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Investigating interleukin 4 during non-allergic asthma

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Abstract

Asthma is a well-known illness of the lower respiratory tract. It has many phenotypes including allergic and non-allergic asthma. The mechanism of non-allergic is still not entirely understood. The aim of the study is to investigate IL-4 response during non-allergic asthma. Blood from non-allergic asthma patients and aged matched healthy controls were collected complete blood count (CBC) was performed on each sample and the serum level of IgE and IL-4 were estimated by enzyme linked immunosorbent assay (ELISA). The results of the current study showed that the majority of the asthmatic patients were between 51-60 years old and with a dominance of females in compare to the healthy control group. The serum level of total IgE was significantly ($p=0.02$) elevated in asthma patients in comparison to healthy controls (122.4 ± 6.9 ul/ml and 91.9 ± 11.1 ul/ml, respectively). The serum level of total IL-4 was non-significantly ($p=0.818$) elevated in asthma patients in comparison to healthy controls (35.4 ± 6.5 ul/ml and 30.9 ± 7.7 ul/ml respectively). A strong significant and positive correlation was found between IgE and IL-4 in asthma patients ($R_2 = 0.7$, $p=0.00$) in compare to no correlation found between them in healthy controls ($R_2 = 0.34$, $p=0.24$). Finally, the ROC curve showed that IL-4 did not give a good performance in the discrimination of the disease in non-allergic asthma. In conclusion, the study suggest that IL-4 have a slight contribution in the mechanism of non-allergic asthma.

Keywords: Non-allergic asthma, IgE, IL-4, Basrah

Introduction

Asthma is a common chronic respiratory illness with a high prevalence worldwide, it affects 350 million people international. There were 1,177,000 asthma patients in Japan in 2014, with a 9–10% prevalence and in 2015, asthma-related causes claimed the lives of roughly 383,000 people (Fukutomi *et al.*, 2010; Nakamura *et al.*, 2020) ^[9, 21]. According to numerous research, there is a higher probability that children with an asthmatic family member would get asthma in the future (Liu *et al.*, 2020) ^[19]. Alem *et al.*, (2020) ^[3] stated that, in asthma patients, the odd ratio (OR) was 1.89 according to the family history.

Allergic asthma occurs via early exposure to environmental allergens such as pollen, cockroaches, animal dander, and house dust mites (HDM), but it can also be brought on later in life by exposure to a novel allergen, such as an occupational allergen. When allergen-specific Th2 cells identify an allergen, they release type 2 cytokines such as IL-4, IL-5, IL-9, and IL-13, which cause an excess of mucus to be produced, an accumulation of eosinophils in the airway wall, and the production of immunoglobulin E (IgE) by allergen-specific B cells (Lambrecht and Hammad, 2017) ^[18].

Non-allergic asthma, in contrast to allergic asthma, typically manifests later in life, is more prevalent in women, and is associated with obesity (Pakkasela *et al.*, 2020) ^[25].

Mast cells and Th2 cells produce IL-4 in addition to other cytokines like IL-5, IL-9, and IL-13 (Byrne *et al.*, 2004) ^[24]. IL-4, in particular, is thought to play a major role in the early stages of asthma development by controlling T cell survival and proliferation as well as IgE synthesis. On the other hand, IL-13 is mostly engaged in the later stages of allergic reactions, which include mucus hypersecretion and airway remodeling (Gour *et al.*, 2015) ^[13].

TNF-alpha and IL-8 are thought to be secreted by the effect of IL-4; these substances raise the number of neutrophils in lung tissue. Neutrophil chemotaxis, which produces O₂, matrix metalloproteinase-9 (MMP-9), leukotrienes-4 (LTB-4), and platelet activating factor (PAF), is mediated by IL-8. As a result, eosinophils accumulate inside and around the airway (Gao *et al.*, 2017) ^[11].

It is also thought that IL-4 increases mucus secretion, which obstructs airways more frequently. Through preventing T-lymphocytes and eosinophils from undergoing apoptosis, IL-4 indirectly preserves the acute allergic response (Steinke, 2004) [30]. The presence of eosinophils is linked to asthma persistence. Asthmatics see an improvement in their symptoms when their interleukin-4 level decreases. There are additional cytokines that have an asthmatic effect besides IL-4, and these other cytokines are also crucial in the pathophysiology of asthma (Khan *et al.*, 2022) [17]. The aim of the study is to investigate IL-4 response during non-allergic asthma.

Methods

The study included individuals who were 18 years of age or older, of both genders. The study comprised a total of 150 participants, divided into two groups: the Asthmatic Patients (AS group, n=75) and the Healthy Controls (HC group, n=75), who were matched in terms of age and randomly selected. All asthma patients sought treatment at a private allergy clinic in Basrah, where they were diagnosed with asthma by a specialized physician. Each participant completed a relevant questionnaire that included demographic information such as age, sex, symptoms, and body mass index (BMI). Additionally, participants provided their assent by signing a consent form.

Study subjects were subjected to blood sample collection, with 5 ml of blood being extracted. The sample was divided into two portions: the initial portion of 4 ml was placed in an SST tube containing gel for the purpose of serological analysis. The serum was isolated using centrifugation at a speed of 3000 revolutions per minute for a duration of 20 minutes. Four portions of serum were prepared for each sample, and all serum samples were stored at a temperature of -20 °C until they were ready for further analysis. Subsequently, a portion of 1 ml was transferred into an EDTA tube for the purpose of conducting a physiological investigation, specifically focusing on complete blood count (CBC) analysis.

The serum total IgE and IL-4 levels in the study participants were measured using ELISA kits, specifically the Human IgE ELISA Kit and Human IL-4 ELISA Kit from Shanghai YL Biont, China. The data was subjected to statistical analysis, beginning with a normality test, followed by a non-parametric test (Mann-Whitney U test). Additionally, Spearman and ANOVA tests were employed to analyze the associated studies, utilizing the SPSS program. Furthermore, the study parameters underwent ROC curve analysis.

Results

The age distribution of patients with AS revealed that the largest proportion of individuals with AS was observed in the 51-60 age group, accounting for 28.3% of the total. The findings of the present study revealed that the proportion of females in the AS group was 70.14%, whereas in the HC group it was 66.66%. Similarly, the percentage of men in the AS group was 29.85%, compared to 33.33% in the HC group.

The results of the present study showed there was an overlap of asthma symptoms among patients, so the overlap rate was between coughing, wheezing, and shortness of breath 32 (47.8%), while the overlap between coughing and wheezing was 2 (3%). The overlap between coughing and shortness of breath 22 (32.8%), While there was no overlap between

shortness of breath and wheezing. The percentage of coughing among patients was 4.5% (n=3), shortness of breath 7.5% (n=5) and wheezing 4.5% (n=3) as shown in the figure 1.

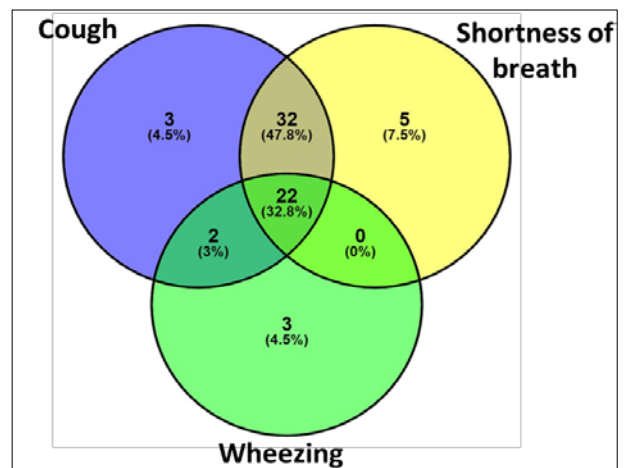


Fig 1: Frequency and overlaps of Asthma symptoms among AS group

The current findings of the present study revealed a significant elevation ($p=0.02$) of IgE level in AS group (122.4 ± 6.9) in compare to HC group (91.9 ± 11.1) as shown in Figure 2.

The current findings of the present study revealed a non-significant elevation ($p=0.818$) of IL-4 level in AS group (35.4 ± 6.5) in compare to HC group (30.9 ± 7.7) as shown in Figure 3.

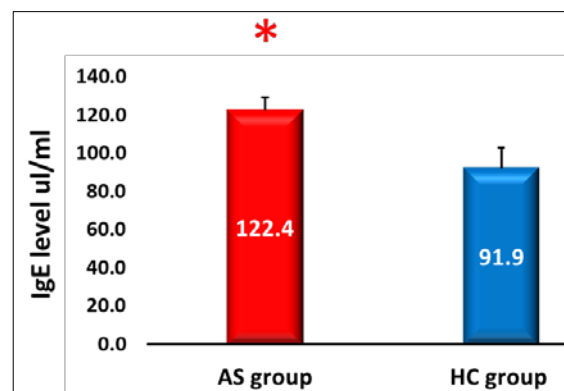


Fig 2: Serum IgE level in AS and HC groups. There is a significant elevation of IgE in asthma patients

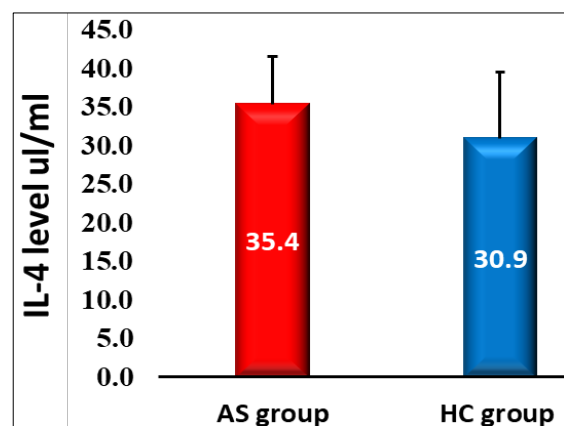


Fig 3: Serum IL-4 level in AS and HC groups. There is no significant elevation of IL-4 in asthma patients

Spearman correlation results showed a very positive and significant correlation between the IgE level and IL-4 levels in AS group ($R^2 = 0.7$, $p=0.00$), while a weak non-

significant correlation was found between the two parameters in HC group ($R^2 = 0.34$, $p=0.24$) as shown in Figure 4 A & B.

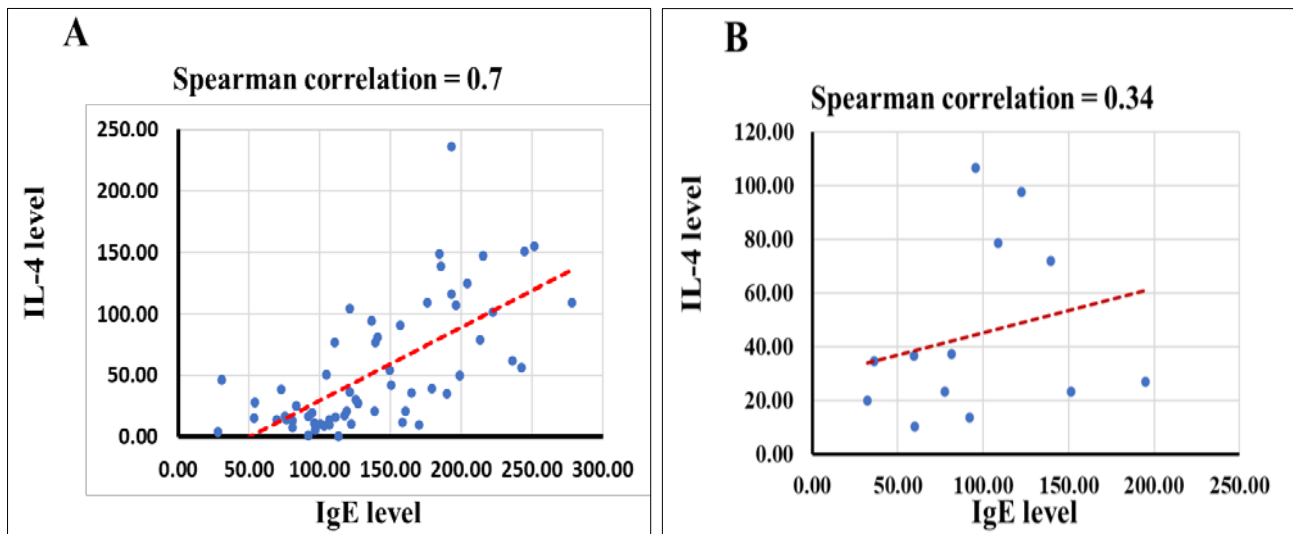


Fig 4: Spearman correlation between IgE and IL-4 in study groups. A. Spearman correlation between IgE and IL-4 in AS group. B. Spearman correlation between IgE and IL-4 in HC group

The receiver operating characteristic (ROC) curve for IgE and IL-4 is shown in Figure 5 A & B. The area under the curve (AUC) values for the level IgE in AS and HC groups were 0.694 (Figure 5 A), indicating good overall

performance of IgE. The area under the curve (AUC) values for the level IL-4 in AS and HC groups were 0.522 (Figure 5 B), indicating a poor overall performance of IL-4.

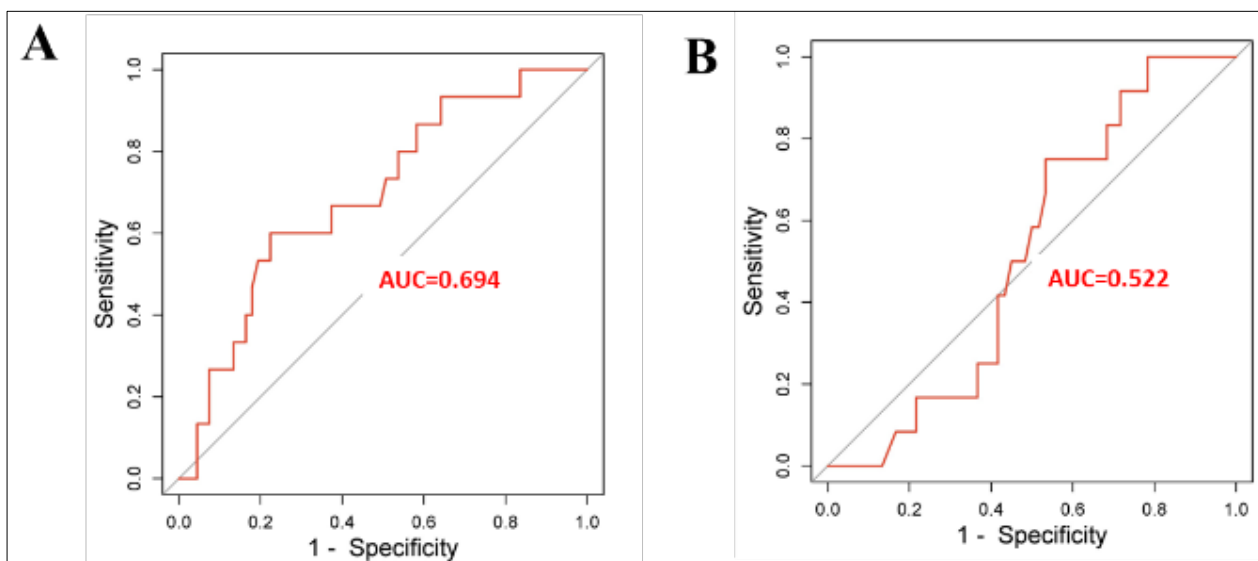


Fig 5: The receiver operating characteristic (ROC) curve of the four study parameters. A. ROC curve for IgE. B. ROC curve for IL-4.

Discussion

It is known that asthma is a heterogeneous disease, with various prevalence and severity in males versus females and through various age ranges as well. The current study showed a predominant of females. The current findings are similar to several studies which also reported a predominant of females in asthma patients (Alsaïmay *et al.*, 2022 and Abdul-Jabbar and Rashid, 2021) [5, 1]. As adults, women have an increased prevalence and severity of asthma, while in children males are more affected. These differences might be caused by several factors such as sex hormones, genetic and epigenetic variations, social and environmental factors, and responses of patients to asthma treatments (Chowdhury *et al.*, 2021) [2].

One of the major factors that causes the differences between males and females is the sex hormones (Fuhlbrigge *et al.*, 2002; Dharmage *et al.*, 2019) [8, 32]. During puberty, sex hormone levels in women is fluctuated, additionally, the menstrual cycle and pregnancy might be associated with asthma pathogenesis, which make females more predisposed to asthma (Fuseini and Newcomb, 2017; Shah and Newcomb, 2018) [10, 29].

Another study showed increased asthma in female (Alkhaled *et al.*, 2022) [4]. There are many factors that may increase this situation such as low level of education or low income (Woods *et al.*, 2016) [31]. In another study which showed that because cardiovascular disease affects males more frequently than women, men have shorter life

expectancies than women which also may affect the prevalence of asthma in females (Harper *et al.*, 2021) [14].

Asthma patient in the current study showed a significant increase in IgE level, IgE is essential in the classification of asthma as asthma can be divided into allergic asthma and non-allergic asthma depending on the present or absent of IgE, or if its levels are normal (Garcia *et al.*, 2013, Loureiro *et al.*, 2018 and Qiu *et al.*, 2020) [12, 20, 28]. In Iraq, Hashim *et al.* (2022) [15] showed that high level of IgE was a significant in asthma patients as well. Due to its essential role in interacting with the allergen, IgE is at the core of type I hypersensitivity. Several trials also look at blocking IgE with a monoclonal antibody called omelimumab as a way to treat asthma, which effectively lessens symptoms of the condition (Palomares *et al.*, 2017, Novosad and Krčmová, 2020, Pelaia *et al.*, 2020 and Cheng, 2021) [26, 22, 27, 7].

The present study showed an increase in serum levels of IL-4 despite the insignificance these results are agreed with many previous studies in regard of their findings of elevated of serum IL-4 levels in asthmatics compared to healthy controls (Antczak *et al.*, 2016; Nuhaier, 2020) [6, 23]. IL-4, an epithelial mediator, was shown to be considerably greater in bronchial asthma patients when compared to control subjects (Kalinauskaite-Zukauske *et al.*, 2019) [16].

In conclusion, despite the elevated level of IL-4 in non-allergic asthma it still not significant suggesting no/or a slight contribution in the mechanism of it and it dose not discriminate the patients from healthy controls according to thus parameter.

Ethics approval

This is an observational study. The Research Ethics Committee has confirmed that no ethical approval is required.

Conflict of interest: The authors confirmed there is no conflict of interest.

Author contributions

Contributors: Aliaa Abood was responsible for material preparation and data collection, Shereen Al-Ali contributed to design the study, analysis of the collected data, and writing the draft Iqbal Abdul Aziz contributed to write the manuscript.

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