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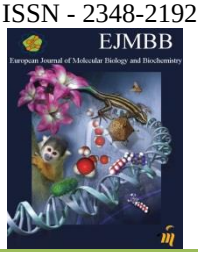

EXPLORING HMGB1 PROTEIN EXPRESSION IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS: A COMPREHENSIVE IMMUNOHISTOCHEMICAL STUDY

Dr. Naveen Vasireddy*

Assistant Professor, Department of ENT, Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India.

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ABSTRACT

Background: Chronic rhinosinusitis with nasal polyps (CRSwNP) is a multifactorial inflammatory condition of the nasal and sinus mucosa, often associated with persistent immune responses. High-mobility group box 1 (HMGB1), a nuclear protein implicated in inflammation and immune modulation, has been proposed as a key player in CRSwNP pathogenesis. This study aimed to investigate the role of HMGB1 in CRSwNP and its association with eosinophilic and non-eosinophilic inflammation. Methods: A collaborative study was conducted at Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India, between 2018 and 2019. Forty-two nasal polyp tissue samples were collected from CRSwNP patients and compared with healthy controls. Patient diagnosis was confirmed using medical history, nasal endoscopy, computed tomography (CT) scans, and atopic status assessments. Immunohistochemical analysis was performed to evaluate HMGB1 expression alongside inflammatory markers, including IL-5, IL-8, and TNF-α. Eosinophilic infiltration was quantified using hematoxylin staining, and statistical analyses were conducted to determine correlations between inflammatory markers and clinical features. Results: HMGB1 was detected in epithelial nuclei, cytoplasm, and subepithelial regions of nasal polyp tissues. CRSwNP patients exhibited significantly higher HMGB1 expression than controls, particularly in inflammatory cells. Eosinophilic CRSwNP cases demonstrated lower HMGB1 expression in the epithelial cytoplasm and higher levels in inflammatory infiltrates compared to non-eosinophilic CRSwNP. Significant correlations were observed between HMGB1 and key inflammatory mediators, including IL-5 and TNF-α, suggesting its role in immune regulation and chronic inflammation. Conclusion: This study highlights the involvement of HMGB1 in CRSwNP pathogenesis and its potential as a biomarker for inflammatory processes in nasal polyposis. The differential expression of HMGB1 in eosinophilic and non-eosinophilic CRSwNP suggests its role in immune cell recruitment and mucosal inflammation. These findings provide new insights into CRSwNP pathophysiology and may inform future therapeutic strategies targeting HMGB1-mediated inflammation in ENT disorders. Further research is warranted to explore its potential as a therapeutic target in chronic rhinosinusitis.

INTRODUCTION

Chronic rhinosinusitis (CRS) can develop due to various underlying conditions, including the presence of nasal polyps. Rhinosinusitis, whether caused by viral, bacterial, or fungal infections, may emerge as a complication of disorders such as cystic fibrosis or primary ciliary dyskinesia [1]. Factors such as allergies, medication sensitivities, gastroesophageal reflux disease (GERD), environmental pollutants, and adverse drug reactions can trigger an exaggerated immune response. This response is characterised by subepithelial swelling,

Corresponding Author
Dr. Naveen Vasireddy

epithelial disruption, and infiltration of inflammatory cells, including eosinophils, mast cells, macrophages, and neutrophils, with eosinophils being predominant [2].

The activation and release of cytokines and mediators by inflammatory cells represent a fundamental biological response to infections and tissue damage, underscoring inflammation as a crucial protective mechanism. However, an excessive inflammatory reaction results from the prolonged recruitment and accumulation of immune cells. The high-mobility group box 1 (HMGB1) protein has been implicated in several inflammatory disorders [3]. Certain membrane receptors contribute to the activation of necrotic, injured, or immunologically stimulated tissues, leading to the secretion of inflammatory mediators. These processes enhance eosinophil protection by fostering endothelial activation and prolonging their lifespan [4].

As a conserved molecular signal in leukocytes and epithelial cells, HMGB1 serves as an "alarmin," residing within granules, the cytoplasm, and the nucleus. It is recognised as a late-stage inflammatory mediator actively secreted by macrophages and monocytes into the extracellular space. Additionally, external inflammatory stimuli such as endotoxins can prompt macrophages and monocytes to release HMGB1. Furthermore, damaged or necrotic epithelial cells can passively release HMGB1, leading to the widespread activation of endothelial cells [5].

Recent research has highlighted that nasal polyp (NP) tissue demonstrates elevated nuclear factor-kappa B (NF-kB) expression compared to non-polyp nasal mucosa. NF-kB plays a pivotal role in regulating the transcription of cytokines, chemokines, and adhesion molecules, significantly influencing disease progression and therapeutic strategies. Beyond its role in NP pathophysiology, transcription factors are also essential in modulating corticosteroid activity. HMGB1 is believed to contribute to both allergic and non-allergic forms of nasal mucosal inflammation. The persistence of sinus and nasal cavity inflammation, driven by chronic exposure to HMGB1, tumour necrosis factor (TNF), and interleukins (ILs), can establish a prolonged inflammatory condition.

MATERIALS AND METHODS

This collaborative study, conducted across multiple institutions and countries, explored the role of a nuclear protein implicated in inflammatory pathogenesis. The research initially focused on a common otolaryngological condition, with ongoing investigations into inflammatory processes affecting the ENT region. Between 2018 and 2019, 42 nasal polyp tissue samples were obtained from patients diagnosed with chronic rhinosinusitis with nasal polyps (CRSwNP) at Sri Lakshmi Narayana Institute of Medical Sciences, Puducherry, India. The study cohort included healthy controls, individuals with asthma, and patients with allergic rhinitis. Diagnosis was established based on a comprehensive evaluation involving medical history, nasal endoscopy, and computed tomography (CT) scans, following the guidelines specified in the European Position Paper on Rhinosinusitis and Nasal Polyps 3. Atopic status was determined through skin prick testing for aeroallergens. Symptom severity was graded on a 0–10 scale, while CT scan findings were classified using the Lund-Mackay system. Patients with conditions that could interfere with study outcomes were excluded. Preoperative biopsy specimens were assessed using the Lund-Kennedy scoring system and subsequently underwent immunohistochemical analysis to detect HMGB1, IL-5, and IL-16 expression. Additionally, frozen tissue samples were examined to provide a comprehensive evaluation of the clinical characteristics of both patients and controls [6].

Hematoxylin staining facilitated the identification of 16 control subjects, 20 CRSwNP patients with eosinophilia, and 22 CRSwNP patients without eosinophilia. The extent of eosinophilic infiltration was determined in 20 high-power (HP) fields under x400 magnification. Patients exhibiting eosinophil counts exceeding 20 per HP field were classified as Eos CRSwNP. Comparative findings between eosinophilic and non-eosinophilic CRSwNP groups are summarised in Table II.

Immunohistochemical staining

A cryostat was used to section biopsy samples (7m) after fixation and dehydration. After air drying for 10 minutes, sections were permeabilized in PBS and placed on chromegelatin-coated slides. Endogenous peroxidases were blocked by applying 3% H2O2 to the skin for 10 minutes at room temperature in the dark. To prepare the slides for blocking, the slides were rinsed in PBS three times for 2 minutes each before being blocked for 20 minutes in PBS containing 10% normal horse serum. Incubation was carried out overnight at 4° with rabbit polyclonal antibodies (ab-18256, dilution 1:300), mouse monoclonal antibodies (ab25034, dilution 1:200), mouse monoclonal antibodies (ab1793, dilution 1:200) and goat polyclonal antibodies (ab10769, dilution 1:200). A five-minute rinse in PBS was followed by three rinses in PBS [7]. As an alternative, secondary antibodies were prepared using PV-9003 or PV-9000 kits. ZSJQ kits provided two minutes of diaminobenzidine development. Mayer's haematoxylin was used to counterstain the slides and neutral resin was used to mount them. Using normal rabbit serum as the positive control, PBS was used as the negative control.

SPSS13.0 software was used to perform all statistical analyses. Except where otherwise noted, all using a repeated measures ANOVA with LDS posthoc multiple comparisons. In cases where there were differences between two groups, Mann-Whitney U tests

were applied. Spearman rank correlation and Pearson correlation are both used to evaluate correlations. An analysis of multivariate regression was conducted to determine whether the expression of HMGB1 protein correlates with eosinophils, IL-5, IL-8, and TNF-□ positive cells. The significance of a p value in statistics was defined as less than 0.05.

Table 1: Describe the clinical characteristics of the study participants

	Control subjects			CRSw			NP	p value	
No. of subjects	16			20			22	–	
Sex (male/female)	5/3			8/2			8/3	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	N	U	Y	N	U	Y N	U	NS
	0	8	8	4	10	6	0 10	12	
Asthma	0	16	0	2	18	0	0 22	0	
Increased eosinophils*		0			16		6		0.005
Increased lymphocytes*		0			4		2		NS

Table 2: By comparing CT scans and nasal endoscopic examinations, differences in symptoms between individuals with Eos CRSwNP and those without Eos CRSwNP were determined.

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	NS0
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

RESULTS

A nasal polyp from chronic rhinosinusitis eosinophilic patients, a chronic rhinosinusitis unaffected patient, and a nasal polyp from chronic rhinosinusitis naive patients, The immunohistochemistry staining of sections from patients with eosinophil chronic rhinosinusitis and nasal polyps, and patients without, CRSwNP patients and controls both showed immunohistochemical recognition of HMGB1. The staining was divided into four sections based on HMGB1 protein expression level: epithelial cytoplasm, epithelial nucleus, focal extracellular infiltration, and inflammation. CRSwNP patients with non-eosinophilic CRS expressed HMGB1 at higher levels than controls. The epithelial cytoplasm staining of HMGB1 was significantly reduced in patients with CRSwNP who were eosinophilic and non-eosinophilic. Subepithelial HMGB1 protein infiltration is lower in chronic rhinosinusitis with nasal polyps caused by eosinophils. There was an increase in HMGB1 expression in inflammatory cells associated with eosinophilic chronic rhinosinusitis with nasal polyps as well as non-eosinophilic chronic rhinosinusitis with nasal polyps.

DISCUSSION

It is clinically known as chronic rhinosinusitis if there is inflammation of the nasal mucosa associated with nasal polyps. According to a recently proposed hypothesis, CRS is caused by a defect or excessive immune response to foreign agents, which results in persistent influxes of inflammatory cells, since nasal anatomical variations do

not seem to correlate with CRS incidence [8]. Since inflammation is primarily observed at the interface with the external environment, the incidence of CRS has not been correlated with nasal anatomical variations. It was widely believed that CRSwNP was a multifactorial disease in the past few decades [9], due to a lack of understanding of its aetiology and pathogenic mechanisms. CRS with or without nasal polyposis appears to be less determined by one microbial factor or environmental factor than by host susceptibility [10]. Cells with cilia, goblet cells, and respiratory epithelium form tight junctions to protect the sinus mucosa. Microbes and foreign substances are protected by mucociliary goblet cells. As a result of foreign proteins stimulating the immune system, the epithelial barrier is mechanically broken down, stimulating microbial colonization. Sinonasal epithelial cells 15 are responsible for adaptive immune responses and the physical barrier. Pattern recognition receptors (PRRs) on airway epithelial cells recognize molecular patterns associated with pathogens [11]. Epithelial cells attract innate defenses by releasing chemokines, cytokines, and innate protective agents. DAMPs, as well as PAMPs, can also help cells detect cellular damage [12]. A well-established DAMP, HMGB1, causes inflammation in the lower airways. Patients with CRSwNP have greater HMGB1 protein levels in their epithelial nuclei, or in their subepithelial areas and have smaller HMGB1 proteins in their epithelial cytoplasm [13]. Chronic rhinosinusitis associated with nasal polyps containing Eos did not differ significantly in HMGB1 expression, indicating that the protein plays a role in the pathogenesis of chronic rhinosinusitis regardless of the underlying cause. Positive cells expressing IL-5, IL-8, or TNF-5 can affect the regulation of HMGB1. CRSwNP pathogenesis should be investigated through research on HMGB1.

CONCLUSIONS

This study explored the nuclear protein HMGB1 in chronic rhinosinusitis with nasal polyps (CRSwNP) and provided valuable insight into inflammatory processes within the ENT field. HMGB1 expression was found to differ in various cellular components between CRSwNP patients and controls, particularly in eosinophil infiltration. Clinical assessments, diagnostic criteria, and immunohistochemical analysis were combined in the study to understand CRSwNP pathogenesis. Eosinophilic involvement is particularly important in nasal and paranasal sinus mucosa, where HMGB1 plays a crucial role. In addition to shedlighting the link between HMGB1 and CRSwNP, the study also paves the way for future studies. We are deepening our understanding of ENT pathologies and exploring potential therapeutic targets through collaboration among several countries and universities. Continuing this research and exploring inflammatory processes in the ENT district will provide more nuanced insights that could ultimately result in better diagnosis and treatment strategies for chronic rhinosinusitis with nasal polyps. In order to advance ENT research, the authors continually discuss the results to ensure a robust and comprehensive interpretation.

REFERENCES

1. Passali D, Berstein JM, Passali FM. (2003). Treatment of recur- rent chronic hyperplastic sinusitis with nasal polyposis. *Arch Otolaryngol Head Neck Surg* 129, 656-9.
2. Passali D, Bellussi L. (2007). Revision of the European Position Pa- per on Rhinosinusitis and nasal Polyposis (EP3OS) with par- ticular attention to acute and recurrent rhinosinusitis. *Acta Otorhinolaryngol Ital* 27, 1-21.
3. Fokkens WJ, Lund VJ, Mullol J. (2012). European Position Paper on Rhinosinusitis and Nasal Polyposis group. Euro- pean Position Paper on rhinosinusitis and nasal polyposis. *Rhinology* 23, 1-299.
4. Scaffidi P, Misteli B, Bianchi ME. (2002). Release of chromatin pro- tein HMGB1 by necrotic cells triggers inflammation. *Nature* 418, 191-5.
5. Gardella S, Andrei C, Ferrera D. (2002). The nuclear protein HMGB1 is secreted by monocytes via a non-classical, vescicle- mediated secretory pathway. *EMBORep* 3, 995-1001.
6. Bianchi ME, Manfredi AA. (2007). High-mobility group box 1 (HM- GB1) protein at the crossroads between innate and adaptive immunity. *Immunol Rev* 220, 35-46.
7. Yang D, dela Rosa G, Tewary P. (2009). Alarmins link neu- trophils and dendritic cells. *Trends Immunol* 30, 531-7.
8. Lotze MT, Tracey KJ. (2005). High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 5, 331-42.
9. Valera FCP, Queirozw R, Scrideliw C. (2008). Expression of transcription factors NF-kB and AP-1 in nasal polyposis. *Clin Exp Allergy* 38, 579-85.
10. Ek M, Popovic K, Harris HE. (2006). Increased extracellular levels of the novel proinflammatory cytokine High Mobil- ity Group Box Chromosomal protein 1 in minor salivary glands of patients with Sjogren's syndrome. *Arthr Rheum* 54, 2289-94.
11. Passali D, Kern E, Lei Chen R. (2012). High mobility group box 1 (HMGB 1): a new protein in the pathogenesis of ENT inflammatory and infectious diseases. *Acta Otorhinolaryngol Ital* 32, 46-7.
12. Kern RC, Conley DB, Walsh W. (2008). Perspectives on the eti- ology of chronic rhinosinusitis: an immune barrier hypoth- esis. *Am J Rhinology* 22, 549-59.

13. Schleimer RP, Lane AP, Kim J. (2007). Innate and acquired immuni- ty and epithelial cell function in chronic rhinosinusitis. *Clin Allergy Immunol* 20, 51-78.