposis (EP3OS) document, related recently by the immunology and European Rhinology society, nasal polyposis is considered a subgroup of chronic rhinosinusits (3). The polypoid disease was generally recurrent despite the medical follow up treatment (4).

The cause of nasal polyposis is still unknown,

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regardless of unknown etiology; nasal polyposis is characterized by extensive inflammatory process associated with local production of several mediators and cytokines by both structural and infiltrating cells.

Activated epithelial cells may be the major source of mediators inducing influx of inflammatory cells mostly eosinephils and proliferation and activation of fibroblasts leading to nasal polyp formation (3).

Accumulation of eosinophils, neutrophils, plasma and mast cells, macrophages and lymphocytes is a frequent finding and there is much evidence to the activity and pathogenic role of these cells (5).

Among the cytokines that have role in nasal polyposis, TGFβ may play a significant role in this pathogenesis possibly through fibroblast activation (6, 7). TGFβ may be responsible for recurrent polyposis (8). TGFβ is a family of dimeric polypeptide growth factors which regulate cell activation, proliferation and differential but also embryonic development, wound healing and angiogenesis, play and important role in normal airway morphogenesis and function , and are involved in the pathogenesis of a variety of airway disease (3).

There are three isoforms of TGFβ, $TGF\beta_1$, $TGF\beta_2$, and TGFβ3. The first is mainly synthesized by endothelial, hematopoietic and connective tissue cells, the second by epithelial and neuronal cells and third primarily by mesenchymal cells (3).

All three TGFβ isoforms are expressed at high level during normal airway development, being particularly involved in branching, morphogenesis, and epithelial cell differential and surfactant synthesis: small amount of TGFβ are still present in adult airways, while increases or decreases in the production of three TGFβ isoforms are linked to a variety of disease states (6). Among its many activities, TGFβ is able to induce fibroblast proliferation and differential into myofibroblast (6, 7).

+TGFβ1 and TGFβ2 are strongly expressed in inflammatory nasal mucosa has lead to the hypothesis that they may play a significant role in inducing the structural modifications that characterize this disease (6).

Compared with TGFβ2, TGFβ1 appear to be active for a longer period of time and with a wide concentration range on fibroblast functions in cell proliferation (6).

The studies on TGFβ isoform expression in nasal polyposis so far have yielded different results so in this study; we investigated the roe of cytokines and mediators in polyp development.

Material and Methods

We conducted a case control study on 39 nasal mucosa samples (15 patients and 24 controls) for TGFβ from September 2006 to October 2007.

Patients were randomly selected from patients with nasal polyposis that proved by CT scan (CT scan need for endoscopic surgery) and controls were selected from healthy persons that were undergoing septoplasty or rhinoplasty. Patients and controls were excluded if they had any of the following: oral or nasal corticosteroid therapy in the preceding 30 days, diseases such as vasculitis, rheumatologic and infections that may affect on nasal mucosa. An informed consent was taken form patients before the study.

Nasal polyp samples (n=15) were obtained during endoscopic sinus surgery and control samples were obtained during septoplasty or rhinoplasty. Samples were taken deeply that including mucosa and lamina propria.

TGFβ1 concentrations were measured with ELISA technique in homogenized polyp tissue (n=15) and in control mucosa samples (n=24).

ELISA measurements:

Biopsy materials were weighed, chopped in to pieces of 1mm homogenized in 0.9% sodium chloride solution, 1ml solution was added to 100mg tissue. Suspensions were centrifuged at 40c at 3000 prm for 10 min, and supernatants were stored in refrigerator at -20 ºC until used. $TGF\beta_1$ cytokine concentrations were measured by using sandwich ELISA kit (Human $TGF\beta_1$ kit, Bander med system, Austria, Europe) according to the manufacturer's instructions.

Statistical Analysis:

The difference of the mean concentrations was analyzed with Mann-Whitney test.

Results

A total of 39 patients were enrolled in the study from September 2006 to October 2007 for measuring the $TGF\beta_1$ level in nasal specimens. The patients ages ranged from 19 to 41 years in control group (mean =27) and 19 to 84 in polyp group (mean=38).

There were 24 patients in control group of whom 19 were male, five were female, and 15 in polyp group of whom 10 were male and 5 were female.

$TGF\beta_{1\ level}$ were measured in two group and were measurable with ELISA technique in 11 polyp tissue and 22 control samples (Table 1).

Table1: *patient sample* TGFβ*1 results*

Concentration range	frequency	percent
0	4	26.7
0-1	2	13.3
1-2	4	26.7
2-3	1	6.7
3-4	1	6.7
4-5	1	6.7
5-6	1	6.7
6-7	1	6.7
sum	15	100

Table 2: *Control sample* TGFβ*1 results*

Concentration range	Percent	Frequency
0	8.3	2
o-1	4.2	1
12-	50	12
23-	8. 3	2
34-	12.5	3
45-	0	0
56-	8.3	2
67-	4.2	1
78-	0	0
89-	4.2	1
sum	100	24

The mean $TGF\beta_1$ level in polyp samples was 1.647±0.619 and in control samples was 2.325±0.466 with no statistically significant differences (*P*=0.236).

We did not find significant correlation between mean $TGF\beta_1$ concentration in polyp tissues and control sample by Mann Whitney test (*P*=0.236) [*P*>0.05 was considered significant] (Fig. 1, and Table 3). In this study, we found $TGF\beta_1$ levels in both patient and control groups but we did not find significant differentiation between these levels.

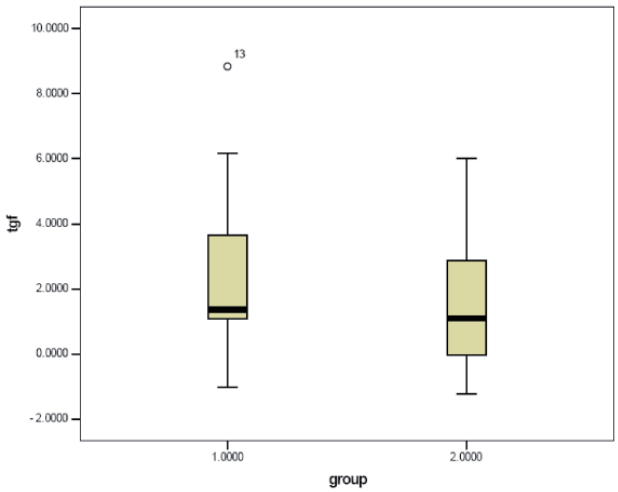

Fig. 1: *The mean concentration of TGFβ1 in two groups*

Table 3 :TGFβ*1 concentration in two groups*

Group	TGFβ1 concentration
Control group	2.325±0.466
Patient group	1.647±0.619
P= 0 .236	

Discussion

Some studies found that $TGF\beta_1$ levels up regulate in nasal polyposis and others found down regulation of $TGF\beta_1$ level in nasal polyps (9, 10).

Beata Rostawaska- Naldoska *et al.* demonstrated that $TGF\beta_1$ mRNA was present at higher levels in all control samples than in polyps (11).

Andre cast *et al.* by using immunohistochemistry

detected no significant difference between $TGF\beta_1$ levels in nasal mucosa from patient polyp samples and control nasal mucosa samples but $TGF\beta_1$ levels was higher in nasal polyp lamina properia and epithelium than controls (12). In our study there was not significant different between the mean level of $TGF\beta_1$ in nasal polyps and normal mucosa sample.

Andre hirshberg *et al.* showed that $TGF\beta_1$ concentration by ELISA measurement significantly higher in control mucosa than in nasal polyps and immunohistochemistrical analysis revealed $TGF\beta_1$ positivity in the lamina propria of polyp samples but non in control specimens, and they described because there is no immunoreactive $TGF\beta_1$ in control specimens in frozen sections but there is a great amount of that in the homogenized tissue, it seems to be evident that normal nasal mucosa has significant latent $TGF\beta_1$ concentration (5). T. Van zele *et al.* found that $TGF\beta_1$ did not regulate in nasal polyps (13).

Tao *et al*. and lee ch *et al* showed that $TGF\beta_1$ expression in nasal polyps was positive (14, 15). This finding confirms our result that showed there was the level of $TGF\beta_1$ in nasal polyp tissue.

Little SC *et al.* showed that $TGF\beta_1$ expression in polyp tissue could have dual effects. One role is act on anti-inflammatory agent shown by the ability to inhibit production. At the same time, $TGF\beta_1$ expression leads to increases in factors involved in fibrosis and angiogenesis, promoting remodeling and cell growth (10).

As we see in those studies with RT-PCR and ELISA technique $TGF\beta_1$ level measured overlay in homogenized solution. Homogenized solution contains all of mucosa layers (epithelium, mucosa membrane and lamina properia). The results of $TGF\beta_1$ levels in nasal polyposis mucosa measured with ELISA and RT-PCR were in controversy. Controversy in those studies, which used IHC technique for measuring TGFβ1 levels in tissue layers of nasal mucosa, was low. It seems that the site of concentration of $TGF\beta_1$ has important role in pathogenesis of nasal polyposis. IHC is best method for measuring $TGF\beta_1$ levels in tissue layers of nasal mucosa.

Conclusion

We recommend further studies on the level of $TGF\beta_1$ in different layers of tissue by IHC because RT-PCR and ELISA technique measured $TGF\beta_1$ levels in homogenized solution not in mucosa layers.

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