A proinflammatory marker in chronic rhinosinusitis: serum calprotectin

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INTRODUCTION

Calprotectin is a heterodimeric complex of S100A8 and S100A9 proteins that are members of the S100 protein family (Kato et al., 2017). It is a cytosolic protein complex of human neutrophils and also has bacteriostatic properties (Vogl et al., 2007). Calprotectin is expressed during myeloid differentiation, is abundant in neutrophil granulocytes and monocytes. Myeloid cells, which are developed in the bone marrow, migrate in large amounts into the blood and further into tissues in many diseases and conditions, like cystic fibrosis, rheumatoid arthritis and many inflammations and malignant diseases. Thus, elevated calprotectin levels in the serum can be used as a strong clinical marker indicating the presence of inflammation. Studies have shown that calprotectin has a strong pro-inflammatory effect. Elevated calprotectin levels in the serum can be used as a strong clinical marker indicating the presence of inflammation. Objective: To investigate serum calprotectin levels in patients with chronic rhinosinusitis (CRS) and to determine the applicability of calprotectin as a potential pro-inflammatory biomarker for CRS.

METHODS: The study consisted of three groups: chronic rhinosinusitis with polyps (CRSwoNP group), chronic rhinosinusitis without polyps (CRSwNP), and healthy control. CRS patients with polyps were further divided into two groups depending on the presence/absence of Samter’s triad. The Nose Obstruction Symptom Evaluation (NOSE) scale score and serum calprotectin value were evaluated in all participants. Results: The mean serum calprotectin value was 79.5±11.8 ng/ml for the CRSwNP group, 71.3±16 ng/ml for the CRSwoNP group, and 61.9±11.6 ng/ml for the control group (p<0.001). The Samter’s triad group had a significantly higher calprotectin value than the non-Samter’s triad group (p=0.03). There was a significant correlation between the NOSE scores and calprotectin levels (rho=0.734, p<0.001). Conclusion: Serum calprotectin values were correlated with the severity of symptoms in patients with CRS; thus, it seems to be a valuable pro-inflammatory biomarker for the diagnosis of the disease and determining its severity. Further studies with larger series are needed to evaluate the preoperative and postoperative serum calprotectin values in patients undergoing surgery.

Key words: chronic rhinosinusitis, nasal poly, calprotectin

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Abbreviations: CALP, Calprotectin; ChT, Chitotriosidase; CRS, chronic rhinosinusitis; CRSwoNP, chronic rhinosinusitis without polyps; CRSwNP, chronic rhinosinusitis with polyps; ESS, endoscopic sinus surgery; ISSHL, idiopathic sudden sensorineural hearing loss; NOSE, Nose Obstruction Symptom Evaluation; S.D., standard deviation; SPARC, serum secreted protein acidic and cysteine-rich

INTRODUCTION

Calprotectin is a heterodimeric complex of S100A8 and S100A9 proteins that are members of the S100 protein family (Kato et al., 2017). It is a cytosolic protein complex of human neutrophils and also has bacteriostatic properties (Vogl et al., 2007). Calprotectin is expressed during myeloid differentiation, is abundant in neutrophil granulocytes and monocytes. Myeloid cells, which are developed in the bone marrow, migrate in large amounts into the blood and further into tissues in many diseases and conditions, like cystic fibrosis, rheumatoid arthritis and many inflammations and malignant diseases. Thus, elevated calprotectin levels in the serum can be used as a strong clinical marker indicating the presence of inflammation (Losse et al., 2010). Studies have shown that calprotectin has a strong pro-inflammatory effect (Dale et al., 1989; El-Rifai et al., 2002). In case of inflammation, increased calprotectin has also been reported in pathological tissue (nasal lavage fluid, exudates, autoimmune diseases, etc.) (Brahian et al., 2009; Tieu et al., 2010). It is also suggested that if a high concentration of calprotectin is present in the body fluid at local inflammatory sites, this might cause a delay in tissue repair and a deleterious effect on the inflamed tissue (Yui et al., 2003).

Chronic rhinosinusitis (CRS) with nasal polyps (CRSwoNP) is a disease accompanied by chronic inflammatory conditions in the upper airways, which seriously impair the quality of life of the patient (Bachert et al., 2010). Various factors, such as viral and bacterial agents (Van Zele et al., 2004; Patou et al., 2008), epithelial barrier disorders, and tissue remodeling (Zhang et al., 2010; Van Bruaene et al., 2011; Van Crombruggen et al., 2013) play a role in the etiology of CRSwoNP. However, the pathophysiology of CRSwoNP has not yet been clearly elucidated (Van Crombruggen et al., 2011). The coexistence of nasal polyps, asthma and aspirin allergy is called Samter’s triad and leads to more severe and persistent complaints related to nasal polyps (Kim et al., 2007). In a randomized, case-control study by Dutu and others (Dutu et al., 2018), the authors focused on the evaluation of serum chitotriosidase (ChT) and 25-hydroxyvitamin D3 (25-OH-D3) as potential biomarkers of CRS associated inflammation, and it was reported that serum ChT activity was significantly increased in CRS patients, compared to the controls, while 25-OH-D3 levels were significantly decreased in patients, versus controls. To the best of our knowledge, there is currently no test that provides information at the onset of complaints about the possible severity during the course of the disease.

There are only a limited number of studies on the role of calprotectin in the mechanism of upper airway inflammation (Van Crombruggen et al., 2016; Kuzucu et al., 2019). The current study aimed to measure the serum calprotectin levels in patients with sinonasal disease and determine the applicability of calprotectin as a potential molecular biomarker for CRS.

MATERIALS AND METHODS

This prospective, cross-sectional, historical cohort study was approved by the local ethics committee and carried out
Table 1. Demographic features of patients

| Variables        | CRSwNP Group (n:26) | CRSwoNP Group (n:24) | Control Group (n:27) | p     |
|------------------|---------------------|----------------------|----------------------|-------|
| Age (years)      | 43±17               | 46±16                | 40±14                | 0.45* |
|                  | (Mean ± S.D.)       |                      |                      |       |
| Gender, n        |                     |                      |                      |       |
| Female           | 12                  | 11                   | 14                   | 0.88**|
| Male             | 13                  | 13                   | 13                   |       |
| Smoking, n       |                     |                      |                      |       |
| Yes              | 10                  | 8                    | 8                    | 19    |
| No               | 16                  | 16                   | 19                   |       |
| Samter’s Triad, n|                     |                      |                      |       |
| Yes              | 6                   | 0                    | 0                    | 0     |
| No               | 20                  | 24                   | 27                   |       |

CRSwNP, chronic rhinosinusitis with nasal polyps; CRSwoNP, chronic rhinosinusitis without nasal polyps; S.D., standard deviation. *One-way analysis of variance. **Kruskal-Wallis analysis

RESULTS

There were a total of 77 participants, 40 males (52%) and 37 females (48%). The age of the participants ranged from 26 to 62 years. The mean age and gender distribution did not significantly differ between the three groups (p=0.45 and p=0.88, respectively). The demographic features of the study sample are shown in Table 1.

The mean serum calprotectin value was 79.5±11.8 ng/ml for the CRSwNP group, 71.3±16 ng/ml for the CRSwoNP group, and 61.9±11.6 ng/ml for the control group. The Welch analysis of variance revealed that the mean calprotectin levels significantly differed between the groups (p<0.001) (Table 2). The post-hoc comparison tests showed that the mean calprotectin level of the CRSwNP group was significantly higher compared to the control group (p<0.001), but did not significantly differ from that of the CRSwoNP group (p=0.13). Additionally, the mean calprotectin level of the CRSwNP group was higher than that of the control group, but this was not statistically significant (p=0.06) (Table 2).

Samter’s triad was detected in six patients in the CRSwNP group. The mean calprotectin value was 88.5±10.1 ng/ml for the patients with Samter’s triad and 76.8±11.1 ng/ml for the remaining patients in the CRSwNP group, which indicated a statistically significant difference between these two subgroups (p=0.03) (Table 3).

There was no significant difference between the mean calprotectin levels of smokers and non-smokers (p=0.4) (Table 4).
Table 2. Comparison of the mean calprotectin levels between groups

|                      | CRSwNP Group\(^1\) (n:26) Mean ± S.D. | CRSwoNP Group\(^2\) (n:24) Mean ± S.D. | Control\(^3\) (n:27) Mean ± S.D. | \(p\)          |
|----------------------|--------------------------------------|----------------------------------------|----------------------------------|----------------|
| Calprotectin (ng/ml) | 79.5±11.8                            | 71.3±16                                | 61.9±11.6                        | 0.001*         |
|                      | \(^{1}p=0.13^{*}\)                    | \(^{2}p=0.06^{*}\)                    | \(^{1}p<0.001^{*}\)             |                |

CRSwNP, Chronic rhinosinusitis with nasal polyps; CRSwoNP, chronic rhinosinusitis without nasal polyps; S.D., standard deviation. *Welch analysis of variance; **Tamhane T2 post-hoc test

Table 3. Calprotectin levels in CRSwNP patients with and without Samter's triad

|                          | Mean calprotectin levels (ng/ml) ± S.D. |
|--------------------------|----------------------------------------|
| With Samter’s Triad (n=6) | 88.5±10.1                              |
| Without Samter’s Triad (n=20) | 76.8±11.1                              |
| \(p\)                     | 0.03**                                 |

CRSwNP, Chronic rhinosinusitis with nasal polyps; S.D., standard deviation; *Student’s t-test

Table 4. Calprotectin levels of smoker and non-smoker patients with CRS

|                          | Mean calprotectin levels (ng/ml) ± S.D. |
|--------------------------|----------------------------------------|
| Smokers (n=18)           | 77.9±14.4                              |
| Non-smokers (n=32)       | 74.3±14.5                              |
| \(p\)                    | 0.40*                                  |

CRS, Chronic rhinosinusitis; S.D., standard deviation; *Student’s t-test

Table 5. Comparison of the mean NOSE scores between groups

|                          | CRSwNP Group (n:26) Mean ± S.D. | CRSwoNP Group (n:24) Mean ± S.D. | Control (n:27) Mean ± S.D. | \(p\)          |
|--------------------------|----------------------------------|----------------------------------|---------------------------|----------------|
| NOSE score               | 75±9.8                           | 63.75±15.0                       | 32.77±6.8                 | <0.001*         |
|                          | *Welch analysis of variance; **Spearman correlation test

DISCUSSION

In recent years, a growing number of studies have been undertaken to investigate the relationship between the serum calprotectin value and inflammatory upper respiratory tract diseases. In a study conducted with 44 participants, the authors compared the serum calprotectin values of idiopathic sudden sensorineural hearing loss (ISSHL) cases with healthy participants. The serum calprotectin levels of patients with ISSHL were found to be higher than healthy individuals. The authors also noted that the serum calprotectin value of the patients who did not recover from the disease was significantly higher compared to those with partial or total recovery (Kuzcu et al., 2019). In another study, the serum calprotectin levels were examined in CRSwNP patients, and it was reported that calprotectin within the cells was transferred to the extracellular matrix due to inflammation; thus, it was elevated in the serum (Van Crombruggen et al., 2016). In the current study, we analyzed the serum calprotectin value in patients with CRSwNP and CRSwoNP and also compared the serum calprotectin values in CRSwNP patients with and without Samter’s triad. Similar to the study by Crombruggen et al., we found that the serum calprotectin levels of the CRSwNP patients were higher than those of the CRSwoNP patients and healthy individuals, and additionally, the serum calprotectin was higher in CRSwNP patients with Samter’s triad compared to those without this condition. We consider that this may be caused by the presence of an additional disease; i.e., asthma triggering inflammation and nasal polyp complaints being more severe in patients with Samter’s triad.

In ultrastructural analysis, mast cell degranulation, which is responsible for high histamine levels in polyp fluids, has been shown to contribute to the formation of nasal polyps. Mast cells have mucosal and connective tissue types. In addition, basophils circulating in blood are transferred to tissues where they are transformed into mast cells (Naclerio et al., 2017). In a previous study that investigated the secreted protein acidic and cysteine-rich (SPARC) value in 26 patients

Figure 1. Scatter plot showing relationship between the NOSE scores and Calprotectin levels of the participants. The many small circles represent the plotted values obtained for each of the variables while the line represents the best fit for the correlation between them.

The mean NOSE score was calculated as 75±9.8, 63.75±15 and 32.77±6.8 in CRSwNP, CRSwoNP and control groups, respectively. Furthermore, a significant positive correlation was found between the NOSE scores and calprotectin levels of the participants (rho: 0.734, \(p<0.001\)) (Table 5; Fig. 1).
with CRSwNP before and after endoscopic sinus surgery (ESS), the postoperative serum SPARC value was decreased by 33%, but the authors noted that it was not possible to use SPARC to predict the course of the disease after ESS (Asmar et al., 2019). In another study, the authors sought an answer to the question of whether the serum periostin value could be used to determine the nasal polyp load in CRSwNP patients and found the level of this protein to be higher in CRSwNP patients compared to the CRSwNP group. The researchers concluded that the serum periostin value could be used as a new molecular biomarker in the presence of nasal polyps (Maxfield et al., 2018). In the current study, we investigated the applicability of serum calprotectin as a biomarker in the presence of nasal polyps in patients with CRS. We also examined the serum calprotectin levels according to the presence/absence of Samter’s triad. Our results showed that the serum calprotectin values were increased in the presence of Samter’s triad compared to the CRSwNP patients without Samter’s triad.

There are studies showing that the NOSE scale provides results consistent with computed tomography and physical examination findings in nasal surgery (Rhee et al., 2003; Kahveci et al., 2012). In a study that included 27 patients, the NOSE scale and septum deviation data on paranasal tomography were compared and found to be consistent (Rhee et al., 2003). Similarly, in another study, 345 patients were screened retrospectively, and the reliability of the NOSE score in revealing nasal obstruction was investigated. As a result, NOSE was shown to be a reliable instrument for the classification of nasal congestion complaints (Lipan et al., 2013). In light of these studies, we decided to standardize the patients’ complaints of nasal congestion using the NOSE scale. We found a correlation between the NOSE scale scores and serum calprotectin values. Among the patients with CRS, nasal obstruction complaints were observed to be increased, particularly in those presenting with nasal polyps, which, in turn, elevated the serum calprotectin levels.

In this study, the serum calprotectin levels of the CRSwNP patients were significantly higher than those of the control group. At the same time, within the CRSwNP group, the serum calprotectin value was higher among the patients with Samter’s triad compared to the remaining patients. In the light of these values, it can be stated that the serum calprotectin value is increased with the increasing severity of CRS. In addition, the serum calprotectin value is elevated in parallel to the increase in the NOSE scale score; i.e., it is higher in patients with the complaints of nasal congestion.

In this study, there are two issues to be addressed. First, the sample was relatively small to reach a widely accepted conclusion. A more extensive survey of cases is needed to be studied. In future work, we aim to investigate whether serum calprotectin values can be used as a prognostic factor for CRS.

CONCLUSION

In conclusion, we observed an increase in the serum calprotectin value in cases with CRS, and the severity of CRS symptoms correlated with the value of serum calprotectin. We recommend that a similar study should be undertaken with a larger case series to evaluate the pre-operative and postoperative serum calprotectin values in patients undergoing endoscopic sinus surgery.

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