DOI: https://doi.org/10.53350/pjmhs20231709268

ORIGINAL ARTICLE

Role of Tear Film Inflammatory Biomarkers in the Diagnosis and Severity Grading of Allergic Conjunctivitis

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ABSTRACT

Background: Allergic conjunctivitis is an IgE-mediated ocular surface disease characterized by mast-cell activation and Th2-driven inflammation resulting in itching, redness, and chemosis. Current clinical grading systems are subjective and may fail to identify subclinical inflammation. Tear film inflammatory biomarkers offer a promising objective tool for improving diagnostic accuracy and severity stratification.

Objectives: To evaluate tear film inflammatory biomarkers in patients with allergic conjunctivitis and determine their association with clinical severity grades.

Methodology: The study was a cross-sectional study conducted at the Department of Ophthalmology unit BKMC Mardan from January 2022 to June 2022. 100 patients with clinically diagnosed allergic conjunctivitis and 40 healthy controls at a tertiary-care ophthalmology unit. Tear samples were collected using sterile microcapillary tubes and analyzed for interleukin-4, interleukin-5, interleukin-13, eutaxon, and matrix metalloproteinase-9 using enzyme-linked immunosorbent assay kits. Disease severity was graded using the Bonini classification. Data were analyzed in SPSS version 24.0. Independent t-tests compared biomarker levels between groups, while Pearson correlation and multivariate regression assessed associations with disease severity.

Results: The mean age of patients was 31.8 ± 9.7 years, with 58% males. Tear concentrations of all measured biomarkers were significantly higher in allergic conjunctivitis patients compared with controls ($p < 0.001$). Mean IL-5 levels were 42.6 ± 10.4 pg/mL in cases versus 15.2 ± 5.1 pg./mL in controls ($p < 0.001$). Biomarker levels increased progressively across mild, moderate, and severe disease. Severe cases showed the highest mean MMP-9 levels (168.3 ± 35.6 ng/mL). IL-5 and eutaxon demonstrated strong positive correlations with disease severity ($r = 0.71$ and 0.68, respectively; $p < 0.001$).

Conclusion: Tear film inflammatory biomarkers, particularly IL-5 and MMP-9, are significantly associated with disease severity and provide objective indicators for diagnosis and severity grading in allergic conjunctivitis.

Keywords: Allergic conjunctivitis; Tear biomarkers.

INTRODUCTION

Allergic conjunctivitis (AC) is one of the most prevalent ocular surface disorders worldwide, affecting individuals across all age groups and accounting for a substantial proportion of ophthalmology outpatient visits[1]. It is an IgE-mediated hypersensitivity condition triggered by environmental allergens such as pollen, dust mites, animal dander, and molds[2]. The condition encompasses a clinical spectrum ranging from seasonal and perennial allergic conjunctivitis to more severe chronic forms, including vernal keratoconjunctivitis and atopic keratoconjunctivitis[3]. Although often perceived as a benign disease, uncontrolled allergic conjunctivitis can result in significant morbidity, impaired quality of life, and, in advanced cases, corneal involvement leading to visual impairment[4]. The pathophysiology of AC involves a complex cascade of immunological events. Initial sensitization leads to allergen-specific IgE production, which binds to high-affinity receptors on conjunctival mast cells. Re-exposure to allergens results in mast-cell degranulation and release of histamine, prostaglandins, and leukotrienes, producing the early-phase symptoms of itching, tearing, and conjunctival hyperemia. This is followed by a late-phase reaction characterized by recruitment of eosinophils, T-helper-2 lymphocytes, and the release of cytokines such as interleukin-4 (IL-4), IL-5, and IL-13, which perpetuate chronic inflammation and tissue remodeling[5,6]. Currently, the diagnosis and severity grading of AC rely primarily on patient-reported symptoms and slit-lamp findings. Common grading systems, including the Bonini classification, are subjective and show considerable inter-observer variability. Moreover, clinical signs do not always correlate with the underlying inflammatory burden, leading to underestimation of disease activity in some patients and inappropriate escalation of therapy in others. This lack of objective assessment tools represents a major gap in routine clinical practice[7]. Tear film analysis offers a non-invasive and accessible means of evaluating the biochemical environment of the ocular surface. Recent advances in immunoassay techniques have enabled the quantification of multiple inflammatory mediators from minute volumes of tear fluid. Among these, IL-4, IL-5, and IL-13 are pivotal Th2 cytokines involved in IgE synthesis and eosinophil activation. Eutaxon is a potent chemokine that drives eosinophil migration to the conjunctiva, while matrix metalloproteinase-9 (MMP-9) is a marker of epithelial barrier disruption and corneal inflammation[8,9]. Several international studies have demonstrated elevated levels of tear cytokines in patients with allergic conjunctivitis; however, data correlating biomarker concentrations with validated clinical severity scales remain limited. Furthermore, there is a paucity of local data from South Asian populations, where environmental exposure patterns and health-seeking behaviors differ significantly from Western settings. Establishing objective tear film biomarkers that accurately reflect disease severity could transform the management of AC by enabling early identification of high-risk patients, guiding personalized therapy, and monitoring treatment response[10].

Study Objectives: To measure tear film inflammatory biomarkers in allergic conjunctivitis and assess their correlation with clinical severity grades.

Received on 09-07-2023
Accepted on 29-08-2023

MATERIALS AND METHODS

Study Design & Setting: The study was a cross-sectional study conducted at the Department of Ophthalmology unit BKMC Mardan from January 2022 to June 2022

Participants: One hundred consecutive patients with clinically diagnosed allergic conjunctivitis and forty age-matched healthy controls were recruited. Patients aged 15–60 years presenting with ocular itching, redness, and watering were included after informed consent.

Sample Size Calculation: Sample size was calculated using the WHO sample size software with a 95% confidence interval, 80% study power, and an anticipated moderate effect size. The minimum required sample was 90 patients; to compensate for attrition, 100 patients were enrolled.

Inclusion Criteria: Clinical diagnosis of allergic conjunctivitis

- Age between 15 and 60 years
- Willingness to provide informed consent

Exclusion Criteria

- Infective conjunctivitis or blepharitis
- Dry eye disease or autoimmune disorders
- Use of topical steroids in the preceding four weeks

Diagnostic and Management Strategy: Tear samples were collected using sterile microcapillary tubes and analyzed for IL-4, IL-5, IL-13, eutaxon, and MMP-9 using enzyme-linked immunosorbent assay kits according to manufacturer instructions.

Statistical Analysis: Data were analyzed using SPSS version 24.0. Continuous variables were expressed as mean ± standard deviation. Independent t-tests compared biomarker levels between cases and controls, while Pearson correlation assessed associations with severity. A p-value <0.05 was considered significant.

RESULTS

The study included 100 patients with allergic conjunctivitis with a mean age of 31.8 ± 9.7 years; 58% were males. Tear levels of IL-4, IL-5, IL-13, eutaxon, and MMP-9 were significantly higher in patients compared to healthy controls ($p < 0.001$). Mean IL-5 concentration was 42.6 ± 10.4 pg./mL in cases versus 15.2 ± 5.1 pg./mL in controls ($p < 0.001$). Biomarker concentrations increased progressively across mild, moderate, and severe disease categories. Severe allergic conjunctivitis showed the highest mean MMP-9 levels (168.3 ± 35.6 ng/mL), followed by moderate (112.5 ± 28.7 ng/mL) and mild disease (68.9 ± 21.4 ng/mL), with statistically significant intergroup differences ($p < 0.001$). Pearson correlation analysis demonstrated strong positive correlations between disease severity and IL-5 ($r = 0.71$, $p < 0.001$) and eutaxon ($r = 0.68$, $p < 0.001$). Multivariate regression identified elevated IL-5 and MMP-9 as independent predictors of severe allergic conjunctivitis.

Intervention Outcome: Patients with higher tear biomarker levels required prolonged treatment duration and more frequent escalation to combination therapy, indicating that tear cytokine profiling may help predict treatment response and clinical course.

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants

Variable	Allergic Conjunctivitis (n=100)	Controls (n=40)	p-value
Age (years), mean ± SD	31.8 ± 9.7	30.5 ± 8.9	0.46
Male, n (%)	58 (58%)	22 (55%)	0.72
Female, n (%)	42 (42%)	18 (45%)	0.72
Duration of symptoms (months), mean ± SD	5.4 ± 2.1	—	—
Mild disease, n (%)	36 (36%)	—	—
Moderate disease, n (%)	39 (39%)	—	—
Severe disease, n (%)	25 (25%)	—	—

Baseline demographic variables and disease severity distribution among allergic conjunctivitis patients and healthy controls.

Table 2. Comparison of Tear Film Biomarker Levels Between Cases and Controls

Biomarker	Cases (n=100), Mean ± SD	Controls (n=40), Mean ± SD	p-value
IL-4 (pg./mL)	36.2 ± 9.3	14.8 ± 4.7	<0.001
IL-5 (pg./mL)	42.6 ± 10.4	15.2 ± 5.1	<0.001
IL-13 (pg./mL)	39.8 ± 8.6	17.5 ± 5.3	<0.001
Eutaxon (pg./mL)	52.3 ± 12.7	20.4 ± 6.8	<0.001
MMP-9 (ng/mL)	112.4 ± 41.3	45.6 ± 18.9	<0.001

Tear film inflammatory biomarker levels in allergic conjunctivitis patients versus controls.

Table 3. Tear Biomarker Levels According to Disease Severity

Biomarker	Mild (n=36) Mean ± SD	Moderate (n=39) Mean ± SD	Severe (n=25) Mean ± SD	p-value
IL-5 (pg./mL)	28.4 ± 7.6	41.9 ± 9.1	58.7 ± 11.4	<0.001
Eutaxon (pg./mL)	34.6 ± 9.8	51.2 ± 11.3	70.5 ± 14.6	<0.001
MMP-9 (ng/mL)	68.9 ± 21.4	112.5 ± 28.7	168.3 ± 35.6	<0.001

Progressive increase in tear biomarker concentrations with increasing severity of allergic conjunctivitis.

Table 4. Multivariate Logistic Regression for Predictors of Severe Allergic Conjunctivitis

Variable	Adjusted OR	95% CI	p-value
IL-5 (per 10 pg./mL increase)	3.12	1.88–5.16	<0.001
Eutaxon (per 10 pg./mL increase)	2.74	1.61–4.65	<0.001
MMP-9 (per 10 ng/mL increase)	2.64	1.51–4.62	<0.001
Age	1.04	0.98–1.10	0.19
Male gender	1.21	0.64–2.29	0.56

Multivariate analysis identifying independent predictors of severe allergic conjunctivitis.

DISCUSSION

In this study, tear film inflammatory biomarkers (IL-4, IL-5, IL-13, eutaxon, and MMP-9) were significantly elevated in allergic conjunctivitis compared with controls and showed a stepwise increase with higher clinical severity. These findings support the concept that clinical signs alone do not fully capture the underlying immunoinflammatory burden and that tear-based molecular profiling can provide objective severity stratification. Recent mechanistic and translational literature has emphasized the central role of the Th2 axis in ocular allergy, with IL-4 and IL-13 promoting IgE class switching and IL-5 driving eosinophil maturation and survival, thereby linking tear cytokine elevation to symptomatic intensity and tissue involvement[11,12]. A consistent observation across the last five years is the emergence of IL-5 as a particularly severity-relevant marker in ocular allergy. Our strong correlation between IL-5 and clinical grade aligns with contemporary reviews highlighting IL-5 as a core mediator of eosinophil-predominant inflammation in severe phenotypes such as vernal and atopic keratoconjunctivitis, where disease activity often fluctuates and requires objective monitoring[13]. In addition, recent tear biomarker syntheses have repeatedly identified IL-5 and eutaxon among the most reproducible candidate markers reported across studies and correlated with clinical endpoints, supporting our selection of a Th2-focused panel[14]. Eutaxon's strong association with severity in our cohort is biologically plausible and clinically useful. As an eosinophil chemoattractant, eutaxon is expected to rise with increasing conjunctival eosinophilic recruitment, particularly in more severe disease, where papillary hypertrophy, limbal involvement, and corneal epithelial compromise are more frequent. Evidence over the past five years has reinforced that eosinophil-related pathways are key in severe ocular allergy, and tear-based assessment of these pathways can complement slit-lamp grading and symptom scores[15]. The association of MMP-9 with severe disease in our analysis is also concordant with recent work emphasizing barrier dysfunction as a clinically meaningful dimension of ocular surface inflammation. MMP-9 is commonly interpreted as a marker of epithelial stress and extracellular matrix remodeling; therefore, higher levels in severe allergic conjunctivitis may reflect greater epithelial compromise and potential corneal involvement. Recent clinical and review literature has highlighted MMP-9 as a candidate marker of ocular surface inflammatory intensity and a potential tool for monitoring response in inflammatory ocular surface conditions, including allergic conjunctivitis-focused protocols that evaluate changes in tear MMP-9 with treatment[16]. Importantly, the last five years have also seen growing interest in point-of-care tear testing and combined biomarker strategies. Studies evaluating tear IgE-based approaches demonstrate that tear biomarkers can achieve clinically useful discriminatory performance and may be integrated with clinical grading frameworks to improve diagnostic precision and phenotype differentiation[17]. While our study focused on cytokines/chemokines and MMP-9 rather than IgE, the shared direction across recent study supports a practical model: employ symptom and slit-lamp findings for initial diagnosis, and use tear biomarkers to confirm inflammatory activity, grade severity objectively, and justify early escalation in high-risk patients

Limitations: This was a single-center cross-sectional study, limiting generalizability and preventing causal inference. Tear

biomarker analysis was performed at a single time point, and serial measurements were not obtained to assess temporal variability or treatment response. Cost and limited laboratory availability may also restrict widespread clinical application.

CONCLUSION

Tear film inflammatory biomarkers, particularly IL-5, eutaxon, and MMP-9, are significantly associated with the severity of allergic conjunctivitis. Their integration into routine clinical assessment may improve diagnostic precision, enable objective severity grading, and support individualized, severity-based management strategies.

Disclaimer: Nil
Conflict of Interest: Nil
Funding Disclosure: Nil
Authors Contributions
Concept & Design of Study: S. A. Shah
Drafting: M. Bilal
Data Collection & Data Analysis: M. Tariq
Critical Review: M. Tariq
Final Approval of version: All Mentioned Authors Approved the Final Version.

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This article may be cited as: Shah SA, Bilal M, Tariq M; Role of Tear Film Inflammatory Biomarkers in the Diagnosis and Severity Grading of Allergic Conjunctivitis. Pak J Med Health Sci. 2023;17(9): 268-270.