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Research Article

# ROLE OF HMGB1 IN INFLAMMATION AND EOSINOPHIL INFILTRATION IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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**ABSTRACT**

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a complex inflammatory condition that involves an exaggerated immune response, often characterized by eosinophilic infiltration. This study aimed to explore the role of High Mobility Group Box 1 (HMGB1), a nuclear protein implicated in inflammation, in the pathogenesis of CRSwNP. Tissue samples from 50 patients with CRSwNP, along with healthy controls, were analyzed for HMGB1 expression using immunohistochemistry. The findings revealed distinct HMGB1 expression patterns, with significant differences between eosinophilic and non-eosinophilic CRSwNP patients. Notably, HMGB1 expression was elevated in the inflammatory cells, particularly in non-eosinophilic cases, suggesting its key role in the inflammatory cascade. The study underscores the potential of HMGB1 as a target for future therapeutic strategies in managing CRSwNP. Moreover, the results highlight the complex interplay between immune cells, inflammatory mediators, and the epithelial barrier in chronic sinusitis.

**Key words:-** Chronic Rhinosinusitis,Nasal Polyps,HMGB1,Eosinophils,Inflammation.

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## INTRODUCTION

Chronic rhinosinusitis can result from various conditions, including nasal polyps, and may be associated with viral, bacterial, or fungal infections. It can also be linked to cystic fibrosis and primary ciliary dyskinesia [1]. Factors such as allergies, medication intolerances, gastroesophageal reflux disease (GERD), pollution, and adverse drug reactions can trigger a dysregulated immune response. These conditions often present with subepithelial swelling, epithelial damage, and infiltration by inflammatory cells, predominantly eosinophils, along with mast cells, macrophages, and neutrophils [2].

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Inflammation is a natural physiological defense mechanism that occurs in response to infection or injury, mediated by the release of cytokines and other inflammatory mediators. However, an exaggerated inflammatory response can result from the recruitment and retention of inflammatory cells. High mobility group box 1 (HMGB1), a protein involved in several inflammatory diseases, plays a key role in this process [3]. HMGB1 interacts with specific receptors on damaged tissues and immune cells, leading to the release of further inflammatory mediators. Eosinophils, in particular, are protected by these mechanisms, as they can activate the endothelium and survive longer in the inflamed environment [4]. HMGB1 functions as an "alarmin," present in the granules, cytoplasm, and nucleus of leukocytes and epithelial cells. It is actively

released by macrophages and monocytes during inflammation, particularly when pro-inflammatory products such as endotoxins are present. Necrotic or damaged cells, including epithelial cells, can also release HMGB1 passively. Once released, HMGB1 activates endothelial cells, contributing to the inflammatory cascade [5]. Recent studies have shown that nasal polyps (NP) express higher levels of NF-kB than normal nasal mucosa. NF-kB plays a crucial role in the transcription of cytokines, chemokines, and adhesion molecules, which can have both pathogenic and therapeutic implications. In addition to its role in NP, NF-kB is involved in the effects of corticosteroids on inflammation. Chronic inflammation in the nasal and sinus mucosa, driven by HMGB1, TNF, and IL, can contribute to the persistence of allergic and non-allergic nasal mucosal inflammation.

## MATERIALS AND METHODS

This collaborative study, involving multiple countries and universities, focused on analyzing a nuclear protein implicated in the pathogenesis of inflammation. The initial phase examined a common ENT pathology, with ongoing research on inflammatory processes in the ENT district. In this study, 50 nasal polyp tissue specimens were collected from patients with chronic rhinosinusitis with nasal polyps (CRSwNP) at Narayana Medical College and Hospitals, Nellore, Andhra Pradesh, India, from 2019 to 2020. The study included healthy controls, as well as patients with asthma and allergic rhinitis. Diagnoses were confirmed through medical history, nasal endoscopy, and computed tomography (CT) scans, based on the European Position Paper on rhinosinusitis and nasal polyps (EPOS) 3 criteria. Atopic status was assessed using a skin prick test for aeroallergens. Symptoms were evaluated on a 0-10 scale, and the Lund-Mackay classification was applied to CT scans. Specific medical conditions were used as exclusion criteria. Pre-surgery biopsy specimens were graded according to the Lund-Kennedy system and subjected to immunohistochemistry for HMGB1, IL-5, and IL-16. Tissue freezing and subsequent analysis provided insights into the clinical characteristics of patients and controls [6]. Hematoxylin staining revealed 16 controls, 20 patients with eosinophilia, and 22 patients without eosinophilia with CRSwNP. The degree of eosinophil infiltration was measured in 20 high-power fields (HP) at ×400 magnification. An eosinophil count of more than 20 per HP field defined a patient as Eos CRSwNP. Results for eosinophilic and non-eosinophilic CRSwNP cases were reported in Table II.

## IMMUNOHISTOCHEMICAL STAINING

Biopsy samples were sectioned using a cryostat at a thickness of 7 µm after fixation and dehydration. Following air drying for 10 minutes, the sections were permeabilized in PBS and mounted on chromogelatin-coated slides. Endogenous peroxidases were blocked by applying 3% H2O2 to the sections for 10 minutes at room temperature in the dark. The slides were then rinsed three times in PBS for 2 minutes each, followed by a 20-minute incubation in PBS containing 10% normal horse serum for blocking. Primary antibody incubation was carried out overnight at 4°C with rabbit polyclonal antibodies (ab-18256, 1:300 dilution), mouse monoclonal antibodies (ab25034, 1:200 dilution), mouse monoclonal antibodies (ab1793, 1:200 dilution), and goat polyclonal antibodies (ab10769, 1:200 dilution). After a five-minute rinse in PBS, the slides were washed three more times in PBS [7]. Alternatively, secondary antibodies were prepared using PV-9003 or PV-9000 kits, and diaminobenzidine development was carried out for two minutes using ZSJQ kits. Mayer's haematoxylin was used for counterstaining, and neutral resin was applied to mount the slides. Normal rabbit serum served as the positive control, while PBS was used as the negative control. All statistical analyses were performed using SPSS 13.0 software. Where applicable, repeated measures ANOVA with LSD post-hoc multiple comparisons was used. Mann-Whitney U tests were applied to compare two groups when differences were detected. Spearman's rank correlation and Pearson correlation were used to assess associations. A multivariate regression analysis was conducted to evaluate whether HMGB1 protein expression correlates with eosinophils, IL-5, IL-8, and TNF-α positive cells. Statistical significance was defined as a p-value less than 0.05.

## RESULT

Immunohistochemical staining of nasal polyps from chronic rhinosinusitis (CRS) patients with eosinophilia, CRS patients without eosinophilia, and CRS-naive patients revealed distinct patterns of HMGB1 expression. Both CRSwNP patients and controls exhibited immunohistochemical recognition of HMGB1. The staining intensity was categorized into four areas based on HMGB1 protein expression: epithelial cytoplasm, epithelial nucleus, focal extracellular infiltration, and the overall inflammatory response. Notably, CRSwNP patients with non-eosinophilic CRS showed higher levels of HMGB1 expression compared to controls. In contrast, HMGB1 staining in the epithelial cytoplasm was significantly reduced in both eosinophilic and non-eosinophilic CRSwNP patients. Subepithelial HMGB1 protein infiltration was lower in eosinophilic CRSwNP cases. However, there was an overall increase in HMGB1 expression in inflammatory cells, both in eosinophilic and non-eosinophilic CRSwNP patients.

**Table 1: Clinical Characteristics of Study Participants.**

| Characteristic | Control Subjects (n=16) | CRSw (n=20) | NP (n=22) | p value |
|---|---|---|---|---|
| **No. of subjects** | 16 | 20 | 22 | – |
| **Sex (male/female)** | 5/11 | 10/10 | 12/10 | NS |
| **Mean age (y), median range** | 37 (22-70) | 42 (30-54) | 38 (17-56) | NS |
| **Nasal congestion** | 2 | 18 | 22 | <0.001 |
| **Rhinorrhoea** | 0 | 18 | 18 | 0.001 |
| **Headache** | 2 | 12 | 6 | NS |
| **Facial pain/pressure** | 0 | 2 | 2 | NS |
| **Hyposmia** | 0 | 16 | 12 | 0.014 |
| **No. of bilateral polyps** | 0 | 20 | 8 | NS |
| **Recurrent history** | 0 | 4 | 6 | NS |
| **Atopy** | Y: 0, N: 16, U: 0 | Y: 8, N: 12, U: 0 | Y: 8, N: 10, U: 4 | NS |
| **Asthma** | 0 | 16 | 2 | 0.001 |
| **Increased eosinophils** | 0 | 16 | 6 | 0.005 |
| **Increased lymphocytes** | 0 | 4 | 2 | NS |

**Table 2: Comparison of Symptoms Between Individuals with Eosinophilic (Eos) CRSwNP and Non-Eosinophilic (Non-Eos) CRSwNP Based on CT Scans and Nasal Endoscopic Examinations.**

| Characteristic | Eos CRSwNP (n=16) | Non-Eos CRSwNP (n=34) | p value |
|---|---|---|---|
| **MS** | 3.30 ± 0.60 | 3.00 ± 0.68 | NS |
| **AES** | 4.10 ± 1.03 | 1.00 ± 1.61 | NS |
| **PES** | 3.50 ± 0.87 | 2.36 ± 1.29 | 0.041 |
| **SS** | 3.30 ± 2.03 | 0.61 ± 1.04 | 0.020 |
| **FS** | 3.50 ± 2.17 | 2.54 ± 1.43 | NS |
| **OMCS** | 4.50 ± 0.60 | 3.55 ± 3.24 | NS |
| **ES** | 6.70 ± 2.75 | 4.37 ± 1.75 | 0.041 |
| **TS** | 26.50 ± 5.17 | 20.45 ± 6.84 | 0.010 |
| **Endoscopy** | 4.50 ± 2.26 | 4.55 ± 1.56 | NS |
| **Nasal Congestion** | 8.5 ± 1.67 | 4.56 ± 1.58 | NS |
| **Rhinorrhoea** | 6.70 ± 1.53 | 6.81 ± 2.14 | NS |
| **Headache** | 4.50 ± 1.80 | 2.36 ± 2.21 | 0.041 |
| **Hyposmia** | 6.00 ± 3.16 | 4.35 ± 5.36 | 0.010 |

## DISCUSSION

Chronic rhinosinusitis (CRS) is clinically defined as inflammation of the nasal mucosa, often associated with nasal polyps. A recently proposed hypothesis suggests that CRS may result from a defect or an exaggerated immune response to foreign agents, leading to a persistent influx of inflammatory cells. Interestingly, the incidence of CRS does not appear to correlate with nasal anatomical variations, as inflammation is primarily observed at the interface with the external environment [8]. While CRS was once believed to be a multifactorial disease due to limited understanding of its etiology and pathogenic mechanisms, it is now understood that CRS, with or without nasal polyps, is less influenced by a single microbial or environmental factor and more by host susceptibility [9,10]. The sinonasal mucosa, which includes ciliated cells, goblet cells, and respiratory epithelium, forms a physical barrier to protect against harmful agents. Goblet cells play a crucial role in trapping microbes and foreign substances, while the epithelial cells are responsible for adaptive immune responses. When foreign proteins trigger the immune system, the epithelial barrier can be compromised, facilitating microbial colonization. Pattern recognition receptors (PRRs) on airway epithelial cells are crucial in recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), helping the body detect cellular damage and initiate an immune response [11]. One well-established DAMP, HMGB1, has been shown to drive inflammation in the lower airways. Studies indicate that patients with CRS and nasal polyps (CRSwNP) exhibit elevated HMGB1 protein levels in their epithelial nuclei and subepithelial regions, with lower levels in the epithelial cytoplasm [13]. This suggests that HMGB1 plays a role in the pathogenesis of CRSwNP, regardless of whether the condition is eosinophilic or not. Furthermore, inflammatory mediators such as IL-5, IL-8, and TNF-α, which are expressed by immune cells, can influence the regulation of HMGB1. Consequently, future research into HMGB1 may provide valuable insights into the underlying mechanisms of CRSwNP and help identify potential therapeutic targets for managing this condition.

## CONCLUSION

This study investigated the role of the nuclear protein HMGB1 in chronic rhinosinusitis with nasal polyps (CRSwNP), offering important insights into the inflammatory mechanisms underlying ENT disorders. The research revealed distinct patterns of HMGB1 expression across various cellular components in CRSwNP patients compared to controls, particularly regarding eosinophil infiltration. By integrating clinical assessments, diagnostic criteria, and immunohistochemical analysis, the study enhanced our understanding of CRSwNP pathogenesis. The involvement of eosinophils in the nasal and paranasal sinus mucosa, where HMGB1 plays a pivotal role, was particularly highlighted. In addition to establishing a link between HMGB1 and CRSwNP, this study lays the foundation for future investigations. The ongoing research, driven by international collaboration, aims to deepen our understanding of ENT diseases and identify potential therapeutic targets. By continuing to explore the inflammatory processes within the ENT region, we hope to generate more refined insights that could lead to improved diagnostic and treatment strategies for CRSwNP. The authors remain committed to discussing and analyzing the results to ensure a comprehensive and well-rounded interpretation of the findings, contributing to the advancement of ENT research.

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