

Tear Levels of Interleukin (IL) 2 & 4 in Patients with Allergic Conjunctivitis

(Levels of cytokines in tear)

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Abstract:- The present comparative cross sectional study was planned to compare cytokine levels in the tear of patients with acute allergic conjunctivitis (AAC), chronic allergic conjunctivitis (CAC) and healthy population. Subjects were divided into Group 1 (without allergic conjunctivitis), Group 2 (with AAC), and Group 3 (with CAC). Tears were collected using Schirmer strips and cytokine analysis & IgE analysis was performed using enzyme linked immune sorbent assay and by fully auto analyser. There were a total of 149 subjects. Cytokine (IL-2,4,5,8) levels & eosinophil count in the group with acute allergic conjunctivitis were approximately two to five -fold higher than in the group with healthy control group where the cytokines, eosinophil & IgE levels were found lowest ($p < 0.05$). Concentration of cytokines i.e. IL-2,4,5,6 & 8 in the CAC were 5.12 ± 1.71 ($p=0.022$), 1.5 ± 0.5 ($p=0.187$), 3.1 ± 1.6 ($p=0.036$), 12.3 ± 3.2 ($p=0.001$) & 20.2 ± 5.3 ($p=0.254$) pg/ml and found significantly high as compare to healthy controls. Although the group with AAC & CAC had the highest levels of IL-8 and eosinophils, no statistically significant inter- difference group (AAC vs CAC) was noted ($p=0.319$). This study demonstrated the presence of raised tear inflammatory cytokines in AAC & CAC patients. AAC patients may be at increased risk of developing CAC.

Keywords:- Cytokine, Eosinophil, IgE, Acute allergic conjunctivitis, Chronic allergic conjunctivitis.

I. INTRODUCTION

Allergic conjunctivitis is a self-limiting, bilateral conjunctival inflammatory disease that affects people who are sensitised and is brought on by an allergen attaching to an immunoglobulin E antibody on local mast cells¹. Depending on whether the inflammation is seasonal (spring, fall) or perennial, there are two types of allergic conjunctivitis (year-round)². During allergic illness, proinflammatory cytokines are probably present in a variety of combinations. Tumor necrosis factor (TNF) α , interleukin-1b, and interferon- γ , which are present in allergic conjunctivitis, have been shown to have synergistic or combination effects on conjunctival epithelial cell activation and produce AAC³, according to a recent study. One of the main characteristics of allergic disorders is the activation of conjunctival epithelial cells, which results in eosinophil-derived mediators in tears and allergen-mediated mast cell activation⁴.

Unlike the self-limiting allergy processes, Acute allergic conjunctivitis (AAC) is a sight-threatening condition. Atopic dermatitis affects 3% of the population, and 15–40% of those people also have eye involvement, typically as AAC⁵. No particular race or location is preferred for the disease's start, which often occurs between 20 and 50 years of age. Depending on the report, the male to female ratio ranges from 2.4:1 to less than 1:1. Mast cells, immunoglobulin E antibody, eosinophils, and other inflammatory cells participation are comparable to allergic conjunctivitis, so it is likely that T-cell involvement is what causes the disease's chronicity and threat to vision⁶. Patients with AKC have conjunctival epithelium that contains mast cells and eosinophils, but normal tissue does not.

People who are predisposed due to their atopic history tend to develop Chronic allergic conjunctivitis (CAC), a sight-threatening bilateral chronic conjunctival inflammatory process⁷. The condition normally begins before the age of 10, lasts between two and ten years, and usually goes away throughout puberty. Young guys who live in dry, hot settings are most at risk⁸.

Due to the frequent negative results of skin testing and the absence of a history of allergy, CAC cannot be regarded as a classic type 1 hypersensitivity reaction. The cytokines in tears have received particular attention in recent investigations. This effort, together with recent developments in our understanding of the intricate workings of the cytokine network, has led to the identification of certain cytokines that distinguish between acute allergic conjunctivitis and chronic allergic conjunctivitis diseases⁹. Discussion of the significance and potential involvement of the cytokines detected in acute and chronic allergic illnesses¹⁰.

In the diagnostic evaluation of individuals with known or suspected allergic disorders, IgE and eosinophil determination is crucial¹¹. Asthma, allergies, parasite infections, granulomatous disorders, fibrotic ailments, and numerous malignant tumours are all known to be characterised with eosinophilia^{4,12}. Estimating serum IgE and eosinophil levels can be used as a quick, affordable, and accurate investigative method to determine whether or not someone has allergic conjunctivitis.

Given the aforementioned context, the present study's goal was to compare the interleukin, serum IgE, and eosinophil levels between patients with allergic conjunctivitis and healthy controls.

II. MATERIAL AND METHODS

Data collection was completed in accordance with the approved study protocol after receiving approval from the Rajshree Medical Research Institute's ethical committee in Bareilly, Uttar Pradesh, India. Each patient gave their written consent after being fully informed. To record demographic information, symptoms, attack frequency, and medical history, including diabetes and hypertension, a google form was used online.

Subjects with allergic conjunctivitis who presented to the Ophthalmology OPD of Rajshree Medical Research Institute in Bareilly, Uttar Pradesh, India during the study period met the inclusion criteria (1st June 2017 to 30th June 2018). Three groups of these participants were created: Group A (N= 48) had no clinical signs of allergic conjunctivitis, Group B (N= 55) had acute allergic conjunctivitis, and Group C (N= 46) had chronic allergic conjunctivitis¹³.

An otorhinolaryngologist diagnosed allergic conjunctivitis based on recommendations made by the World health organisation and its Impact on Asthma (ARIA), which works with the World Health Organization to spread awareness of and implement evidence-based management for allergic conjunctivitis and asthma¹⁴. The guidelines define allergy as a condition of the nose caused by an immunoglobulin E (IgE)-mediated inflammation after allergen contact, marked by symptoms such rhinorrhea, nasal obstruction, itching, and sneezing^{5,9}.

The inclusion criteria for group B and C were, a history of allergic conjunctivitis symptoms such as eye irritation, redness, tears, and photophobia over the preceding month and year.

Severe ocular surface disease (such as shield ulcer), chronic ocular surface inflammation (such as keratoconjunctivitis sicca, glaucoma on topical medication), autoimmune disease, a history of topical antihistamine use within the previous month, a history of ocular trauma, or a history of ocular surgery within the previous six months were all considered exclusion criteria.

Serum sample¹⁵

The blood was allowed to coagulate for about 20 minutes after being drawn into a BD Vacutainer™ serum tube (BD, Franklin Lakes, NJ, USA). The serum was then collected by centrifuging at 130 g for 10 min, and it was kept at 20 C.

Tear Tests¹⁶

A sample was taken from one eye from each patient in each of the three groups. The lower conjunctival cul-de-sac was covered with a Schirmer strip (Contacare, Baroda, India) after local topical anaesthetic (G. proparacaine 0.05 percent) was injected there. Over the course of five minutes, tears were allowed to diffuse into the strip. During this time, the subjects were permitted to make any number of blinks. Within an hour of being collected, the Schirmer strip was then put into a simple tube and frozen to 80°C.

Estimation of interleukins^{17,18,19}: Tear samples were diluted 1:25 in phosphate-buffered saline with 1 mg/mL bovine serum albumin (Immunoconcepts, Sacramento, California) to a final volume of 100 uL, and ELISA was carried out using cytokine detection kits in accordance with the manufacturer's instructions (Biolegend, San Diego, Calif.). The following ELISA kits were used to detect cytokines: Legend Max Human IL-4 and IL-2 Elisa Kits with Pre-coated Plates the LegendMax Human IL-5 Elisa Kit with Pre-coated Plates, the Legend Max Human IL-6 Elisa Kit with Pre-coated Plates, and the Legend Max Human IL-8 Elisa Kit with Pre-coated Plates. The detection sensitivity for IL-2, IL-4, IL-5, IL-6, and IL-8 was 1.5, 0.6, 2.0, 1.2, and 4.0pg/mL, respectively, according to the standard curves.

Statistics: SPSS-16 version 16.0 Inc., Chicago, USA was used to evaluate this investigation. Assessments were made on the contrast between groups A, B & C. Statistics were judged to be significant at P values under 0.05. The mean and standard deviation of the measurement data that followed a normal distribution were expressed, and the comparisons between groups were made using the t-test/z-test, one way analysis of variance, Pearson test, and Mann-Whitney test (p<0.05 was regarded as significant).

III. RESULTS

The study included 149 people in total (81 males and 67 women), with a mean age of 35.11 years. In the current investigation, group B and C had significantly greater levels of the cytokines IL-2, 4, 5, 6, and 8 than group A. Similar results were reported when group B was compared to group C, i.e., significantly higher levels of eosinophils and IgE levels (p=0.001 & p=0.043). The average percentage of eosinophils in controls was 2.081.14 and in groups B and C, the percentage count increased by 2 to 3.5 fold, which was similarly statistically significant when compared to healthy controls with a p value of less than 0.05.

The levels of IL-2, IL-4, IL-6, and IL-8 were higher in the group C than the group B, with the control group demonstrating the lowest level of tear cytokines. A significant intergroup difference (group A vs B and Ge=group A vs C) was noted with these 4 cytokines (p <0.05 for each)

The levels of cytokines (IL-2,4,5,8) and eosinophils were found to be two to five times greater in the group with acute allergic conjunctivitis than in the healthy control group, where they were found to be at their lowest levels (p, 0.05). The concentration of cytokines, specifically IL-2, 4, 5, 6, & 8, in the CAC was considerably higher than that of healthy controls and was 5.12 1.71 (p=0.022), 1.5 0.5 (p=0.187), 3.1 1.6 (p=0.036), 12.3 3.2 (p=0.001), and 20.2 5.3 (p=0.254) pg/ml (table 1). Despite the greatest levels of IL-8 and eosinophils in the AAC & CAC group, there was no statistically significant difference between the these two groups (p=0.319).

IV. DISCUSSION

S.L	Parameters	Group A(N=48)	Group B (N=55)	Group A vs B	Group C(N=46)	Group A vs C
		Healthy controls	Acute conjunctivitis	P Value	Chronic conjunctivitis	P Value
1	Age	31.75 (11.15)	27.56(16.10)	0.005*	46.03(21.36)	0.005 [§]
2	Sex :male/ female	24/24	29/26	0.627*	28/28	0.614*
3	Tear breakup time (sec)	5.4±2.9	4.8±2.6	0.761*	4.1±2.8	0.657*
4	Redness (Numbers)	1.5±1.2	30.4±16.3	0.042*	38.1±8.2	0.038*
5	IL-2 (pg/ml)	1.91±0.93	3.78±1.28	0.032 [#]	5.12±1.71	0.022 [#]
6	IL-4 (pg/ml)	0.8±0.22	1.3±0.3	0.192 [#]	1.5±0.5	0.187 [#]
7	IL-5(pg/ml)	2.2±1.17	2.6±1.9	0.041 [#]	3.1±1.6	0.036 [#]
8	IL-6(pg/ml)	1.6±0.81	9.8±2.8	0.011 [#]	12.3±3.2	0.001 [#]
9	IL-8(pg/ml)	4.5±2.08	16.3±3.1	0.334 [#]	20.2±5.3	0.254 [#]
10	IgE (IU/ml)	81.17±15.07	927.18±218.13	0.001 [#]	1133.53±341.18	0.001 [#]
11	Eosinophils	2.08±1.14	4.95±3.42	0.093 [#]	7.15±4.38	0.076 [#]

Table 1: Demographic with clinical features and Cytokines levels of patients among groups

Note:

§= 1 way analysis of variance test ($p < 0.05$ was considered significant)*= Pearson χ^2 test ($p < 0.05$ was considered significant)#= Mann- Whitney test ($p < 0.05$ was considered significant)

The connection between allergic conjunctivitis, which is a component of a more systemic inflammatory process, and allergies has been the focus of research for a significant amount of time. The fact that the cytokines that were discussed in the beginning of our research work are typically elevated not only in allergic conjunctivitis, but in all other types of conjunctivitis as well, indicates that these cytokines may be useful markers of ongoing inflammation. In light of this, we intended to investigate the levels of interleukin (IL) 2 and IL 4 in the tears of patients who suffered from allergic conjunctivitis. Patients who suffer from allergic rhinitis have been shown to have elevated levels of cytokines both in their serum and in the nasal mucosain the past^{20,21}. On the other hand, to the best of our knowledge, this is the first study to compare tear film cytokine levels among patients with acute allergic conjunctivitis and chronic allergic conjunctivitis with healthy patients.

In type 1 hypersensitivity-mediated disease, activation of mast cells and release of a number of proinflammatory mediators, such as histamine, IL-4, IL-6, and IL-8 result from the crosslinking of the IgE on these receptors in response to an allergen^{1,4,15,22}. In our study, we discovered that AAC and CAC patients had significantly higher levels of IL-4, IL-6, and IL-8 than healthy control patients. Significantly less pronounced in group B and C was this increase (AAC vs CAC). Similar to this, our study discovered that IL-4 levels in tears were two times higher in CAC patients than in healthy controls. Understanding the pathogenesis of allergic conjunctivitis will be aided by IL-4 because it is a significant mediator of allergic conjunctivitis and is linked to T helper cell activation in peripheral blood, which causes B lymphocytes to produce IgE²³⁻²⁵. Furthermore, our research supports the fact that group B and C had significantly higher IgE levels (p 0.001).

In accordance with the clinical signs of ocular inflammation observed in the group, our investigation demonstrated that IL-4, IL-6, IL-8, and TNF levels were predictably greater in the tear film of allergic conjunctivitis patients. Chronic rhinosinusitis patients have nasal mucosa that is increased in IL-8, which has also been shown to control the movement of circulating leukocytes to target sites^{23,26}. Similar findings were made in our investigation, where it was shown that the levels of IL-2, IL-4, IL-6, and IL-8 in the AAC and CAC group were significantly greater than in the healthy control group. Although IL-5 has historically been linked to allergy illness, we found no evidence of a substantial increase in its levels among the various subgroups. It has been suggested that IL-5 is a disease's defining symptom²⁵.

We still need to learn more about the clinical and therapeutic ramifications of these results; for example, we need to figure out if treating individuals with non-symptomatic allergic conjunctivitis just involves focusing on the allergic symptoms' source.

V. CONCLUSION

Patients with AAC & CAC had tear cytokine concentrations that were noticeably greater than those of the general population.

Conflict of interest; NO

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