SIGNIFICANCE OF INTERLEUKIN-17A AND INTERLEUKIN-23 IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

RAHIME KOCA¹, METE EYIGOR², USTUN OSMA¹, HULYA EYIGOR², MUSTAFA DENIZ YILMAZ¹, OMER TARIK SELCUK¹, LEVENT RENDA¹, MERAL GULTEKIN²
¹Antalya Education and Research Hospital, Department of ENT Head and Neck Surgery, Antalya, Turkey - ²Akdeniz University Medical Faculty, Department of Medical Microbiology, Antalya, Turkey

ABSTRACT

Introduction: Several previous studies have investigated the significance of interleukin 17 (IL-17) in the pathogenesis of chronic rhinosinusitis with nasal polyposis; however, the importance of IL-23 has not been studied. We investigated the significance of IL-17 and IL-23 in the pathogenesis of by chronic rhinosinusitis with nasal polyposis measuring the levels of these proinflammatory cytokines in serum and nasal lavage samples.

Materials and methods: Twenty-five patients aged >18 years with chronic rhinosinusitis with nasal polyposis and a control group with 25 healthy subjects were included the study. The levels of IL-17 and IL-23 were measured in the serum and nasal lavage fluids using enzyme-linked immunosorbent assays.

Results: The average serum level of IL-17 was 10.2 pg/ml in the study group, and it was 5.0 pg/ml in the control group. The average nasal lavage level of IL-17 was 16.8 pg/ml in the study group compared with 1.0 pg/ml in the control group. There were no statistically significant differences between the groups in the serum levels of IL-17 or IL-23; however, a significant difference between the two groups was found in the lavage fluid levels of IL-17 (p <0.001).

Conclusion: We found a significant difference in the nasal lavage fluid levels of IL-17, which demonstrates the importance of local inflammation. This supports the hypothesis that IL-17 is significant in the pathogenesis of nasal polyposis.

Keywords: Chronic rhinosinusitis, nasal polyposis, IL-17A, IL-23, serum, nasal lavage, ELISA.

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Introduction

Chronic rhinosinusitis (CRS) is a multifactorial disease; however, the presence of nasal polyposis may be a significant underlying etiological factor affecting the pathophysiology⁵. In the pathophysiology of nasal polyposis, up-regulation of adhesion molecules is induced by interleukin-1 (IL-1) in the vascular endothelium and by IL-8 in epithelial cells and neutrophils. This occurs in addition to effusion, which happens as a result of increased secretion of neutrophil chemoattractants and the accumulation of neutrophils⁶.

Histomorphological analysis of CRC with nasal polyposis (CRSwNP) has revealed eosinophils and mixed mononuclear cells with a suppression of neutrophil activity⁶. IL-17A is a member of the IL-17 cytokine family, which consists of six cytokines, termed IL-17A-F⁷. IL-17A and IL-17F have the highest degree of homology, and IL-17A is also produced in natural killer (NK) cells, neutrophils, eosinophils, and δγT cells⁸. IL-17 is important in host responses to infections and in the regulation the neutrophil response and is significant in the pathogenesis of CRSwNP⁹,10.
IL-23 is a member of the IL-12 family, with a unique p19 subunit and a p40 subunit that it has in common with IL-12. It has structural similarities with IL-12 and plays a significant role in chronic inflammation and the pathogenesis of some autoimmune diseases. IL-23 has a synergistic relationship with transforming growth factor beta (TGF-β), amplifying the expression of rorγt and, consequently, the production of IL-17. The IL-23/IL-17 pathway is related to the local tissue inflammatory response. Therefore, it is thought that IL-23 is significant in the early stages of inflammatory responses to pathogens or injuries, as it directly induces IL-17 production and neutrophil accumulation. However, we were not able to find any studies investigating IL-23 in patients with CRSwNP. Here, we investigate the significance of IL-17 and IL-23 in the pathogenesis of CRSwNP by measuring the levels of these proinflammatory cytokines in serum and nasal lavage samples.

Materials and methods

Patients

The study included patients admitted to the ENT Department of Antalya Education and Research Hospital between July 2011 and November 2012 with complaints of nasal congestion, nasal and/or postnasal drip, and facial pain during the previous 12 weeks. All patients were aged > 18 years, and the presence of nasal polyps was evaluated via endoscopic examination. The study protocol was approved by the Ethics Committee of the Antalya Education and Research Hospital. Informed consent was obtained from each of the patients. Diagnosis of CRS was based on medical history, clinical examination, endoscopic examination, and computed tomography (CT) scanning and was consistent with the guidelines set out by the European Position Paper on Rhinosinusitis and Nasal Polyps, 2007. The study group was selected from the patients who did not obtain medical therapy and/or who underwent surgery at least 8 weeks prior to the study. Patients who previously underwent surgery for nasal polyposis; those with craniofacial malformation, immune deficits, or malignancy; and those who used drugs that affect the immune system were excluded from the study. The control group consisted of 25 healthy volunteers without chronic rhinosinusitis, nasal polyps, allergic rhinitis, or asthma and with no history of drug use. Nasal lavage fluid and serum were collected from both groups.

A visual analogue scale (VAS), on which each symptom was rated on a scale from 0 to 7 (see Table 1), was used to measure severity of symptoms and quality of life. The findings of CT scans were quantified using the Lund-Mackay scale. To create a standard study group, patients with complete opacification of all sinuses on both sides were included in the study (maximum score: 24).

Nasal lavage fluid

Nasal lavage fluid collection was performed as described by Jacobi et al. (13). The patients were in the sitting position with their head tilted back in the horizontal plane by 30° 10 s after applying 5 ml of 30°C saline solution to each nostril. The fluid was drained and collected in a polypropylene container.

Measurement of IL-17 and IL-23

The serum and nasal lavage samples were stored at 20°C. The levels of IL-17 and IL-23 were measured based on human IL-17AF and IL-23 using a Platinum enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, USA) at the Akdeniz University Health Sciences Research and Application Center (HSRAC).

Statistical analysis

Data were analysed using the software package SPSS for Windows (version 11.5, SPSS Inc., Chicago, IL, USA). Continuous variables that were normally distributed were analysed with the Shapiro-Wilk test, and Student’s t-test was used to analyse significant differences between groups in terms of mean age. The Mann-Whitney U test was used for VAS scores that reflected differences
between positive and negative groups relative to the markers. Nominal variables were evaluated using Pearson’s chi-square test or Fisher’s exact test. Spearman’s correlation test was used to investigate the significance of relationships between mean VAS scores and levels of IL-17 and IL-23. Statistical significance was set at p < 0.05.

Results

A total of 50 patients were included: 25 with CRSwNP and 25 controls. The study group consisted of 18 male (72%) and 7 (28%) female patients. The control group consisted of 12 (48%) male and 13 (52%) female subjects. The mean age was 45.9 years (range: 20-69) in the study group and 32.1 years (range: 22-57) in the control group.

No statistically significant differences were found in the serum levels of IL-17 between the study group and the control group (p = 0.247). The mean nasal lavage level of IL-17 in the study group was 16.8 pg/ml, whereas it was 1.0 pg/ml in the control group; this difference was statistically significant (p < 0.0001).

There were no statistically significant differences in the serum levels of IL-23 between the study and control groups (p = 0.077), and IL-23 was found only in the serum of three patients. IL-23 was not found in the nasal lavage fluids of any patients. Table 2 lists the distribution of cases with positive serum and lavage levels of IL-17 and IL-23. Table 3 lists the serum and lavage fluid levels of IL-17 and IL-23.

Although there was no statistically significant correlation between the mean VAS score and the serum and lavage levels of IL-17 in the study group, a statistically significant inverse correlation was found between the VAS score and the serum level of IL-23 (r = -0.398, p = 0.049) (Table 4).

In the study group, there were no statistically significant differences between the mean VAS score and the serum or nasal lavage fluid levels of IL-17 (p > 0.05). However, the mean VAS score was significantly lower in the serum-IL-23-positive group than in the serum-IL-23-negative group (p = 0.046) (Table 5).

<table>
<thead>
<tr>
<th>Control Group</th>
<th>Study Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-17</td>
<td>2 (8%)</td>
<td>6 (24%)</td>
</tr>
<tr>
<td>Lavage IL-17</td>
<td>4 (16%)</td>
<td>19 (76%)</td>
</tr>
<tr>
<td>Serum IL-23</td>
<td>-</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Lavage IL-23</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Distribution of cases with positive serum and lavage levels of IL-17 and IL-23 in study and control groups.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-17</td>
<td>5.1</td>
<td>0</td>
<td>89.8</td>
<td>0.153</td>
</tr>
<tr>
<td>Control Group</td>
<td>10.2</td>
<td>0</td>
<td>81.5</td>
<td></td>
</tr>
<tr>
<td>Study Group</td>
<td>16.8</td>
<td>0</td>
<td>79.4</td>
<td></td>
</tr>
<tr>
<td>Lavage IL-17</td>
<td>1</td>
<td>0</td>
<td>13.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Control Group</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Study Group</td>
<td>4.8</td>
<td>0</td>
<td>90.7</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Serum and lavage levels of IL-17 and serum levels of IL-23 in the study and control groups.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-17</td>
<td>3.5</td>
<td>2.3</td>
<td>4.9</td>
<td>0.975</td>
</tr>
<tr>
<td>Negative</td>
<td>3.4</td>
<td>3.2</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3.9</td>
<td>3.3</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Lavage IL-17</td>
<td>3.3</td>
<td>2.3</td>
<td>4.4</td>
<td>0.08</td>
</tr>
<tr>
<td>Negative</td>
<td>3.5</td>
<td>2.3</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
<td>2.8</td>
<td>3.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Correlation coefficients between VAS and serum and lavage levels of IL-17 and IL-23 in the study group, including significance levels.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Mean</th>
<th>Min</th>
<th>Max</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-23</td>
<td>0.299</td>
<td>0.368</td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Lavage IL-17</td>
<td>0.398</td>
<td>0.049</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: VAS scores and serum and lavage levels of IL-17 and IL-23 in the study group.
Discussion

The prevalence of CRSwNP is 4% in the population as a whole\(^{(14)}\). The following cytokines have been found to be related to nasal polyps and tissue eosinophilia: IL-1, IL-3, IL-4, IL-5, IL-6, IL-8, IL-11, IL-16, granulocyte-macrophage colony-stimulating factor (GM-CSF), RANTES, eotaxin, TGF-\(\beta\), and tumour necrosis factor alpha (TNF-\(\alpha\))\(^{(15)}\).

Molet et al. reported increased expression of IL-17 in nasal polyps compared with normal nasal tissues using in situ hybridisation\(^{(11)}\). Wang et al. reported the presence of IL-17 and IL-17 receptors in nasal polyps using Western blot analysis and immunohistochemical staining\(^{(17)}\). Du et al. found elevated IL-17 expression in the nasal tissues and blood of patients with nasal polyposis; however, significant IL-17 expression was observed in patients with allergic rhinitis only in the nasal mucosa\(^{(18)}\). Hu XD et al. found that IL-17A was associated with the pathogenesis of CRS and polyp remodelling\(^{(19)}\). Shen et al. reported an imbalance in the ratio of T17 levels to Treg levels, which was associated with inflammation and the formation of an atopic reaction\(^{(20)}\). They found increased levels of Th17 in mononuclear cells in the peripheral blood in all atopic and non-atopic patients with nasal polyposis, as well as reduced levels of Treg. These results show that patients with nasal polyposis and atopy have an abnormal Th17/Treg ratio and suggest that this is important in the pathogenesis of nasal polyposis\(^{(21)}\). Ono et al. found a significant increase in the mean number of IL-17A+ cells in eosinophilic chronic rhinosinusitis compared with non-eosinophilic chronic rhinosinusitis. IL-17A is expressed by a group of inflammatory cells, including both eosinophils and lymphocytes. They found a significant correlation between eosinophils and IL-17A, which is also produced by macrophages as well as eosinophils, in eosinophilic chronic rhinosinusitis\(^{(21)}\). Van Bruaene et al. reported no significant differences in the levels of IL-17 mRNA between patients with chronic rhinosinusitis (with or without polyps) and a control group\(^{(20)}\).

Makihara et al. found significant positive correlations between the radiological severity of sinusitis and both the total number of IL-17A+ cells and the degree of eosinophilia in sinonasal tissue samples from patients with CRSwNP\(^{(22)}\). However, Saitoh et al. reported that CT and symptom scores were not correlated with the number of IL-17A+ cells in CRS\(^{(23)}\). We did not find any significant differences between the two groups (\(p = 0.153\)) in this regard; however, levels of IL-17AF in the nasal lavage fluid reflected significant differences between the control group and the study group (\(p < 0.001\)). These results are consistent with the literature and support the hypothesis that IL-17A is synthesised locally in the nasal polyp tissue. Although the patient group was homogeneous in terms of the staging of polyps, this significant difference in the lavage fluid levels of IL-17A indicates the importance of this cytokine in local inflammation.

IL-23 is a member of the IL-12 family and features a p19 subunit that is unique to IL-23, as well as a p40 subunit that it has in common with IL-12\(^{(24)}\). Despite its structural similarity to IL-12, IL-23 is significant in chronic inflammation, as well as in the pathogenesis of some autoimmune diseases\(^{(24)}\). McGeachy et al. reported that IL-23 is necessary for the differentiation of Th17 cells and for proper effector function. These results suggest that the IL-23–Th17 axis is critical in the pathogenesis of inflammatory diseases\(^{(25)}\).

Wakash et al. found that IL-23 is significant in the antigen-dependent activation of both Th2 and Th17 cells and important in the active phase of allergic respiratory tract inflammation. They reported that Th17 mediates neutrophil migration and that Th2 mediates eosinophil migration in the respiratory tract\(^{(26-28)}\). Wang et al. investigated the immunomodulator effect of IL-23 and IL-17 on allergic rhinitis using a mouse model and suggested that IL-17A and (especially) IL-23 are significant in the pathology of allergic rhinitis\(^{(29)}\).

Despite numerous studies on the significance of IL-23 in allergic respiratory tract and inflammatory diseases, there have been no investigations of its importance in chronic sinusitis with nasal polyposis. Here, we investigated levels of IL-23 and IL-17 in the serum and nasal lavage fluids of CRSwNP patients and a control group. IL-23 was not found in the lavage fluids of any patients. Although serum IL-23 levels were higher in the patient group than the control group, the difference was not statistically significant (\(p = 0.077\)). IL-23 is important in the production of IL-17 and its effector functions, and it is elevated in other inflammatory diseases. We found decreased levels in patients with nasal polyposis, in which IL-17 is important.

We suggest that more sensitive and larger-scale studies on the importance of IL-23 are warranted to improve our understanding of the inacti-
viation of IL-17 during earlier stages of the progression of CRSwNP; the results of such studies may provide useful information about the treatment options for nasal polyposis. In particular, IL-23 levels in nasal polyp tissue may be significant for the pathogenesis of CRSwNP.

We found no significant differences between VAS scores and serum levels of IL-17; however, a statistically significant inverse correlation was found between VAS scores and serum levels of IL-23 (r = -0.398; p = 0.049). In the study group, no statistically significant differences were found between mean VAS levels and serum levels of IL-17 or nasal lavage levels of IL-17 (p > 0.05); however, the mean VAS level was significantly lower in the serum-IL-23-positive group than in the serum-IL-23-negative group (p = 0.046).

Hu XD et al. reported a significant difference between CRS IL-17 expression and disease symptom scores, as determined using endoscopic and radiological methods(19). Ciprandi et al. reported a positive correlation between symptom scores and serum levels of IL-17 in patients with allergic rhinitis(20). It is difficult to obtain information about the severity of the disease without the use of imaging methods, and the required medical treatment can be determined indirectly based on symptoms and evaluations of quality of life. Based on the literature, it appears that the relationships between therapeutic responses and the inflammatory mediators involved in the pathogenesis are unclear; however, it also appears that there is potential to develop more effective drugs with fewer side effects than corticosteroids (which are frequently used at present) based on investigations of the importance of inflammatory cytokines.

We believe that, by developing new medical treatments related to IL-17 and IL-23 (which is an IL-17-promoting factor) and depending on the levels of IL-17 detected in nasal lavage materials, localised anti-interleukin treatment options may be effective alternatives to nasal corticosteroid sprays. Suppression of IL-17 at a systemic level may provide new medical treatments for nasal polyposis.

References


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Corresponding author
Assoc. Prof. Dr. ÖMER TARIK SELÇUK, MD
FEB-ORLHNS
Antalya Education and Research Hospital, ENT Department
Antalya (Turkey)