ORIGINAL RESEARCH

To explore the levels of calprotectin in the serum of patients diagnosed with chronic rhinosinusitis (CRS) and to discover whether or not calprotectin might serve as a possible molecular proinflammatory biomarker for CRS

[1]Dr.Tarun Kumar, [2]Dr. Ranjan Kumar

[1]Senior Resident, Department Of Biochemistry AIIMS, Patna, Bihar, India
[2]Associate Professor, Department of ENT, Bhagwan Mahavir Institute of Medical Sciences, Pawapuri, Bihar, India

Corresponding Author
Dr. Ranjan Kumar
Associate Professor, Department of ENT, Bhagwan Mahavir Institute of Medical Sciences, Pawapuri, Bihar, India

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ABSTRACT

Aim: The purpose of this study is to explore the levels of calprotectin in the serum of patients diagnosed with chronic rhinosinusitis (CRS) and to discover whether or not calprotectin might serve as a possible molecular proinflammatory biomarker for CRS. **Materials and methods:** The volunteers were separated into the following categories: The first group was made up of patients who were diagnosed with CRS and found to have nasal polyps on endoscopic nasal examination (CRSw NP); the second group was made up of patients who were diagnosed with chronic rhinosinusitis without nasal polyps (CRSwoNP); and the third group was made up of healthy controls. Participants' medical records, age, gender, smoking status, presence of asthma, aspirin-induced respiratory status, and Samter's triad were documented, as well as the demographic and clinical information that was acquired from them. The Nasal Obstruction Symptom Evaluation (NOSE) scale was verbally delivered to the participants. **Results:** The group that received CRSwNP had a mean serum calprotectin value of 80.14±7.58 ng/ml, whereas the group that received CRSwoNP had a value of 72.58±5.85 ng/ml, and the control group had a value of 62.14±6.36 ng/ml. There were six patients in the CRSwNP group who were found to have Samter's triad. The patients who had Samter's triad had a mean calprotectin value of 89.85±6.37 ng/ml, whereas the other patients in the CRSwNP group had a mean calprotectin value of 77.88±7.19 ng/ml. The mean levels of calprotectin in smokers and non-smokers did not vary significantly from one another ($p = 0.33$). The mean NOSE score was determined to be 76.11±3.96 in the CRSwNP group, 64.18±3.69 in the CRS wo NP group, and 33.48±5.01 in the control group, respectively. In addition to this, there was found to be a substantial positive association between the NOSE scores and the calprotectin levels of the subjects (<0.001) **Conclusion:** In conclusion, we found that instances of CRS were associated with an increase in the value of serum calprotectin, and there was a correlation between the value of serum calprotectin and the severity of CRS symptoms.

Keywords: Calprotectin, Chronic Rhinosinusitis, Proinflammatory

INTRODUCTION

Calprotectin is a heterodimer complex that is made up of the proteins S100A8 and S100A9, which are both part of the family of S100 proteins. [1]In addition to having bacteriostatic capabilities, it is a protein complex that is found in the cytoplasm of human neutrophils. During the process of myeloid differentiation, calprotectin is expressed. It is found in high levels in neutrophil granulocytes and monocytes. In many diseases and conditions, such as cystic fibrosis, rheumatoid arthritis, and many inflammations and malignant diseases, myeloid cells, which are developed in the bone marrow, migrate in large quantities into the blood and then further into the

tissues. This process is known as myeloid cell migration. Hence, a higher level of calprotectin in the serum may be employed as a powerful clinical diagnostic that indicates the existence of inflammation. [2] Many studies have shown that calprotectin contributes significantly to the inflammatory response. Increased levels of calprotectin have been seen in diseased tissue in cases of inflammation (nasal lavage fluid, exudates, autoimmune diseases, etc.) It has also been hypothesised that if there is a high concentration of calprotectin present in the bodily fluid at local inflammatory areas, this might create a delay in the process of tissue healing in addition to having a detrimental impact on the tissue that is inflamed. [3]

Chronic rhinosinusitis (CRS) with nasal polyps (CRSwNP) is a disease that is characterised by persistent inflammatory diseases in the upper airways, which severely degrade the patient's quality of life. CRS is an abbreviation for chronic rhinosinusitis with nasal polyps. The pathogenesis of CRSwNP is complicated and involves a number of different causes, including viral and bacterial pathogens, abnormalities of the epithelial barrier, and tissue remodelling. The pathogenesis of CRSwNP, on the other hand, has not yet been completely explained. Samter's trio refers to the phenomenon in which nasal polyps, asthma, and an allergy to aspirin coexist in a patient. This phenomenon results in nasal polyp-related symptoms that are both more severe and more chronic. [4] In a case-control study that was carried out by Dutu and others, the authors focused on the evaluation of serum chitotriosidase (Cht) and 25-hydroxyvitamin D3 (25-OH-D3) as potential biomarkers of CRS associated inflammation. It was reported that serum Cht activity was significantly increased in CRS patients, in comparison to the controls, while 25-OH-D3 levels were significantly decreased in patients, versus controls. There is presently no test that, to the best of our knowledge, can offer information at the beginning of the complaints about the likely severity that may occur over the course of the illness.

There have only been a few of research conducted on the part that calprotectin plays in the inflammatory process that occurs in the upper airways.[5] The purpose of this research was to evaluate whether or not calprotectin may be used as a possible molecular biomarker for CRS by measuring the levels of calprotectin in the serum of individuals who were diagnosed with sinonasal illness.

MATERIALS AND METHODS

The regional ethics committee gave its blessing to the prospective, cross-sectional, and historical cohort research that was proposed. The volunteers were separated into the following categories: The first group was made up of patients who were diagnosed with CRS and found to have nasal polyps on endoscopic nasal examination (CRSwNP); the second group was made up of patients who were diagnosed with chronic rhinosinusitis without nasal polyps (CRSwoNP); and the third group was made up of healthy controls. Participants' medical records, age, gender, smoking status, presence of asthma, aspirin-induced respiratory status, and Samter's triad were documented, as well as the demographic and clinical information that was acquired from them.

Patients in the CRSwNP group were tested individually as well by further subdividing them into groups based on whether or not they had Samter's triad. This was done so that each subgroup could be studied independently (nasal polyps, aspirin allergy, and asthma).

The Nasal Obstruction Symptom Evaluation (NOSE) scale was verbally delivered to the participants. This scale consists of five questions that quantify the impact of the condition on quality of life based on a scale that ranges from 0 to 4. The findings of the scale were multiplied by five, and the maximum possible score on the NOSE was therefore one hundred.

People who were under the age of 20 or over the age of 70, as well as those who had inverted papillomas, cystic fibrosis, sinonasal granulomatosis, or any autoimmune disease, a history of acute or chronic infection or symptoms of infection, hypertension, angina pectoris, myocardial infarction, diabetes mellitus, metabolic syndrome, chronic obstructive pulmonary disease (COPD), amyloidosis, During an eight-hour fasting period, venous blood was drawn in the morning for the purpose of determining the serum calprotectin value prior to the commencement of therapy. A Human CALP (calprotectin) ELISA kit was used on a Chemwell 2900 (Al-gen) analyzer to carry out the calprotectin measurement. The measurements were given in terms of ng/mL. The coefficient of variation (CV%) values for both the intraassay and the interassay range were determined to be 3.92 and 4.76, respectively. Every sample was evaluated twice, and the results that were obtained from both analyses were averaged for the statistical analysis.

STATISTICS EXAMINATION

The findings were summarised using the mean together with the standard deviation (S.D.). By using the Kolmogorov Smirnov normality test ($p=0.2$), we were able to demonstrate that the data followed a normal distribution. We used one-way analysis of variance to compare the groups' mean ages, and we used Kruskal-Wallis analysis to analyse the groups' gender distributions. Both of these analyses are described in more detail below. We conducted the Levente test to determine whether or not the variances were similar (the result was $p=0.012$).

The Welch analysis of variance was used to make a comparison between the calprotectin levels of the three groups. As a kind of post-hoc analysis, the Tamhane T2 was used for more sophisticated pair wise comparisons. A Student's t-test was carried out

in order to compare the differences between patients who had Samter's triad and those who did not have it with those that existed between smokers and non-smokers. The Spearman correlation test was used in order to investigate the degree to which there is a connection between the NOSE score (an ordinal variable) and calprotectin levels. We conducted the statistical analysis using the SPSS 25.0 programme for Windows (which was developed by SPSS Inc. in Chicago, Illinois), and we determined that a result was statistically significant if it had a P value that was lower than 0.05.

RESULTS

There were a total of 150 participants, with 80 men accounting for 53.33 percent of the group and 70 females accounting for 46.67 percent. The participants' ages varied from 20 all the way up to 70 years old. There was not a significant difference in terms of the mean age or gender distribution across the three different groups ($p = 0.11$ and $p = 0.21$, respectively). Table 1 presents the demographic characteristics of the research sample for your perusal. The group that received CRSwNP had a mean serum calprotectin value of 80.14±7.58 ng/ml, whereas the group that received CRSwoNP had a value of 72.58±5.85 ng/ml, and the control group had a value of 62.14±6.36 ng/ml. The results of the Welch analysis of variance showed that the average levels of calprotectin in each group were substantially different from one another ($p<0.001$) (Table 2). In the post-hoc comparison analyses, the results indicated that the mean calprotectin level of the CRSwNP group was considerably greater than that of the control group ($p<0.001$), but that it did not significantly vary from that of the CRSwoNP group ($p = 0.17$). In addition, the mean level of calprotectin in the CRSwoNP group was greater than the level in the control group; however, this difference did not reach the threshold of statistical significance ($p = 0.14$). (Table 2).

There were six patients in the CRSwNP group who were found to have Samter's triad. The patients who had Samter's triad had a mean calprotectin value of 89.85±6.37 ng/ml, whereas the other patients in the CRSwNP group had a mean calprotectin value of 77.88±7.19 ng/ml. This revealed that there was a statistically significant difference between these two groupings ($p = 0.02$) (Table 3). The mean levels of calprotectin in smokers and non-smokers did not vary significantly from one another ($p = 0.33$). (Table 4). The mean NOSE score was determined to be 76.11±3.96 in the CRSwNP group, 64.18±3.69 in the CRSwoNP group, and 33.48±5.01 in the control group, respectively. In addition to this, there was found to be a substantial positive association between the NOSE scores and the calprotectin levels of the subjects (<0.001) (Table 5)

Table 1: Demographic profile of patients

Variables	CRSw NP Group	CRSwoNP Group	Control Group	p
Age	44.11±5.36	47.19±6.74	41.25±7.15	0.21
Gender				
Female	20	24	26	
				0.11
Male	30	26	24	
Smoking				
Yes	20	15	18	0.25
No	30	35	32	
Samter’s Triad				
Yes	10	0	0	0.14
No	40	50	50	

Table 2: Comparison of the mean calprotectin levels between groups

	CRSwNP Group	CRSwoNP Group	Control	p
Calprotectin (ng/ml)	80.14±7.58	72.58±5.85	62.14±6.36	0.001

Table 3: Calprotectin levels in CRwNP patients with and without Samter’s triad

	Mean calprotectin levels (ng/ml) ± S.D.	P value
With Samter’s Triad	89.85±6.37	0.002
Without Samter’s Triad	77.88±7.19	

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

	Mean calprotectin levels (ng/ml) ± S.D.	P value
Smokers	81.58±5.55	0.33
Non-smokers	76.39±4.71	

Table 5: Comparison of the mean NOSE scores between groups

	CRSwNP Group	CRSwoNP Group	Control	p
NOSE score	76.11±3.96	64.18±3.69	33.48±5.01	0.001

DISCUSSION

In recent years, a rising number of research have been carried out to study the association between the serum calprotectin value and inflammatory upper respiratory tract illnesses. These investigations have been conducted both in humans and in animals. The authors of the research compared the blood calprotectin levels of those who had idiopathic sudden sensorineural hearing loss (ISSHL) with those of healthy patients, who participated in the study with a total of 44 participants. Patients diagnosed with ISSHL were found to have elevated amounts of calprotectin in their blood compared to healthy persons. The scientists also found that the serum calprotectin value of the patients who did not recover from the condition was considerably greater when compared to those who had either a partial or whole recovery from the illness. [5] In a separate piece of research, the serum calprotectin levels of patients with CRSwNP were analysed. The researchers found that due to inflammation, the calprotectin found within the cells was transferred to the extracellular matrix, leading to an increased concentration of the protein in the serum. [6] In the present research, we investigated the serum calprotectin value in patients with CRSwNP and CRSwoNP. Also, we compared the serum calprotectin values in CRSwNP patients who had Samter's triad to those who did not have the condition. We discovered that the serum calprotectin levels of CRSwNP patients were significantly higher than those of CRSwoNP patients and healthy individuals. In addition, we discovered that the serum calprotectin levels of CRSwNP patients with Samter's triad had significantly higher levels than those of CRSwNP patients who did not have this condition. In individuals who have Samter's triad, nasal polyp complaints tend to be more severe, which leads us to believe that this may be the result of the existence of a third illness. Specifically, asthma may be the condition that sets off the inflammation. Mast cell degranulation, which is responsible for high amounts of histamine in polyp fluids, has been demonstrated to contribute to the creation of nasal polyps by ultrastructural analysis. This finding supports the hypothesis that nasal polyps are caused by allergies. Both mucosal and connective tissue types may be found in mast cells. In addition, basophils that are circulating in the circulation may become mast cells after being transported to tissues and undergoing the necessary transformation. [7]. A previous study investigated the serum secreted protein acidic and cysteine-rich (SPARC) value in 26 patients with CRSwNP before and after endoscopic sinus surgery (ESS). The authors noted that it was not possible to use SPARC to predict the course of the disease after ESS. The postoperative serum SPARC value was decreased by 33%. [8]. In a different piece of research, the authors sought to find an answer to the question of whether the serum periostin value could be used to determine the nasal polyp load in CRSwNP patients. They discovered that the level of this protein was higher in CRSwNP patients when compared to the CRSwoNP group. In their study, the researchers came to the conclusion that the value of serum periostin might be employed as a novel molecular biomarker to detect the existence of nasal polyps. [9] In the present investigation, we studied whether or not serum calprotectin may be used as a biomarker to determine whether or not a patient with CRS had nasal polyps. In addition to this, we investigated the levels of serum calprotectin in relation to the presence or absence of Samter's triad. In the presence of Samter's triad, the serum calprotectin levels were found to be higher than in the CRSwNP patients who did not have Samter's triad. This was shown by the findings of our study.

Studies have shown that the NOSE scale delivers results that are congruent with the findings of computed tomography and physical examination in nasal surgery.[10] The NOSE scale and septum deviation data on paranasal tomography were compared in a research that comprised 27 individuals, and the results showed that they were consistent with one another. [10] In a separate research that followed a similar format, the accuracy of the NOSE score in determining whether or not a patient had nasal obstruction was evaluated using 345 participants who had had a retrospective screening. As a consequence of this, the NOSE has been shown to be a trustworthy tool for the categorization of nasal congestion symptoms. [11] In light of these research, we came to the conclusion that the NOSE scale would be the best tool to use in order to standardise the patients' complaints of nasal congestion. We observed that there was a connection between the scores on the NOSE scale and the serum calprotectin levels. Complaints of nasal obstruction were shown to be more common in patients diagnosed with CRS. This was notably the case in individuals who presented with nasal polyps, which, in turn, led to an increase in serum calprotectin levels.

Compared to the levels found in the control group, the CRSwNP patients' serum calprotectin levels were noticeably greater than those seen in the other participants. At the same time, within the CRSwNP group, the patients who had Samter's triad had a greater serum calprotectin value compared to the other patients in the group. In light of these data, it is possible to make the assertion that the serum calprotectin value rises together with the progression of CRS severity. In addition, the level of serum calprotectin rises in tandem with an increase in the score on the NOSE scale. This means that the level of

serum calprotectin is greater in individuals who report experiencing symptoms of nasal congestion.

CONCLUSION

In conclusion, we found that instances of CRS were associated with an increase in the value of serum calprotectin, and there was a correlation between the value of serum calprotectin and the severity of CRS symptoms. In order to analyse the preoperative and postoperative serum calprotectin levels in patients having endoscopic sinus surgery, we suggest that another research with a bigger case series be conducted along the same lines as the one we have just described.

REFERENCES

1. Kato T, Kouzaki H, Matsumoto K, Hosoi J, Shimizu T (2017) The effect of calprotectin on TSLP and IL-25 production from airway epithelial cells. Allergol Int 66: 281–289.
2. Loser K, Vogl T, Voskort M, Lueken A, Kupas A, Nacken W, et al (2010) The Toll-like receptor 4 ligands Mrp8 and Mrp14 are cru- cial in the development of autoreactive CD8+ T cells. Nat Med 16: 713–717.
3. Yui S, Nakatani Y, Mikami M (2003) Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull 26: 753–760.
4. Kim JE, Kountakis SE (2007) The prevalence of samter's triad in pa- tients undergoing functional endoscopic sinus surgery. Ear, Nose Throat J 86: 396–399.
5. Kuzucu I, Candar T, Baklaci D, Guler I, Kum RO, Arslan H, et al (2019) A prognostic marker in idıopathic sudden sensorineural hear- ing loss: serum calprotectin. Clin Exp Otorhinolaryngol 13: 36–40.
6. Van Crombruggen K, Vogl T, Pérez-Novo C, Holtappels G, Bachert C (2016) Differential release and deposition of S100A8/A9 proteins in inflamed upper airway tissue. Eur Respir J 47: 264–274
7. Bachert C, Zhang N, Holtappels G, De Lobel L, van Cauwenberge P, Liu S, Lin P, Bousquet J, Van Steen K (2010) Presence of IL-5 protein and IgE antibodies to staphylococcal enterotoxins in nasal polyps is associated with comorbid asthma. J Allergy Clin Immunol 126: 962–968.
8. Asmar MH, Gaudreau A, Maniakas A, Mfuna Endam L, Desrosiers M (2019) An evaluation of SPARC protein as a serum biomarker of chronic rhinosinusitis. Otolaryngol Head Neck Surg 160: 158–164
9. Maxfield AZ, Landegger LD, Brook CD, Lehmann AE, Campbell AP, Bergmark RW, Stankovic KM, Metson R (2018) Periostin as a bio- marker for nasal polyps in chronic rhinosinusitis. Otolaryngol Head Neck Surg 158: 181–186
10. Rhee JS, Book DT, Burzynski M, Smith TL (2003) Quality of life as- sessment in nasal airway obstruction. Laryngoscope 113: 1118–1122
11. Lipan MJ, Most SP (2013) Development of a severity classification sys- tem for subjective nasal obstruction. JAMA 15: 358–361