Interlukin-25 and Nasal Polyps, a Novel Target to Hit

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ABSTRACT

Background: Chronic Rhinosinusitis (CRS) can be subdivided into 2 major categories; CRS with or without nasal polyps (CRSwNP or CRSsNP). IL-25 has been implicated in promoting Th2 responses in airway inflammation. However, the clinical relevance of local IL-25 in CRS patients remains relatively uncharacterized.

Objective: The aim of this work is to evaluate the relation between IL-25 and types of CRS.

Subjects and Methods: This is a prospective study on 120 patients (60 CRSwNP, 60 CRSsNP) and 20 controls. All underwent clinical and radiological assessment. Biopsies from their nasal mucosa were taken for pathological assessment to detect mucosal changes, tissue eosinophilia and percentage and intensity of immunohistochemical IL-25 staining in tissues, and for correlation with clinical and radiological criteria.

Results: CRSwNP patients are more associated with asthma, higher nasal endoscopy score, more severe inflammatory pathology, partial epithelial degeneration and ulceration and IL-25 tissue levels than CRSsNP and control group. 46% of patients with CRSwNP had tissue eosinophilia which is significantly higher than CRSsNP and control groups. We also found that the total nasal symptom score (TNSS), CT score and basement membrane thickness had a statistically significant positive correlation with immunohistochemical score of IL-25.

Conclusion: This study shows increase in IL25 expression and tissue eosinophilia in CRSwNP when compared to CRSsNP and control group. Thus, IL-25 may be a promising therapeutic target and an indicator for the management of CRSwNP patients.

Key Words: Chronic sinusitis, immunohistochemistry, interleukin-25, nasal polyp.

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INTRODUCTION

Chronic rhinosinusitis (CRS) is one of the most common diseases in the upper respiratory tract[1]. It is defined as an inflammatory condition of the paranasal sinuses lasting at least 12 weeks in duration. According to endoscopic examination of visible nasal polyps (NP) in the nasal meatus, CRS can be subdivided into CRS with NP (CRSwNP) and CRS without NP (CRSsNP)[2]. CRSwNP is usually characterized by type 2 T-helper (Th2) response and tissue eosinophilia, in contrast to CRSsNP that shows Th1 cell predominant response[3].

Although CRS is highly prevalent and is associated with quality of life impairment and cost burden, the key inflammatory mechanisms that drive inflammation and persistence in this disease remain unclear. The current treatment options for CRS are limited, especially medical treatment which frequently fails to sufficiently achieve relief of symptoms, leading to the frequent use of endoscopic sinus surgery to achieve improvement[4].

There is an urgent unmet clinical need to understand the immunopathology of CRSwNP. Several studies have indicated regional variation in CRSwNP endotypes. Western countries show a predominance of eosinophilic TH2-associated polyps, and Staphylococcus aureus superantigens have been implicated in driving the TH2 response[5]. Conversely, CRSwNP in patients from southern Asia is associated with neutrophilic infiltration and a local TH1/TH17 signature[6].

IL-25 (also known as IL-17E) is a member of the IL-17 cytokine family and plays a variety of roles in different inflammatory process, such as asthma and atopic dermatitis. Recently, the epithelial cell-derived cytokines IL-25 and IL-33, acting through their respective receptors IL-17RB and ST2, have been implicated in promoting TH2 responses in animal models of allergic inflammation responsible for CRSwNP[7].

In our study we aimed to further understand the pathogenesis of CRSwNP by investigating the relation between IL-25 and CRSwNP by comparing IL-25 tissue level and histopathological examination of tissue biopsies in CRSwNP, CRSsNP and control groups. This understanding is needed to further advance diagnostic and treatment strategies for CRSwNP patients.
PATIENTS AND METHODS

The study includes three patients’ groups:

1. Study group 1: includes 60 patients diagnosed as CRSwNP
2. Study group 2: includes 60 patients diagnosed as CRSsNP
3. Study group 3: includes 20 control patients without rhinosinusitis.

Inclusion criteria

Adult patients with chronic rhinosinusitis with and without nasal polyps diagnosed clinically according to Fokkens et al. (2012) and radiologically according to Lund-Mackay CT scoring and control group.

Exclusion criteria

Acute sinusitis, Invasive acute or chronic fungal sinusitis, Chronic granulomatous inflammation and use of oral or nasal steroids within the last 2 weeks.

All patients were subjected to:

1. full history taking (Allergy history, Asthma, Aspirin sensitivity, and tobacco use) and otorhinolaryngological examination according to Fokkens et al.
2. Disease specific quality of life was defined by total nasal symptom score (TNSS), where an overall score was obtained through averaging all components. It consists of 4 nasal symptoms (rhinorrhea, itching, obstruction and sneezing) each taking a score of 0-3 according to the symptom intensity. The possible overall score ranges from 0 (no symptoms) to 12 (maximum symptom intensity).
3. Endoscopic examination of the nasal cavity using Meltzer et al. for grading of nasal polyps where grades ranged from 0-4 beginning from polyps which are endoscopically nonvisible, small polyps confined to the middle meatus (MM), multiple occupying MM, extending beyond MM and finally obstructing the nasal cavity.
4. Computed tomography (CT scan) of Paranasal sinuses and grading according to Lund-Mackay CT Scoring where each of the maxillary, anterior and posterior ethmoid, sphenoid, frontal sinuses and osteomeatal complexes were given 0-2 scores with zero denoting no abnormality and 1 and 2 denoting partial and total opacification respectively.
5. Biopsies were obtained from the patients during Functional Endoscopic Sinus Surgery (FESS) from ethmoidal polyps in the 1st group patients and uncinate process in 2nd group patients and from turbinate or septal mucosa in the control group.
6. Specimens were fixed in 10% formalin, paraffin embedded, sectioned and processed for routine Hematoxylin-eosin (HE) stain for histological examination, and degree of tissue eosinophilia and basement membrane thickness.
7. Immunohistochemical staining were performed using peroxidase labeled streptavidin- biotin technique using polyclonal anti IL 25 antibody (biopsies Co, Ltd catalog number YPA2008).

Interpretation of IL 25 staining

IL 25 was present in the inflammatory cells, positive staining was scored quantitatively microphotographs of stained histological slides were taken using the C-5060 and a software Olympus soft imaging solution analysis starter. The percentage of stained cells was graded as 0 (no staining), 1(staining of 1-10% of cells), 2 (staining of 11-50% of cells), 3(staining of 51-80% of cells), 4(staining of 81-100% of cells). Staining intensity was seen microscopically and graded accordingly into no staining (0), mild(1), moderate(2) or strong (3). The overall score was expressed as the multiplication of the intensity and percentage scores with a product ranging from 0-12. Based on the final score, it was categorized as negative (0), mild (1-4), moderate (5-8) or marked (9-12). Immunohistochemical staining of epithelium was expressed based on intensity into no staining (0), mild (1), moderate (2) and strong (3)

Statistical Analysis

Descriptive statistics

• Mean, Standard deviation (± SD) and range were used for parametric numerical data, while Median and Inter-quartile range (IQR) were used for non-parametric numerical data.
• Frequency and percentage were used for non-numerical data.

Analytical statistics

• Kruskal-Wallis test was used to assess the statistical significance of the difference of a non-parametric variable between more than two study groups.
• Chi-Square test was used to examine the relationship between two or more qualitative variables.
• Correlation analysis (using Spearman’s method): To assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically “r” defines the strength and direction of the linear relationship between two variables.
RESULTS

Clinical data

Comparing the clinical data of the three groups, there was highly statistically significant difference in age in group-1 (CRSwNP); compared to the other 2 groups ($p < 0.01$) (Table 1, Figure 1). Smoking was statistically significantly higher in group 2 (CRSsNP) compared to other groups ($p = 0.018$). History of Asthma and TNSS score were statistically significantly higher in group 1 patients in comparison to the other two groups ($P = 0.01$ and $<0.0001$ respectively), (Table 1).

Table 1: Comparison between the 3 groups as regards basic clinical data using Kruskal-Wallis and Chi square tests.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group-1 (60)</th>
<th>Group-2 (60)</th>
<th>Group-3 (20)</th>
<th>Kruskal-Wallis test</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>42 (34-48)</td>
<td>37 (30-45)</td>
<td>30 (23-34)</td>
<td>= 0.000007</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Group-1 (60)</td>
<td>Group-2 (60)</td>
<td>Group-3 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Female</td>
<td>23 (39.3%)</td>
<td>16 (26.7%)</td>
<td>9 (45%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>37 (61.7%)</td>
<td>44 (73.3%)</td>
<td>11 (55%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>+ve</td>
<td>14 (23.3%)</td>
<td>27 (45%)</td>
<td>4 (20%)</td>
<td>= 0.018</td>
</tr>
<tr>
<td>History of asthma</td>
<td>+ve</td>
<td>24 (40%)</td>
<td>7 (11.6%)</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Total nasal symptom score</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>$&lt;0.0001$</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: (A) case of chronic rhinosinusitis with nasal polyp (A) the subepithelial tissue shows mild degree of immunohischemical staining of (IL 25 antibody 40x) (red star). The surface epithelium show partial ulceration (yellow arrow) and partial degeneration (red arrow). (B, C, D) CT scan coronal cuts show total opacification of all sinuses (Lund and Mackay score 24).
Comparing CT scan data

The median Lund and McKay score was 20 in group-1 which was significantly higher than that in groups 2 and 3 (10 and 2 respectively) \( (p < 0.01) \) (Table 2).

Table 2: Comparison between the 3 groups as regards CT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group-1 (60)</th>
<th>Group-2 (60)</th>
<th>Group-3 (20)</th>
<th>Kruskal-Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>Median (IQR)</td>
<td>( P ) value</td>
</tr>
<tr>
<td>Lund and McKay score</td>
<td>20 (18 – 20)</td>
<td>10 (8 – 12)</td>
<td>2 (0-4)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Histopathological results

Tissue biopsies were examined histologically after staining with Hematoxylin-eosin (HE) stain, epithelial degeneration (a change consisting of disintegration of tissue), ulceration (discontinuity or break in surface epithelium), tissue eosinophilia and severity of inflammation were subjectively recorded by the pathologist.

In group 1 (CRSwNP) patients had significantly higher partial epithelial degeneration and ulceration \( (p= 0.0001) \) (Figures 1,8) and a higher number of patients had tissue eosinophilia (46% of patients) \( (p= 0.001) \) when compared to the other 2 groups. Regarding severity of inflammation in group 1 70% of patients had moderate and 20% had severe degree of inflammation (Figure 2) while in group 2 the majority of the patients had mild inflammation (63% of patients in the group) denoting significantly higher moderate and severe inflammation in CRSwNP group when compared to CRSsNP and control \( (p < 0.01) \) (Table 3).

**Fig. 2:** A case of chronic rhinosinusitis with nasal polyps (a) the surface epithelium is markedly hyperplastic (black arrow). The subepithelial tissue shows marked inflammatory cellular infiltrate (red star) (IL 25 antibody 100x). (B,C,D) CT coronal cuts show complete opacification of all sinuses frontal, maxillary, ethmoid and sphenoid (Lund and Mackay score 23).
Table 3: Comparison between the 3 groups as regards Histopathology data using Chi square test. (PU:Partial Ulceration, PD:Partial Degeneration, SM:Squamous Metaplasia)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group-1 (60)</th>
<th>Group-2 (60)</th>
<th>Group-3 (20)</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD</td>
<td>0 (0%)</td>
<td>2 (3.3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>PD/PU</td>
<td>31 (51.7%)</td>
<td>24 (40%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>PU</td>
<td>20 (33.3%)</td>
<td>31 (51.7%)</td>
<td>18 (90%)</td>
<td>≈ 0.0001</td>
</tr>
<tr>
<td>PU/PD/SM</td>
<td>5 (8.3%)</td>
<td>1 (1.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>PU/SM</td>
<td>4 (6.7%)</td>
<td>2 (3.3%)</td>
<td>2 (10%)</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0_No</td>
<td>28</td>
<td>7</td>
<td>3</td>
<td>≈ 0.001</td>
</tr>
<tr>
<td>1_Mild</td>
<td>6 (10%)</td>
<td>38 (63.3%)</td>
<td>15 (75%)</td>
<td></td>
</tr>
<tr>
<td>2_Moderate</td>
<td>42 (70%)</td>
<td>16 (26.7%)</td>
<td>0 (0%)</td>
<td>≈ 0.0001</td>
</tr>
<tr>
<td>3_Severe</td>
<td>12 (20%)</td>
<td>6 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

**Basement membrane thickness**

Patients with CRSwNP (group 1) had statistically significantly higher subepithelial fibrosis (moderate and severe degree) (Figure 3), higher interstitial fibrosis (moderate and severe degree) and higher blood vessel wall thickness (Figure 4) when compared with patients with CRSsNP (group 2) and control group (p<0.0001) (Table 4).

Fig. 3: A case of chronic sinusitis with nasal polyp, ethmoidal polyp (Masson Trichrome 100x) red arrow show thick basement membrane.

Fig. 4: A case of chronic sinusitis with nasal polyp, ethmoidal polyp (Masson Trichrome 100x) yellow arrow show thick blood vessel wall, red arrow show marked interstitial fibrosis.
Table 4: Comparison between three groups regarding Basement membrane thickness

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group-1 (60)</th>
<th>Group-2 (60)</th>
<th>Group-3 (20)</th>
<th>Chi square test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subepithelial fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0_Negative</td>
<td>0 (0%)</td>
<td>4 (7%)</td>
<td></td>
<td>Negative</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>1_Mild</td>
<td>5 (9%)</td>
<td>46 (76%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2_Moderate</td>
<td>44 (73%)</td>
<td>10 (17%)</td>
<td></td>
<td>Negative</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>3_sever</td>
<td>11 (18%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td>0 (0%)</td>
<td>13 (22%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intertitial fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1_Mild</td>
<td>12 (21%)</td>
<td>38 (63%)</td>
<td></td>
<td>Negative</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>2_Moderate</td>
<td>40 (66%)</td>
<td>9 (15%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3_sever</td>
<td>8 (13%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood vessel wall thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td>65%</td>
<td>30%</td>
<td></td>
<td>Negative</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>negative</td>
<td>35%</td>
<td>70%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immunohistochemical results

Patients in group 1 showed a significantly higher Immunohistochemical score of inflammatory cells (moderate and marked degree) (Figures 7,8,10) and higher intensity of epithelium staining (moderate and strong degree) (Figures 5,6,7) when compared to the two other groups (p <0.01) (Table 5).

Fig. 5: Box and whisker graph show Comparison between the 3 groups as regards Immunohistochemical score of inflammatory cells where in group 1, the IQR was (4-12) and median was 8, group 2 show IQR (2-4) and group 3 was (0 – 1).

Fig. 6: A case of chronic rhinosinusitis with nasal polyps (A) the surface epithelium shows marked immunohistochemical staining (IL 25 antibody 40x) (red arrow). (B,C,D) CT coronal cuts show opacification of frontal ethmoidal maxillary and sphenoid sinus (Lund and Mackay score 23).
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Fig. 7: A case of chronic sinusitis with nasal polyps (A) The epithelium is hyperplastic with marked immunohistochemical staining (blue arrow), also marked degree of immunohistochemical staining of inflammatory cells was noted (IL 25 antibody 100x) (yellow star). (B,C,D) CT scan coronal cuts show total opacification of sinuses with closure of osteomeatal complex. (Lund and Mackay score 24).

Table 5: Comparison between the 3 groups as regards Immunohistochemical staining using Kruskal-Wallis and Chi square tests

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group-1 (60)</th>
<th>Group-2 (60)</th>
<th>Group-3 (20)</th>
<th>Kruskal-Wallis test</th>
<th>Chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemical score of inflammatory cells</td>
<td>8 (4 – 12)</td>
<td>2 (2 – 4)</td>
<td>0 (0 – 1)</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemical Score of inflammatory Cells</td>
<td>0_Negative</td>
<td>0 (0%)</td>
<td>7 (11.7%)</td>
<td>12 (60%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1_Mild</td>
<td>9 (15%)</td>
<td>47 (78.3%)</td>
<td>8 (40%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2_Moderate</td>
<td>17 (28%)</td>
<td>6 (10%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3_Marked</td>
<td>34 (56%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>IL epithelium intensity</td>
<td>0_No staining</td>
<td>2 (3.3%)</td>
<td>20 (33.3%)</td>
<td>14 (70%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1_Mild</td>
<td>20 (33.3%)</td>
<td>36 (60%)</td>
<td>6 (30%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>2_Moderate</td>
<td>37 (61.7%)</td>
<td>4 (6.7%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3_Strong</td>
<td>1 (1.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

(IQR): interquartile range

Correlation analysis

Spearman's correlation analysis shows that; Lund and Mackay score, grade of polyp, total nasal symptom score (TNSS) and basement membrane thickness had a highly significant positive correlation with immunohistochemical score. (Table 6)

Table 6: Spearman's correlation analysis for some Factors associated with Immunohistochemical score

<table>
<thead>
<tr>
<th>Associated Factor</th>
<th>Immunohistochemical score</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lund and McKay score</td>
<td>0.621</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Grade of polyp</td>
<td>0.458</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>TNSS</td>
<td>0.438</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Basement membrane thickness</td>
<td>0.453</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Rho; spearman’s (correlation coefficient)
DISCUSSION

The epithelial cell-derived cytokine IL-25, acting through the receptor IL-17R, has been implicated in promoting Th2 responses in airway inflammation. However, the clinical relevance of local IL-25 in CRSwNP patients remains relatively uncharacterized[13].

In the present study, a link was identified between the local IL-25 expression and the Th2-biased inflammatory profiles in nasal tissues of CRSwNP patients. These findings might expand our understanding on the pathophysiology of CRSwNP and contribute to the treatment of CRSwNP.

CRS with nasal polyps (CRSwNP) is a Th2-biased, eosinophilic mucosal inflammation. Th2 cytokines, in particular IL-5 and IL-13, are implicated in the pathogenesis of the eosinophilic airway inflammation such as asthma, allergic rhinitis, and CRSwNP[14].

The triad of epithelial derived cytokines, IL-25, IL-33, and TSLP, has provoked considerable interest as potential therapeutic targets, as they initiate the Th2-biased inflammation profiles by activating Th2 and ILC2 cells[15].

Concerning demographic and personal data of the patients, in our study there was highly significant increase in age in CRSwNP group compared to other groups, but non-significant difference was found regarding sex of the patients. On the contrary, in Lam M et al. (2015) there was a significant increase in age in the control group compared to CRSwNP group because he used patients with sinonasal tumors as controls which may provide an inexact baseline of comparison[16]. Hong et al. (2017) found no significant difference in age between the CRSwNP and control group, this comes in agreement with Chen et al. (2017) who provided evidence that IL-25 expression and control group, this comes in agreement with Chen et al. (2017) who provided evidence that IL-25 expression was significantly increased in CRSwNP group. This is because IL-25 promotes tissue remodeling by acting directly on human fibroblasts to induce collagen secretion, moreover IL-25 induces recruitment of endothelial progenitor cells and subsequent neovascularization[17].

In our study, the number of asthmatic patients was significantly higher in CRSwNP group than CRSsNP and control group, this comes in agreement with Chen et al. (2017) who provided evidence that IL-25 expression is significantly enhanced in polyp tissues of patients with CRSwNP and was significantly increased in the asthmatic subgroup compared with the non-airway hypersensitivity (NAHR)[18]. These findings, therefore, expand our understanding of the concept of united airway diseases and show that IL-25 may play a crucial role in initializing concomitant asthma in CRS which makes it a potential therapeutic target in concomitant Asthma and CRS[19].

Regarding disease specific quality of life, there was a positive correlation between total nasal symptom score (TNSS) and immunohistochemical score of IL-25. Hong et al. (2017) used (TNSS) to express quality of life and was consistent with high IL-25 group[20]. Lam et al. (2013) found that disease specific quality of life (SNOT 22) did not correlate with expression of IL-25. This may be related to objective and subjective methods which potentially measure different constructs of the same disease[21]. Symptoms, physical functioning and wellbeing have poor correlation with objective measures of disease severity in almost all chronic diseases like diabetes and chronic heart disease[22].

By using Meltzer et al. grading system[23] for endoscopic grading of the nasal polyps, we found a positive correlation between immunohistochemical score of IL-25 and grade of the nasal polyps. This was consistent with Hong et al. (2017) who used nasal endoscopic score to record the size of nasal polyps on both sides, this score was greater in IL-25 high subgroup than that in IL-25 low subgroup in the CRSwNP patients[17], which highlights the expression of IL-25 as a risk factor in CRSwNP pathogenesis.

By increasing the severity of the nasal polyps denoted by higher scores in Lund-Mackay[24] CT Scoring, tissue IL-25 immunohistochemical score showed a statistically significant positive correlation with the CT score, which highlights the association between IL-25 and severity of the disease. This was a common observation with many authors as Hong et al. (2017)[17], Shen et al. (2011)[25] and Lam et al. (2013) (19) who also found that CT scores significantly correlated to IL-25 high subgroup.

However, Ozturan et al. 2017 found a statistically non-significant correlation between IL25 level and Lund-Mackay score, the reasons underlying these correlations may be caused by different immune cells infiltrating the tissue[26].

We also found a positive correlation between the severity of inflammatory response and the presence of nasal polyps, where in our study the number of patients with moderate or severe inflammatory pathology was significantly higher in CRSwNP group compared to other groups, these results correlated with Lam et al. (2015) where patients with severe degree of inflammation had increased expression of IL25[27].

In the present study, subepithelial fibrosis (indicator of basement membrane thickness) in CRSwNP group was greater compared to CRSsNP group. Hong et al. (2017) also found that basement membrane thickness increases with IL 25 high group. This is because IL 25 promotes tissue remodeling by acting directly on human fibroblasts to induce collagen secretion, moreover IL 25 induces recruitment of endothelial progenitor cells and subsequent neovascularization[28].

Park et al. was the first to report that IL 25 activates nasal polyp derived fibroblasts (NPDFs) for the release of extracellular matrix (ECMs) and matrix metalloproteinases (MMPs), he suggested that IL 25 induced release of α SMA, fibronectin, collagen, MMP-1 and MMP-13 from NPDFs is mediated by activation of the Mitogen activated protein kinase (MAPK) and nuclear factor KB (NF-KB) pathways, thereby providing new clues for fibroblasts mediated inflammation by changing the ECM composition in nasal polyps[29].

This is consistent with the study by Shin et al which demonstrated that IL-25 was significantly increased in...
both human polyp tissues and murine model, and anti-IL-25 treatment reduced the number of polyps, mucosal edema thickness, collagen deposition, and infiltration of inflammatory cells[31].

The involvement of allergy in CRS has been controversial. Such speculation was prompted when significant eosinophilia was noted in CRS mucosa, similar to allergic disease of the lower airways[18].

CRS with nasal polyps (CRSwNP) is a Th2-biased, eosinophilic mucosal inflammation. In our study we found a significant increase in eosinophils in CRSwNP group compared to CRSsNP group. This was supported by Chen et al. (2017)[19], Hong et al. (2017)[17] and Linuma et al. (2015)[27] who reported that the IL-25 level in polyps was increased in patients with eosinophilic CRS, and elevation of IL5 and IL-9 production was found in polyp mononuclear cells from patients with eosinophilic CRS by stimulation with IL-25 under T-cell receptor stimulation. Liao et al. (2015) found that IL-25 and IL-17RB were enhanced in epithelial cells in both eosinophilic and non-eosinophilic CRSwNP compared to the control[27].

We also found 46% of CRSwNP patients had high tissue eosinophilia. These patients had poorer CT score and TNSS compared to non-eosinophilic CRSwNP patients. This comes in agreement with Cho et al. (2017)[28] who found that eosinophilic CRSwNP when compared to non-eosinophilic CRSwNP, had higher CT score and worse preoperative and postoperative endoscopy scores.

Linuma et al. (2015)[27] reported that epithelial cells, mast cells, eosinophils and endothelial cells express IL-25, however the major source of IL-25 in ECRS was most likely eosinophils due to large numbers of IL-25 in eosinophilic cation protein (ECP) cells. They also found that IL-25 levels were significantly increased and were correlated with IL-5 and IL-9 levels in eosinophilic CRS (ECRS) samples but not in non-eosinophilic CRS (NECRS) samples, moreover, IL-25 levels in NPs were significantly correlated with the number of tissue eosinophils and CT scores[29]. Accordingly acting with IL-17RB expressing TH2 cells, IL-25 could induce a vicious cycle that accelerates eosinophil infiltration[27].

After immunohistochemical staining, a score was made from the product of multiplication of the intensity and percentage of staining of inflammatory cells denoting the presence of IL 25, the score ranged from 0-12. Based on the final score, expression was categorized as negative (0), mild (1-4), moderate (5-8) or marked (9-12)[29]. IL-25 expression was greater in epithelial cells of nasal polyps compared with those of uncinate process in patients with CRSsNP and turbinate or septal mucosa in control subjects, moreover, we documented a significant increase of immunohistochemical score of inflammatory cells in nasal polyps of patients with CRSwNP compared with those in uncinate process from patients with CRSsNP, this comes in agreement with shin et al. (2015) (25) and Lam et al. (2015)[29] where tissue levels of IL-25 were demonstrated to be significantly higher in CRSwNP patients compared to CRS patients without nasal polyp and healthy controls.

Hong et al. (2017) revealed that the expression of IL-25 and its receptor IL-17RB were significantly increased in patients with CRSwNP, and IL-25 was distributed in both epithelium and the inflammatory cells of the subepithelial area[17].

Unlike previous literatures, Ozturan et al. (2017) mentioned that they did not find statistically significant difference in tissue IL-25 levels between CRSwNP, CRSsNP, and control groups (p = 0.698). The same study declared that the mean tissue IL-33 level in the CRSwNP group was found to be significantly lower than those of CRSsNP and control groups. They stated a negative correlation between the severity of CRS and IL-25 and IL-33 levels[22].

Miljkovic et al. (2014)[29] showed that IL-25 mRNA was decreased markedly in nasal polyps versus the ethmoid sinuses of control and CRSsNP patients furthermore, Kato et al. (2015)[20] reported that IL-25 was not an important modulator of type 2 inflammation in CRS; their data showed that mRNA levels of IL-25 were very low in sinuses and the level of IL-25 mRNA did not differ much between nasal polyps and healthy sinus tissues. Different factors could explain these discrepancies, including different sites of sampling, inclusion/ exclusion criteria and preoperative treatment between various studies.

Differences in mRNA expression of these cytokines in the context of CRS also could thus potentially be attributed to differences at the level of the sinonasal microbiome, Mice lacking commensal bacteria (e.g. due to antibiotic treatment) exhibit reduced expression of IL-25, IL-33 and TSLP in intestinal epithelial cells[31]. This supports an important immunomodulatory role for bacteria, and their products, in the pathophysiology of CRS and possibly in the regulation of expression of Th2-inducing cytokines, differences in mRNA expression of these cytokines in the context of CRS could thus also potentially be attributed to differences at the level of the sinonasal microbiome[32].

Although levels of IL-25 in CRSwNP remain controversial most studies have shown that IL-25 is upregulated in patients with CRSwNP, and that it plays a role in the pathogenesis of nasal polyp formation. Thus, blocking IL-25 could be an effective therapeutic strategy for nasal polyp patients with high levels of IL-25[17].

To further validate the clinical potential of nasal IL-25 in patients with CRSwNP, we next compared the sensitivity and specificity of nasal interleukin 25 to predict patients with CRSwNP and the cutoff tissue level of IL 25 by applying ROC curve analysis consequently when the cutoff tissue IL25 protein level was >20%. The sensitivity was 70% and specificity was 85%.

Oral corticosteroids are commonly used in the treatment of chronic rhinosinusitis with nasal polyps (CRSwNP). However, the efficacy of oral-corticosteroid
therapy varies between different patients with CRSwNP\(^1\). IL-25–mediated TH2-biased response is reported to be associated with corticosteroid sensitivity in airway inflammatory response, while their value for predicting oral-corticosteroid response to CRSwNP has not been completely evaluated\(^3\). 

IL-25, an important epithelial-derived proinflammatory cytokine, plays various roles in different inflammatory murine models, such as asthma, atopic dermatitis, and pulmonary fibrosis, and orchestrates type 2 responses at the mucosa site\(^3\). Cheng et al reported that the IL-25 high patients with asthma have better improvements in FEV\(_1\) following inhaled corticosteroid treatment than do the IL-25 low subgroup, and suggested serum IL-25 level as a predictor for corticosteroid sensitivity in asthma therapy. This study thus aimed to characterize the inflammatory profiles of patients with CRSwNP, and to evaluate the predictive biomarkers for oral-corticosteroid responses in a prospective clinical study\(^3\). 

Recently, Hong et al. (2018) evaluated the factors that may affect oral-corticosteroid treatment outcome in CRSwNP and declared that IL-25 levels in the nasal polyp tissue and to a certain extent blood serum could be used to predict the clinical efficacy of oral-corticosteroid sensitivity\(^3\). Further studies are needed to investigate predictive tissue markers for prognosis and recurrence.

**CONCLUSION**

This study shows increase in IL25 expression and tissue eosinophilia in CRSwNP patient with more severe inflammatory pathology, mucosal changes and basement membrane thickness. Thus, IL-25 may be a promising therapeutic target and an indicator for the management of CRSwNP patients.

**CONFLICT OF INTERESTS**

There are no conflicts of interest.

**REFERENCES**

28. Cho SW, Dae WK, Jeong WK, Chul HL, and Chae SR: Classification of chronic rhinosinusitis according to a nasal polyp and tissue eosinophilia: limitation of current classification system for Asian population. Asia Pac Allergy. (2017):121-130
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