ORIGINAL ARTICLE

Correlation of Serum Levels of Interleukin-1β and CCL 24 with Severity of Persistent Allergic Rhinitis in Children

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ABSTRACT

Background: Allergic rhinitis (AR) is a distressing clinical presentation especially in pediatric age which affects quality of life and may predispose to bronchial asthma. Various inflammatory biomarkers might be involved in allergic rhinitis pathogenesis and correlated with its severity such as IL1 β and CCL 24. Objectives: This study aims to detect serum level of IL 1 β and CCL 24 in studied pediatric patients and correlate their levels with the severity of allergic rhinitis and with comorbid asthma in children. Methodology: Peripheral blood eosinophil count was detected and serum level of IgE, IL1 β and CCL24 were assayed in pediatric patients with allergic rhinitis using ELISA. In addition, correlation of their levels with the severity of AR. Results: Eosinophil count and serum levels of IL1 β and CCL24 were significantly elevated in (AR) patients compared with the controls (P=0.0001*), (P=0.026*) and (P=0.017*) respectively. Parental smoking and Associated Asthma of high statistical significance when correlated with severity of allergic rhinitis (P=0.005) and (P<0.001) respectively. Family history of allergy& allergy in other sibling were also significantly correlated with severity of AR (P=0.093) and (P=0.02) respectively. Conclusion: This study clarifies the evidence that IL-1β is an important inflammatory biomarker for pathogenesis and severity of allergic rhinitis and it might predispose to other allergic diseases subsequently it may be investigated as a therapeutic target especially in severe cases.

INTRODUCTION

Allergic rhinitis (AR) is an immunoglobulin E-mediated type of inflammation of the upper airway, which is induced by allergens and regulated by T cells. Worldwide, the prevalence of allergic rhinitis has raised; approximately 10 to 30% of adults and 40% of children are affected 1. AR has a major impact on quality of life by causing symptoms of sneezing, nasal congestion, nasal pruritus, rhinorrhea and obstruction of the nasal passages. Furthermore, it may contribute to secondary complications and co-morbidities (acute and chronic sinusitis, asthma exacerbations, eustachian tube dysfunction and chronic otitis media) by speech delay in pediatric populations and in rare cases permanent hearing damage. Moreover, in children, rhinitis can lead to craniofacial abnormalities due to chronic mouth breathing 2.

The Allergic Rhinitis and its Impact on Asthma (ARIA) 2016 update recommends introducing the terms “intermittent” and “persistent” on classification of allergic rhinitis instead of “seasonal” and “perennial” depending on persistence period of symptoms. The severity of allergic rhinitis differentiated into, mild, moderate/severe based on symptoms and their effect on social life, school and work performance 3.

The pathophysiology of AR is a multifactorial process including; effector cells enhancement and migration, inflammatory cells release of mediators, chemokines and cytokines, and eventually damage to the nasal epithelium and nerve endings 4. Inflammatory biomarkers play fundamental role not only in diagnosis and staging AR but also in monitoring progression, and response to therapy. For instance, IL-1β is a proinflammatory cytokine linked to the promotion of inflammation in severe allergic rhinitis, development of chronic obstructive pulmonary disease (COPD), asthma and its exacerbations due to infection 5,6,7.

Regarding chemokines, C-C Motif Chemokine Ligand 24 (CCL24) (also known as eotaxin-2 or myeloid progenitor inhibitor factor-2), is a chemokine involved in the pathogenesis of asthma and rhinitis. It contributes to the fibrocytes recruitment from blood to the airway in atopy 8,9. Estimation of inflammation biomarkers level offers insight on the mechanisms of allergic rhinitis, monitoring its severity, and may provide target for novel therapy. The previous studies targeting various
biomarkers for AR have been published over the past decades based on their detection in nasal mucosa. Our study was directed to assess their role in serum.

**METHODOLOGY**

**Study design:**
This case-control study was conducted at the outpatient clinic of Otolaryngology, Clinical pathology departments, Allergy Unit of Zagazig University Hospitals and Microbiology & Immunology Departments, Faculty of Medicine, Zagazig University and It was carried out between Mars 2021 and August 2021.

**Ethical approvals**
The study protocol was reviewed and approved by the Institutional Review Board (IRB) (no. 6784), Faculty of Medicine, Zagazig University, Egypt. The study complied with the guidelines of the Declaration of Helsinki 1975. The parents of all participants agreed and signed the informed consent.

**Subjects:**
A total of 200 participants were enrolled in the study. They included 100 pediatric patients with allergic rhinitis and 100 apparently healthy non-atopic child as controls. The two groups were age and gender matched.

The patients were clinically diagnosed as persistent allergic rhinitis (rhinorrhea, nasal obstruction, nasal itching, and sneezing that persist for more than 4 days per week or persist for more than 4 weeks). Patients were classified into 3 groups mild, moderate and severe; according to the AR and its impact on asthma (ARIA) depending on presence or absence of impairment in sleep, impairment of normal daily activities (work performance or school activity), troublesome symptoms. Patients were also examined for associated asthma.

**Exclusion criteria:**
Exclusion criteria included children <6 years old, those on medications as steroids, antihistaminic and unconsented patients.

**Methods:**

**Samples**
Five ml of blood were obtained from each participant and divided into; 2 ml blood in the EDTA tube for eosinophils counting and 3 ml placed into plain tube for serum collection for ELISA. Serum was obtained by allowing samples to clot for 30 minutes followed by centrifugation for 10 minutes at approximately 3000g, and stored at -20 °C until used.

**Peripheral blood eosinophils counting**
Two ml whole blood in the EDTA tube was utilized for complete blood counts using an XS500i Hematology analyzer (Sysmex, Kobe, Japan). In addition, peripheral blood differential cell analysis was conducted, using the blood film.

**Skin prick test (SPT)**
Skin prick test (SPT) to case group was performed at the Allergy and Immunology Unit, Faculty of Medicine, Zagazig University. Allergen panel used (homemade extracts of pollen, house dust, smoke, wool, cotton, mixed fungi, hay rice dust, clover and maize). POSITIVE SKIN TEST HISTAMINE (port Washington, NY, USA) was used as a positive control and saline as a negative control. The test was interpreted by measuring the size of the wheal after 20 min. The patient was considered sensitized when wheal diameter for the tested allergen was 3 mm or more and associated with erythema.

**Measuring of total IgE by Enzyme linked immunosorbent assay (ELISA)**
Quantitative determination of total IgE in serum by ELISA according to manufacturer’s instructions (IMMUNOSPEC Corporation, Canoga Park, CA 91303, USA). The optical density of each well was immediately determined using a micro plate reader (Stat Fax® 303 Plus) set to 450 nm.

**Measuring of Serum Level of IL-1β and CCL24**
Quantitative determination of Serum Level of IL-1β and CCL24 were done using double-antibody sandwich enzyme-linked immunosorbent assay. Human IL-1β ELISA kit (USA. Catalogue No.201-12-0144) and Human CCL24ELISA kit (USA. Catalogue No. 201-12-0059) according to the manufacturer’s instructions. The optical density of each well was immediately determined using a micro plate reader (Stat Fax® 303 Plus) set to 450 nm.

**Statistical analysis:**
After data collection, data were coded, entered and analyzed using SPSS (Statistical Package for Social Science) version 25. Qualitative data were presented as frequencies and percentages while, quantitative data were presented as mean, standard deviations (SD). Quantitative data of two independent normally distributed groups were compared using student t test while Qualitative independent data were compared using Chi square test. P value (≤ 0.05) was considered statistically significant difference and <0.001 is considered highly significant difference

**RESULTS**
A total 200 participants were enrolled in this study. They were (100) pediatric patients with allergic rhinitis with mean age of 8.5±1.5, boys/girls: 66/34 and 100 apparently healthy non-atopic child with mean age of 8.8±2.2, boys/girls: 63/37

There were statistically significant differences in IgE serum level and eosinophils count among the studied groups (P<0.001) and (P<0.00001) as shown in table 1. Twenty-six (26) AR patients had normal total IgE (26%).
Table 1: The characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (No=100) Patients Mean±SD</th>
<th>Group 2 (No=100) Control Mean±SD</th>
<th>T test</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>8.5±1.5</td>
<td>8.8±2.2</td>
<td>1.127</td>
<td>0.519</td>
</tr>
<tr>
<td>IgE IU/ml Mean±SD</td>
<td>380.5 ± 125.2</td>
<td>31 ± 9.5</td>
<td>27.835</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>Eosinophils count Mean±SD</td>
<td>983.3 ± 234.6</td>
<td>177.7 ± 83.3</td>
<td>9.706</td>
<td>&lt;0.0001 **</td>
</tr>
<tr>
<td>Eosinophils % Mean±SD</td>
<td>6.2 ±1.6</td>
<td>1.5 ± 0.7</td>
<td>7.7992</td>
<td>&lt;0.0001 **</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>66 (66%)</td>
<td>63 (63%)</td>
<td>0.197</td>
<td>0.658</td>
</tr>
<tr>
<td>Girls</td>
<td>34 (34%)</td>
<td>37 (37%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T test (Student t test), X²=Chi-Square test, **=Highly significant

Serum level of IL1β and CCL24 were assessed by ELISA in all our studied groups and they were significantly elevated in allergic rhinitis (AR) patients were compared with the controls (P=0.026*) and (P=0.017*), respectively as shown in table 2, 3.

Table 2: Serum level of IL1β in the studied participants

<table>
<thead>
<tr>
<th>Serum level (pg/ml)</th>
<th>Group 1 (No=100) Patients Mean±SD</th>
<th>Group 2 (No=100) Control Mean±SD</th>
<th>T test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>1825.12±523.54</td>
<td>1653.13±452.51</td>
<td>2.486</td>
<td>0.026*</td>
</tr>
</tbody>
</table>

T test (Student t test)

Table 3: Serum level of CCL24 in the studied participants

<table>
<thead>
<tr>
<th>Serum level (pg/ml)</th>
<th>Group 1 (No=100) Patients Mean±SD</th>
<th>Group 2 (No=100) Control Mean±SD</th>
<th>T test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>318.45 ± 112.35</td>
<td>279.65 ± 95.22</td>
<td>2.635</td>
<td>0.017*</td>
</tr>
</tbody>
</table>

T test (Student t test)

We classified the patients depending on symptoms severity into 2 subgroups: subgroup 1 including children with intermittent allergic rhinitis and mild persistent allergic rhinitis and subgroup 2 including those with moderate to severe persistent allergic rhinitis. There were 64 patients in subgroup 1 and 36 patients in subgroup 2. By comparing the different assayed markers among the two subgroups, there was high statistically significant difference among them as regard serum level of IL1β and IgE (P<0.001) while serum level of CCL24 was not significantly different (P=0.491) as shown in table 4

Table 4: Comparison of IL1β, CCL24 and total IgE level according to severity of allergic rhinitis

<table>
<thead>
<tr>
<th>Laboratory Tests</th>
<th>Subgroup 1 (No.=64) Mean±SD</th>
<th>Subgroup 2 (No.=36) Mean±SD</th>
<th>T test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE (IU/ml)</td>
<td>276.52 ± 37.01</td>
<td>354.25 ± 90.72</td>
<td>4.916</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>IL1β (pg/ml)</td>
<td>2375.3 ± 182.4</td>
<td>2641.3 ± 351.5</td>
<td>4.2314</td>
<td>&lt;0.001 **</td>
</tr>
<tr>
<td>CCL24(pg/ml)</td>
<td>397.2 ± 115.3</td>
<td>456.1 ± 291.8</td>
<td>1.161</td>
<td>0.491</td>
</tr>
</tbody>
</table>

When the correlation of different factors with the severity of allergic rhinitis in both groups were evaluated, we found that parental smoking and associated asthma showed high statistically significant correlation (P=0.005) and (P<0.001) respectively, in addition, family history of allergy& allergy in other sibling were also significant (P=0.093) and (P=0.02) respectively. Meanwhile when we analyzed associated urticaria; it was statistically insignificant (P=0.630) as shown in table 5
DISCUSSION

Allergic rhinitis (AR) is a major atopic inflammatory condition worldwide. AR is the result of environmental exposure of a genetically predisposed persons to allergen. Allergic rhinitis is diagnosed by medical history, clinical examination and detection of allergen-specific IgE (skin prick tests or serum-specific IgE)\(^{11}\).

In our study, the case group (AR patients) had significantly higher mean level of serum IgE as compared to the control group. Similar results were revealed in the study conducted by Sachdeva and his colleagues\(^ {12}\). Moreover, another study performed by Abdelmaksoud and his coworkers\(^ {13}\) but in different age groups had similar results.

Normal total IgE was detected in 26% of cases and elevated in 74% of them in current study. Concerning measuring total IgE, Ganesan and coworkers\(^ {14}\) in their study reported only 45.3% of children had elevated serum total IgE levels, while Refaat and his colleagues\(^ {15}\) found that 80% of allergic rhinitis patients had elevated total IgE. The threshold for diagnosing allergic disease and the ability to discriminate between allergic conditions by total IgE assessment are lacking\(^ {16}\).

Different countries adopt different reference values of total IgE for allergy diagnosis\(^ {11}\). Moreover, normal total IgE cannot exclude significant allergy specially in pediatric group and other IgE-independent pathways might be responsible for mediating allergy in those patients\(^ {17,18,19}\).

The correlation between eosinophils counts and allergic disease has been known for many years and they were previously investigated in nasal smear and in peripheral blood in patients of allergic rhinitis\(^ {20}\). Concerning eosinophils count peripheral blood, it was significantly elevated in group 1 than group 2 \( (P=0.017*)\). This was in accordance with Gupta and his colleagues\(^ {21}\) in their case control study which evaluated olfaction in allergic rhinitis patients. Yoon and his colleagues\(^ {22}\) also in their study on AR in preschool children had found similar result as eosinophils count was significantly increased in AR patient compared to healthy ones\(^ {23}\). On the other hand, Mikolajczyk and his colleagues\(^ {24}\) reported that 94.29% of their AR patients had no eosinophilia and majority of patients in another study had shown blood absolute eosinophil count levels of <500 cell per cu.mm regardless of the severity of clinical disease\(^ {25}\).

Jagdeeshwar and his colleagues\(^ {26}\) explained and concluded that a normal blood absolute eosinophil count levels were detected in AR with nasal symptoms only, whereas they were increased in AR patients with nasal and respiratory symptoms\(^ {27}\).

The previous studies of allergic rhinitis highlighted the role of inflammatory biomarkers assessment in diagnosis conformation, especially in children. A study done by Sim and his colleagues\(^ {28}\) reported that IL-1β produced by nasal epithelial cells were increased in nasal lavage fluids from patients with allergic rhinitis especially after allergen challenge and this supported the results of our study concerning serum total IL 1 β which was significantly higher in patients with allergic rhinitis than control group. This is concordant with the results obtained by Han and his coworkers\(^ {7}\).

Allergic rhinitis is linked to a systemic inflammatory response leading to upregulation of proinflammatory mediators’ expression. This associates allergic rhinitis to comorbid conditions such as asthma, chronic hyperplastic eosinophilic sinustitis, nasal polyps, and exudative otitis media. This systemic inflammatory condition was proved by the finding that local nasal corticosteroids efficiently decrease the local inflammation in the nares, but it failed to control inflammation in tissues involved in the comorbid conditions\(^ {29}\).

Serum level of CCL24 was elevated in allergic rhinitis (AR) patients in comparison with the controls \( (P=0.017*)\) and this result was the same adopted by lee\(^ {27}\) in 2002 who reported that CCL24 was significantly higher in the allergic rhinitis group than in the non-allergic rhinitis and normal control group \( (p<0.01, p<0.01)\). In addition, De Corso and his colleagues\(^ {30}\) showed that high statistical difference in the levels of eotaxin-2 (CCL24) in nasal secretions in patients with persistent allergic rhinitis (PAR) in comparison to healthy participants \( (128.9±51.7 \text{ pg/mL in comparison to } 16.4±10.7 \text{ pg/mL})\).

| Table 5: Correlation of different factors with severity of allergic rhinitis |
|---------------------------------|------------------|------------------|------------------|------------------|
|                                 | Subgroup1        | Subgroup2        | Odds             | X²               | P value         |
|                                 | (No.=64) No. (%) | (No.=36) No. (%) | ratio(95% CI)    |                  |                |
| Family history of allergy      | 55(85%)          | 26(72.2%)        | 2.35 (0.85-6.48) | 2.816            | 0.093           |
| Parental smoking               | 19(29.7%)        | 21(58.3%)        | 0.3(0.13-0.71)   | 7.878            | 0.005**         |
| Allergy in other sibling       | 25(39%)          | 6 (16.7%)        | 3.2(1.17-8.8)    | 5.402            | 0.02*           |
| History of associated urticaria| 15(23%)          | 10(27.8%)        | 0.79(0.31-2.02)  | 0.231            | 0.630           |
| History of associated asthma   | 4(6%)            | 17(47%)          | 0.075(0.022-0.25)| 23.313           | <0.001**        |

**Note:** The data presented above are based on a study by Abdallah et al., and the results are consistent with previous research on the correlation of different factors with the severity of allergic rhinitis. The findings highlight the role of inflammatory biomarkers and eosinophil counts in the diagnosis and management of allergic rhinitis. Further research is needed to confirm these findings and to explore potential therapeutic interventions.
The main sources of CCL24 in the human body are fibroblasts, cutaneous epithelial, nasal epithelial cells, and macrophages. Both serum or plasma concentration and tissue expression of eotaxins is high in allergic and inflammatory processes, it can be suspected that the activation of cells secreting these chemokines occurs not only in tissues, but also in the blood stream. The case group was classified into 2 subgroups based on the duration of symptoms and severity of the condition. Group 1 were children with intermittent and mild persistent allergic rhinitis versus subgroup 2 including those with moderate to severe persistent allergic rhinitis. Different assayed markers were compared between the two subgroups, and there was high statistically significant difference among them as regard Serum level of IL1 β and IgE (P<0.001) while serum level of CCL24 was not significantly different (P=0.491) and this was agreed to some extent with Han and his coworkers in significant difference of IL1 β among two groups (p = 0.003). On contrary, CCL-24 (an eosinophils activation marker), were significantly increased in Group 2 (p = 0.039) and total IgE level was not significant different between two groups (p = 0.258). This difference can be explained that patients in subgroup 2 in our study were stable regarding lower airway hypersensitivity, while in Han and coworker study the patients had active asthma. Different studies showed that eotaxins are linked to poor asthma control.

Association of nasal symptoms with the total IgE was not statistically significant by study performed by Ganesan and his colleagues while Azid et al. who perform their study on Malaysian population to detect relevance of total IgE and allergic diseases and they concluded correlation of elevated total IgE levels with disease severity. Also, Karli et al. who concluded that the determination of IgE in allergic rhinitis is a supportive method. However, it cannot be recommended for routine use because of the time loss and high cost.

At the end of our study, we tried to trace the correlation of different factors to severity of allergic rhinitis in two subgroups, we found that paternal smoking and patient associated asthma had high statistical difference (P=0.005) and (P<0.001) respectively. In addition, family history of allergy & allergy in other sibling were also significant (P=0.093) and (P=0.02) respectively. This was agreed with Han et al. who evaluated the correlation of different factors including perinatal conditions, environmental aspects, socioeconomic factors and family history of allergic disease with the severity of AR and found that perinatal, environmental, and socioeconomic factors had no correlation with moderate and severe AR. They reported that parental history of allergic rhinitis and increased expression of IL-1β is a significant risk factors of moderate to severe AR (p = 0.011 and p = 0.030) respectively.

CONCLUSION

This study clarifies the evidence that high level of IL-1β may promote inflammation in severe allergic rhinitis and could be used as a marker of severity, subsequently it may be investigated as a therapeutic target.

Recommendations:

We recommend further studies on different age groups with larger sample size to ensure role of CCL24 and investigate role of IL1 β as a therapeutic target.

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