Interferon Gamma and its Correlations with IGE and NLRP3 in Eosinophilic and Non-Eosinophilic Asthma

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Abstract

Asthma is a chronic inflammation of the lower respiratory tract with a range of symptoms including coughing, whistling and shortness of breath. The aim of the study is to investigate the correlation of interferon gamma (INF γ) and its correlation with the levels of IgE and NLRP3 in asthma phenotypes of patients in Basrah. The study included 112 participants (69 AS patients and 43 healthy controls). Complete Blood Count (CBC) was performed for asthma's phenotype discrimination, and the level of INF γ was measured using enzyme linked immunosorbent assay (ELISA). Additionally, with the use of data from previous study (unpublished data) the correlations between INF γ , IgE and NLRP3 were investigated in asthma phenotypes using Pearson's correlation. The results of the current study showed that the majority of asthma patients were non-eosinophilic asthma, moreover, no obvious differences in the level of INF γ among the study groups. Furthermore, positive and significant correlation were found between INF γ and NLRP3 in non-eosinophilic asthma. In conclusion, the non-eosinophilic asthma was predominant among patients living in Basrah. In addition, the positive and significant correlation between INF γ and NLRP3 suggest a role of these two parameters in non-eosinophilic asthma.

Key words: Asthma, Phenotypes, INFy, NLRP3, Basrah

Introduction

Asthma is a common chronic disease that affects 1-18% of the population in various countries. Asthma is characterized by variable symptoms including wheezing, shortness of breath, chest tightness coughing, and variable restrictions of exhaled airflow. Importantly, both Symptoms and airflow restrictions characteristically vary in their severity with time. Asthma is usually associated with airway hyperresponsiveness to direct or indirect stimuli and chronic airway inflammation. Airway hyperresponsiveness (AHR) is a key feature of asthma (Lauzon et al., 2016).

Interferons (IFNs) play a substantial role in many reactions including mediating early antiviral responses, the induction of type I IFNs plays a significant role in RSV-bronchiolitis. The data around the role of IFNs in defining susceptibility to asthma following bronchiolitis are contradictory. Interferon gamma (INF- γ) has a role in the susceptibility to asthma following bronchiolitis are contradictory. It has been related with the recurrent of wheezing during asthma. The high IFN responses during acute disease are linked to asthma development. Although the role of INF was controversial, there are reports of the involvement of INF in the pathogenesis of asthma. Furthermore, IFN γ , the principal Th1

effector cytokine, has been found to be vital for the resolution of allergic related immunepathologies (Naumov et al., 2019 and Figueiredo et al., 2012). Additionally, IFN γ reduces recruitment of lymphocytes and eosinophils, and inhibits airways hyperresponsiveness and mucus, but promotes airway neutrophils and overall lung inflammation (Mitchell et al, 2011).

IgE was correlated to asthma in many studies. It has been reported that the assessing of cumulative IgE levels specific for respiratory allergens can be of benefit in the screening of allergic phenotype of severe asthma and might be considered as biomarker useful for asthma therapy (Naumova et al., 2022). IgE involved in the mechanism of type I hypersensitivity disorders. It is clear that allergic asthma has an elevated levels of IgE and it also link sensitive people with others have IgE- mediated diseases such as allergic rhinitis (Flashner et al., 2021). Furthermore, IgE was determine as a biomarker of type 2 asthma (Woo et al., 2021).

Inflammasomes are a collection of cytosolic protein complexes which have the ability to recognize various signals including stress signals, exogenous microorganisms, and endogenous signals. The response of Inflammasomes to the various signals is mediated by the activation of caspase-1, which produce IL-1 β , IL-18 and starting the inflammatory response. The role of NLRP3 in asthma has been investigated in many studies. Reports have been found regarding the close association of NLRP3 inflammasomes to a variety of lung diseases, such as asthma, chronic obstructive pulmonary disease (COPD) and lung cancer. In addition, many of these reports have shown that NLRP3 inflammasomes contributed in the chronic airway inflammation process in both asthma and COPD (Lachowicz et al., 2019 and Kim et al., 2017). The aim of the study is to investigate the correlation of INF γ and its correlation with the levels of IgE and NLRP3 in asthma phenotypes of patients in Basrah.

Materials and methods

The current study is a case control study, consist of 112 participants (AS group=69 ans HC group=43), patients participate in the current study were asthma patient who attend a private clinic in Basra, southern Iraq. The age of participants which ranged from 20-85 years and from both sexes. All patients have already been diagnosed with asthma by specialist. In addition, all participants filled out a relevant questionnaire and signed the consent form.

Approximately 3ml placed into sterile SST gel tubes for immunological study, and 2ml of blood was placed in an EDTA tube for CBC. The level INF- γ of was estimated using Human INF- γ ELISA Kit (Elabscience, USA, Cat# E-EL-H0108). All data were statistically analyzed using normality test and Mann-Whitney U test and Pearson's correlation using MINITAB®19 statistical software.

Results

The results of the current study showed that among asthma patients, the majority of them have shortness of breath (62.32%) followed by coughing (56.50%) then whistling (49.30%), as shown in Figure 1. Moreover, the overlapping among the symptoms showed in the majority of the patients as shown in Figure 2.



Figure 1: The percentage of asthma symptoms in AS group. A. The result showed that only 62.32% of AS patients had shortness of breath while 37.68% have no difficult breathing. B. The result showed that only 56.50% of AS patients had cough, while 34.50% had no cough. C. The result showed that 49.30% of AS patients had whistling, while 50.70% did not have whistling.



Figure 2: Venn diagram representing the overlap of asthma symptoms

The CBC results revealed that eosinophilic As group composed 26.4% of the AS group and non-eosinophilic asthma composed 73.6% of the AS group (Figure 3). The non-eosinophilic asthma group showed a significant elevation of neutrophils (p=0.000), while there was a significant elevation in the number of lymphocytes, eosinophils and basophils in eosinophilic As group (p value = 0.03, 0.000 and 0.004 respectively) more details can be found in Table 1.



Figure 3: The percentage of eosinophilic and non-eosinophilic asthma groups

Blood cells	Non- Eosinophilic AS group	Eosinophilic AS group
	(mean±S.E. or median)	(mean±S.E. or median)
Neutrophils	63*	46.4
Eosinophils	1	5.6*
Basophils	0.1	0.3*
Monocyte	6.35	6.30
Lymphocyte	29.4	34.1*

Table 2: The blood cells count between eosinophilic and non-eosinophilic asthma groups

*Means significant results

The current study results of the INF γ level showed that it was non-significantly (p=0.101) elevated in AS group (30.8±0.5 pg/ml) in compare to HC group (31.0±0.4 pg/ml) as shown in figure 4A. The INF γ levels was also non-significantly elevated (p=0.273) in the eosinophilic asthma group (32.1±1.1 pg/ml) in comparison with non-eosinophilic asthma group (30.7±0.5 pg/ml) and HC group (31.0±0.4 pg/ml) as shown in Figure 4B.



Figure 4: A. The INFγ levels in Asthma and healthy controls. B. The INFγ levels in non-Eosinophilic, Eosinophilic Asthma and healthy controls

The level of IgE and NLRP3 were measured in a previous study (unpublished data) where the level of IgE was elevated in AS group in compare to HC group and significantly elevated in the Eosinophilic AS group in compare to non-eosinophilic AS group. The NLRP3 levels showed an elevation in non-eosinophilic as group in compare to eosinophilic AS group. The results of the correlations among the three serological parameters (IgE, INF γ and NLRP3) in eosinophilic asthma group in the current study showed no significant correlations. The value of Pearson's correlation between IgE and NLRP3 was - 0.374 and the p value = 0.188. The value of Pearson's correlation between IgE and INF γ was -0.247and the p value = 0.375, while the value of Pearson's correlation between NLRP3 and INF γ was 0.180 and the p value = 0.539 as illustrated in Figure 5.

The results of the correlations among the three serological parameters (IgE, INF γ and NLRP3) in non-eosinophilic asthma group in the current study showed significant correlations between IgE and NLRP3 and between NLRP3 and INF γ . The value of of Pearson's correlation between IgE and NLRP3 was 0.602 and the p value = 0.000. The value of Pearson's correlation between IgE and INF γ was 0.106 and the p value = 0.494, while the value of Pearson's correlation between NLRP3 and INF γ was 0.625 and the p value = 0.000 as illustrated in Figure 6.



Figure 5: Pearson' correlations of IgE, INFy and NLRP3 in eosinophilic asthma



NLKP5 level

Figure 6: Pearson' correlations of IgE, INFy and NLRP3 in non-eosinophilic asthma

Discussion

Asthma (AS) is a chronic airway inflammatory disease is characterized by intermittent attacks of breathlessness, wheezing, and cough accompanied by variable airflow obstruction. (Jartti et al., 2020). Airways obstruction, which is the primary symptom of asthma, is caused by a decrease in airway diameter leading to narrowing of the airways. The current results showed females dominancy in asthma cases. Reports showed a clear sex difference in asthma. The reason of this differences results from the role of sex hormones inside the female's body making them more susceptible to developing asthma. These physiological changes that any women go through during her life include maturation,

marriage, menarche, pregnancy, breast feeding and menopause (Fuhlbrigge et al., 2002 and Zein & Erzurum, 2015)

In the presence study different phenotypes of asthma were investigated, including eosinophilic asthma or non-eosinophilic asthma. Regarding Eosinophilic asthma (EAs) is a type of asthma that is characterized by high levels of white blood cells called eosinophils in the airways of the lungs. Recent studies have suggested that sputum and blood eosinophil counts are important factors for predicting asthma exacerbation. (Nakagome & Nagata, 2018).

The results of the present study showed no obvious changes in the level of INF- γ . The mechanism of INF- γ has a dual function, in animal models of asthma, the action of IFN- γ is to reduce the recruitment of both lymphocytes and eosinophils, in addition, it might inhibit AHR and the production of mucus. In contrast, IFN- γ might promote the presence of neutrophils in the airway and inclusive lung inflammation. Moreover, IFN- γ might stimulate the activation of eosinophil (Mitchell et al., 2011).

The results of correlation showed positive correlation between INF γ and NLRP3 and IgE and NLRP3 which suggest a role of INF γ and NLRP3 in non-eosinophilic asthma patients living in Basrah.

In conclusion, although INF γ did not show differences within asthma phenotypes but it showed positive correlations with NLRP3 in non-eosinophilic asthma proposing a role of INF γ and NLRP3 in non-eosinophilic asthma patients living in Basrah/Iraq.

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