Study of immunological parameters associated with asthma with and without bacterial infection in Thi-Qar Province

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ABSTRACT

Background. Asthma is a prevalent and persistent respiratory condition that impacts more than 300 million individuals globally, leading to substantial illness and death.

Objective. The current study aims to evaluate the level of immune parameters in asthma patients infected with and without pathogenic bacteria in Thi-Qar province south of Iraq.

Material and method. The present study was conducted at Al-Nasiriyah teaching hospital October 2023 to June 2024 and involved 130 individuals, of which 100 asthmatic patients 70 have bacterial infections and 30 without bacterial infections, and 30 as control group. The age ranges from 3-80 years. The immune parameters were evaluated by ELISA technique.

Result. This study was recorded the IL-5 and IgE increased significantly in asthmatic patients with bacterial infections than other groups, while the IL-13 and TNFα not scored a significant difference between patients according to bacterial infection. the results also, showed that the bacterial infection patients had positive IL-5 (51.43%,) while 20% in asthmatic patients without bacterial infection, the patients with bacterial infection had positive IL-13 (17.14%,) and 13.33% in asthmatic patients without bacterial infection, the TNFα scored 50% of bacterial infected patients and 40% in patients without bacterial infection. Finally, the IgE was positive 67.14% in bacterial infected patients, and 50% in patients without bacterial infection.

Conclusion. This study concluded that the asthmatic patients with and without bacterial infection had high significant level of immune parameters than control group, also, showed that the bacterial infection induce level of IL-5, and IgE in asthmatic patients.

Keywords: asthma, IL-5, IL-13, TNFα, IgE, bacterial infection

INTRODUCTION

Asthma is a persistent inflammatory condition affecting the airways, resulting in symptoms such as coughing, wheezing, difficulty breathing, and a feeling of heaviness in the chest. Asthma symptoms arise from the inflammation of the airways, which leads to processes including mucus production, alteration of the airway wall, and bronchial hyperresponsiveness (BHR), which is the tendency of smooth muscle cells to react to nonspecific stimuli such as cold air [1]. Asthma frequently manifests during childhood (childhood-onset asthma), while it is possible for individuals to develop asthma later in life (late-onset asthma). Childhood-onset and late-onset asthma exhibit distinct variations, adult-onset asthma is characterized by greater severity and a weaker association with allergies compared to asthma that develops in childhood. For children, having atopy (a genetic tendency to develop allergic diseases), reduced lung function, and respiratory tract infections, particularly those caused by rhinovirus, are significant variables that increase the likelihood of asthma per-

sisting [2]. The causative relationship between the underlying inflammation in asthmatic children and the pathogenicity of respiratory viruses, as well as the role of numerous viral infections in early childhood in the development of asthma, remains unclear [3].

Given the significant role of inflammation in the development of asthma, it is logical that the main objective of asthma treatment is to effectively manage symptoms and address the underlying inflammation to prevent recurrent episodes of the condition [4]. An unsupervised clustering methodology was used to examine the disease progression and clinical characteristics of asthma. The results showed that asthma is a diverse disease, with significant variations among patients in terms of age of onset, associated risk factors, severity, presence of other medical conditions, and response to treatment [5]. Recently, the condition has been categorized into two groups: Th2-high asthma and Th2-low asthma, based on the level of Th2 inflammation. Th2-high asthma is defined by the presence of eosinophilic airway inflammation, which is linked to higher levels of eosinophils in the blood or increased fractional exhaled nitric oxide (FeNo). On the other hand, Th2-low asthma encompasses neutrophilic asthma and paucigranulocytic asthma [6]. Cytokines of the Type-2 variety, specifically interleukin IL-4, IL-5, and IL-13, have a role in the progression of bronchial asthma [7]. IL-5 is essential for the development of eosinophils in the bone marrow, their stimulation, and their ability to survive in inflamed areas, such as the airways. IL-4 plays a role in the development of T-cells, activation of B-cells, differentiation of B-cells into plasma cells, and the synthesis of IgE. This contributes to allergic responses by increasing the binding of allergens to mast cells or basophils. While IL-13 shares similarities with IL-4 in terms of its actions, the significant clinical impacts of IL-13 are observed on structural cells, including epithelial cells, smooth muscle cells, and fibroblasts [8]. For instance, IL-13 stimulates the development of goblet cells that generate mucus and contribute to restructuring, including the enlargement of smooth muscle [8,9]. However, IL-13 does not play a role in the differentiating of T-cells, as immature T-cells do not possess IL-13 receptors. Certain bacterial lysates and herbal remedies can inhibit the expression of IL-4/IL-13 and reduce eosinophilic airway inflammation [10]. Following the activation of the immune system, macrophages, T cells, and other cells come together and release a variety of substances, including interleukin-1b, 4, 5, 10, and 13, as well as tumor necrosis factor (TNF)-a. These substances disrupt the anti-inflammatory equilibrium and worsen the evolution of asthma [11]. TNF-like cytokine 1A (TL1A) is a transmembrane protein encoded by the TNFSF15 gene. It possesses a stable trimer structure like TNF-a [12]. Human lung myofibroblasts, when stimulated by TNF-a, exhibited a significant increase in TL1A expression and collagen formation. However, the researchers did not investigate the specific processes by which TL1A affects TNF-a-stimulated myofibroblasts [13].

MATERIALS AND METHODS

Patients group

A one hundred and thirty individual were included in this investigation, 70 patients diagnosed with asthma by a Respiratory specialty infected with bacteria, consisting of 40 males and 30 females, with age ranging from 3-80 years, and 30 patients non-infected with bacteria.

Control group

The control group included 30 healthy people consisting of 15 males and 15 females, with an age between 3 and 73 years.

Exclusion criteria

People with asthma who suffer from chronic diseases such as diabetes, high blood pressure, and autoimmune diseases.

Samples collection

Sputum samples

Sputum samples were obtained from patients with asthma and (controls 1 asthma patients without bacterial infection) by clean containers then transported directly to the laboratory within one hour after collection for examination.

Blood samples

A five ml of blood were collected from both patients and control group, two ml of blood was putted in gel tube and left about half hour for coagulation, after that the samples were centrifuged 4000 RPM for five minutes, for serum obtained. The serum was stored at -20°C until using a immunological parameters evaluation, the immunoglobulin IgE and cytokines IL-5, IL-13 and TNFα were evaluated by Enzyme-Linked-Immuno-Sorbent-Assay Technique (ELISA).

Bacteria culture

Blood agar, MacConkey agar, Mannitol salt agar, Brain heart infusion broth, and Urease activity test media were included for diagnosis the gram-positive bacteria.

Approval of the ethical committee

The presented revision has been permitted by the Directorate of Health in the Thi-Qar Committee (No. 210 ∕ 2023 in 26/10/2023). The patient's consent was taken verbally in hospitals and clinics, when visiting them in their homes, or by mobile phone conversation for an invitation for blood and sputum samples.

Statistical analysis

The data of the current study was statistically analysis by using SPSS version 26, based on using Kruskal-Wallis H for mean and range and Chi-square for independent at p. value <0.05.

RESULTS

Evaluation levels of IL-5, IL-13, TNFα and IgE in asthmatic patients and control group

This study was conducted that a high significant difference at p. value <0.05, in the levels of immune parameters studies groups, this study recorded the level of IL-5 and IgE increased significantly in asthmatic bacterial infected patients compared with other asthmatic patients and control group, while the levels of both IL-13 and TNFα increased significantly in both groups of asthmatic patients compared with control group, as in Table 1.

TABLE 1. *Evaluation levels of IL-5, IL-13, TNFα and IgE in asthmatic patients and control group*

	IL5	IL13	TNFα	IgE
	Mean + (Range)			
With infection	56.4 (21.5-121.3)[a]	40.1 (26.3-87.3)[a]	53.3 (28.6-106.7)[a]	155.5 (8.90-746.2)[a]
Without infection	44.8 (22.4-130.8)[b]	40.9 (28.6-87.3)[a]	48.7 (28.0-87.39)[a]	112.6 (47.0-211.0)[b]
Control	30.7 (21.2-40.83)[c]	29.4 (25.7-37.9)[b]	31.2 (24.6-45.62)[b]	48.26 (32.1-68.30)[c]
Kruskal-Wallis H	<0.001	<0.001	<0.001	<0.001

Estimate result of IL-5 of asthmatic patients (with and without bacterial infection) and control group

This study was conducted that a high significant difference between studies groups, and between asthmatic patients groups according to result of IL-5 at p. value <0.05, was noted 36 (51.43%) of asthmatic patients with bacterial infection was positive for IL-5, 6 (20%) positive in asthmatic patients without bacterial infection, while the lowest positive recorded in control group 5 (16.67%), according to odds ratio, the probability of positive result of IL-5 in asthmatic patients had bacterial infection increased 4 times than same patients without bacterial infection (Table 2).

Estimate result of IL-13 of asthmatic patients (with and without bacterial infection) and control group

This study was conducted that a high significant difference between studies groups, while a non-significant difference between asthmatic patients groups according to result of IL-13 at p. value <0.05, was noted 5 (17.14%) of asthmatic patients with bacterial infection was positive for IL-13, 4 (13.33%) positive in asthmatic patients without bacterial infection, while non-recorded positive result in control group 0 (0.00%), according to odds ratio, the probability of positive result of IL-13 in asthmatic patients had bacterial infection increased more than one times than same patients without bacterial infection as in Table 3.

Estimate result of TNFα of asthmatic patients (with and without bacterial infection) and control group

This study was conducted that a high significant difference between studies groups, while a non-signifi-

TABLE 2. *Estimate result of IL-5 of asthmatic patients (with and without bacterial infection) and control group*

IL-5	Positive		Negative		Total	
	No.	%	No.	%	No.	%
With infection	36	51.43	34	48.57	70	53.84
Without infection	6	20.00	24	80.00	30	23.08
Control	5	16.67	25	83.33	30	23.08
Total	47	36.15	83	63.85	130	100
All groups CalX^2= 34.1　　TabX^2= 5.99　DF= 2　p. value <0.001Sig Patients groups CalX^2= 20.9　TabX^2= 3.84　DF= 1　p. value <0.001Sig OR infected/non-infected 4.16 (2.22-7.79)						

TABLE 3. *Estimate result of IL-13 of asthmatic patients (with and without bacterial infection) and control group*

IL-13	Positive		Negative		Total	
	No.	%	No.	%	No.	%
With infection	5	17.14	65	82.86	70	53.84
Without infection	4	13.33	26	86.67	30	23.08
Control	0	0.00	30	100	30	23.08
Total	9	6.92	121	93.08	130	100
All groups CalX^2= 17.5　　TabX^2= 5.99　DF= 2　p. value <0.001Sig Patients groups CalX^2= 0.627　TabX^2= 3.84　DF= 1　p. value 0.482Non-sig OR infected/non-infected 1.37 (0.62-2.99)						

cant difference between asthmatic patients groups according to result of TNFα at p. value <0.05, was noted 35 (50%) of asthmatic patients with bacterial infection was positive for TNFα, 12 (40%) positive in asthmatic patients without bacterial infection, while 5 (16.67%) positive result in control group, according to odds ratio, the probability of positive result of TNFα in asthmatic patients had bacterial infection increased more than one times than same patients without bacterial infection (Table 4).

Estimate result of TNFα of asthmatic patients (with and without bacterial infection) and control group

This study was conducted that a high significant difference between studies groups, and between asthmatic patients groups according to result of IgE at p. value <0.05, was noted 47 (67.14%) of asthmatic patients with bacterial infection was positive for IgE, 15 (50%) positive in asthmatic patients without bacterial infection, while 6 (20%) positive result in control group, according to odds ratio, the probability of positive result of IgE in asthmatic patients had bacterial infection increased more than two times than same patients without bacterial infection as in Table 5.

DISCUSSION

Evaluation of IL-5 in asthmatic patients (with and without bacterial infection) and control group

The present study showed that the level of IL-5 increases significantly in asthmatic patients than control group, also cytokine level increased significantly in asthmatic patients with bacterial infection in compari-son with asthmatic patients without bacterial infection. In addition, (51.43%), of bacterial infected asthmatic patients were positive IL-5 while the percentage was 20% in asthmatic patients without bacterial infection. Furthermore, the IL-5 positive result in asthmatic patients increased as 4.16 times than asthmatic patients without bacterial infection.

The present study aligns with the findings of Bessa et al. [14] and Lun et al. [14], which demonstrated a significant elevation in IL-5 levels in the serum or plasma of untreated asthma patients compared to controls. These elevated levels of IL-5 exhibited positive associations with risk score, total eosinophil count, and total serum eosinophil count. Also, the study of Dimitrova [16], recorded the IL-5 increased with severity of disease, was recorded the sever patients had high level than all moderate, mild, and control group. In contrast this study disagreed with study of Alturaiki, [17], which was noted a non-significant difference in IL-5 between two groups, also it was disagreed with study John et al. [18], their study found a non-significant difference between asthmatic patients and control group. The discrepancy between the current study and prior researches may arise from the usage of corticosteroid inhalation by these individuals. It has been shown that corticosteroids can reduce IL-5 by inhibiting NF-κB-dependent transcription [19]. Nevertheless, in a mouse model of allergic airway disease, IL-5 was the target of antibody-induced reductions in airway inflammation, indicating that IL-5 plays a significant role in asthma severity assessment [20].

The interactions between microbial factors and host cells play a crucial role in the immune response of the

TABLE 4. *Estimate result of TNFα of asthmatic patients (with and without bacterial infection) and control group*

TNFα	Positive		Negative		Total	
	No.	%	No.	%	No.	%
With infection	35	50.00	35	50.00	70	53.84
Without infection	12	40.00	18	60.00	30	23.08
Control	5	16.67	25	83.33	30	23.08
Total	47	36.15	83	63.85	130	100
All groups CalX^2= 24.9 $\quad$ TabX^2= 5.99 $\quad$ DF= 2 $\quad$ p. value <0.001Sig Patients groups CalX^2= 2.20 $\quad$ TabX^2= 3.84 $\quad$ DF= 1 $\quad$ p. value 0.155Non-sig OR infected/non-infected 1.50 (0.87-2.62)						

TABLE 5. *Estimate result of IgE of asthmatic patients (with and without bacterial infection) and control group*

TNFα	Positive		Negative		Total	
	No.	%	No.	%	No.	%
With infection	47	67.14	23	32.86	70	53.84
Without infection	15	50.00	15	50.00	30	23.08
Control	6	20.0	24	80.0	30	23.08
Total	68	52.30	62	47.70	130	100
All groups CalX^2= 78.8 $\quad$ TabX^2= 5.99 $\quad$ DF= 2 $\quad$ p. value <0.001Sig Patients groups CalX^2= 5.92 $\quad$ TabX^2= 3.84 $\quad$ DF= 1 $\quad$ p. value 0.015Sig OR infected/non-infected 2.03 (1.16-2.59)						

airways. The earliest interactions between microorganisms and the host immune response mostly involve epithelial cells and dendritic cells, although airway macrophages, intraepithelial lymphocytes, and other leukocytes also contribute to this process. Dendritic cells have a crucial function in connecting the innate and adaptive immune responses and act as a central controller of allergic inflammation [21]. In addition, the study of Ramos-Martínez et al. [22], in line with current study was investigated that asthmatic patients with bacterial infection had high IL-5 level compared asthmatic patients only and control group, also, their study showed IL-5, IL-9, and IL-13 decreased significantly and L-10, IFNγ increased after treatment the patients with Vit-D3. In the investigation of Son et al. [23] found evidence that both viruses and bacteria can activate nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs). This activation leads to the formation of inflammasomes and the subsequent proteolytic activation of IL-1β, IL-18, and IL-5. Streptococci can enter epithelial cells, and the first attachment of oral Streptococcus to these cells is probably facilitated by the antigen I/II adhesion family, stringent starvation protein A (SspA), and SspB. [24].

Evaluation of IL-13 in asthmatic patients (with and without bacterial infection) and control group

This study showed that the level of IL-13 increases significantly in asthmatic patients than control group, while non-significantly difference between asthmatic patients according to bacterial infection. Also, 17.14% of asthmatic patients with bacterial infection were positive IL-13, while in asthmatic patients without bacterial infection they were 13.33%. Furthermore, the IL-13 positive result in asthmatic patients increased 1.37 times than asthmatic patients without bacterial infection but non-significantly.

The current study was in line with recent study conducted by Kazaal et al. [25], with regard level of IL-13 in asthmatic patients, while disagreed with regard result of IL-13 was showed all patients had positive IL-13 result 100%, and 0% in control group. In addition, it showed a positive correlation with the count of eosinophils. Also, the study of Cai et al. [26], recorded the IL-13 highly increased in asthmatic patients. Moran et al. [27] demonstrated a notable correlation between IL-4 and IL-13 in individuals with asthma. IL-4 is regarded as a crucial regulator of Th2 cells, which are responsible for allergic responses. It not only triggers the production of other pro-allergic cytokines like IL-5 and IL-13, but also plays a key role in the development of allergic disorders. IL-4 facilitates the formation of myeloid dendritic cells (mDCs) and has a role in the movement of Th2 cells and eosinophils to the site of inflammation. Both interleukin-4 (IL-4) and interleukin-13 (IL-13) stimulate B cells to produce immunoglobulin E (IgE), promote an increase in the number of goblet cells, initiate an exaggerated response of the airways, and stimulate excessive production of mucus [28, 29]. Also, our result came agreed with the study of Son et al. [23], that recorded the level of both IL-5 and IL-13 had a positive correlation with eosinophil count, while our finding disagreed with the same study when showed that the IL-13 increased significantly in asthmatic patients with bacterial infection. The reason for the difference with Son study may be due to the type of bacteria isolated Porphyromonas pasteri and V. rogosae, in their study. Innate lymphoid cells of Group 2 (ILC2) can be stimulated by microorganisms that interact with Toll-like receptors (TLR), without the need for IgE-dependent activation [30]. In addition, when epithelial cells are exposed to various harmful stimuli, they become activated and stimulate the production of ILC2, IL-5, and IL-13. This process may play a crucial role in the initial phases of the allergic immune response [31].

Evaluation of TNFα in asthmatic patients (with and without bacterial infection) and control group

In this study the level of TNFα was increased significantly in asthmatic patients in comparison with control group, while the level was non-significant between asthmatic patients according to bacterial infection, in addition 50% of bacterial infected patients were positive for TNF, and the cytokine positivity was 40% in patients without bacterial infection, furthermore the bacterial infected patients increased 1.5 times than other patients group.

As in the research of Kubysheva et al. [32], that involved three groups of patients, our results come agreed with it in finding of patients cohorts had high level of TNFα than control group, they found the highest level of cytokine was observed in individuals with asthma and COPD, followed by asthma and chronic obstructive pulmonary disease, and lastly in people with bronchial asthma. The study of Bazan-Socha et al. [33], included two cohorts of asthma patients, one with allergies and one without allergies. The results showed that both groups had elevated levels of TNFα compared to the control group. However, there was no statistically significant difference between the two patient groups. Furthermore, it is worth mentioning The NFα may serve as a standalone indicator for prolonged CLT in both asthma and control groups, despite the absence of a clear correlation between these variables in linear univariate regression models. Numerous studies have documented that IL-18 acts as a coenzyme in individuals with asthma, contributing to the formation of both Th1 and Th2 cells. Additionally, IL-18 can enhance the activity of natural killer (NK) cells and the expression of Fas-ligand in cells. Evidence demonstrates that this cy-

tokine possesses the characteristics of a potent facilitator, augmenting the stimulation of diverse proinflammatory cytokines, such as IFN-g, IFN-γ, GM-CSF, TNF-α, IL-13, IL8, IL-17, and IL-5 [34, 35].

The study of Wardzyńska et al. [36], showed the genetic expressing micro-MRN of TNF-α in asthmatic patients, also, recorded the disease severity has positive correlation with BMI. So yet, the exact function of systemic inflammation in the development of asthma remains unclear. In both atopic and non-atopic individuals, blood levels of soluble tumor necrosis factor receptors were observed to rise during episodes of asthma [37]. Adipose tissue can also serve as a significant origin of proinflammatory cytokines, Furthermore, Giuffrida et al. [38] found that the level of TNF-α was considerably higher in asthmatic patients compared to the control group. They also observed a non-significant difference in TNF-α levels between asthmatic patients and non-asthmatic patients who were infected with germs and/or viruses. TNF- α and IL8 have important roles in lung dysfunction [39]. Previous studies, such as the study conducted by Tirado et al. [40] and the study conducted by Fujitsuka et al. [41], have indicated that patients with microbial lung infections exhibit elevated levels of pro-inflammatory cytokines (IL-1b and TNF-a), Th2 cytokines (IL-4 and IL-5), and chemokines (MCP-1 and RANTES). These findings suggest an intricate immune interaction during microbial respiratory infections. Cytokines in question may originate from cells within the lung tissue. Epithelial cells, mast cells, basophils, monocyte/macrophages, and lymphocytes could release various types of cytokines and chemokines (such as IL-1, TNF, IL-4, IL-5, IL8, MCP-1, RANTES) when interacting with bacteria in lung tissue. Nevertheless, it is unable to exclude the involvement of other cells in different tissues. Previous reports conducted by Chung, [42], demonstrated disparities in cytokine patterns between asthma and other respiratory illnesses. Studies have documented differences in the cytokine profiles of bronchoalveolar lavage between individuals with chronic obstructive pulmonary disease (COPD) and asthma. The cytokine expression profile in COPD differs from that reported in asthma. Asthma typically exhibits infiltration of eosinophils and Th2-cells, which is accompanied by the production of IL-4, IL-5, and IL-13. IL-8, IL-1, and TNF-a have more significant involvement in COPD.

Evaluation of IgE in asthmatic patients (with and without bacterial infection) and control group

The IgE increased significantly in asthmatic patients than in control group, and the antibody is increased significantly in bacterial infected patients in comparison with other patients group, in addition the level of IgE was positive in 67.14% in bacterial infected patients,

and in 50% of patients without bacterial infection, furthermore the bacterial infected patients increased 1.5 times than other patients group. Clinicians commonly utilize total IgE levels as a biomarker to ascertain the allergic phenotype of asthma. Nevertheless, it is frequently the case, and our research findings confirm this hypothesis, that measuring total IgE alone is inadequate, and further examination for allergen-specific IgE is necessary, which ultimately incurs higher expenses compared to utilizing the Phadiatop™ as an initial screening test. The cost of skin tests with allergens is generally lower than the cost of determining specific IgE levels in vitro. However, skin tests can only assess a limited number of allergen sources and the process is time-consuming, often necessitating numerous visits to the patient. Additionally, in vivo experiments can be conducted on asthma patients who have a forced expiratory volume in one second (FEV1) level greater than 70%, however it may pose a higher risk for patients with severe asthma [43,44].

The above mentioned finding of current study is agreed with recent study of Naumova et al. [45] that recorded the IgE level increased significantly in patients with mixed asthma, then in allergic asthma, then in non-allergic asthma than control group. Also, the study of Crespo-Lessmann et al. [46], reported the asthmatic patients had higher level of serum IgE than non-allergic patients, while non-significantly in level of IgE in sputum. A study has demonstrated the presence of localized synthesis of IgE in the nasal passages of patients who have tested negative for skin prick tests and had undetectable levels of IgE in their blood serum [47]. Since there is currently no established physiological cause for non-allergic asthma, it is reasonable to assume that individuals with non-atopic asthma may also have a local allergic response due to the shared features of the two conditions. Airway inflammation with eosinophilia, increased production of Th2 cytokines, and airway-induced exacerbations are common features of both allergic and nonallergic asthma, despite certain distinctions between the two [48,49]. Researchers found that SE-IgE levels are again linked to severe disease in atopic dermatitis, and that skin colonization with S. aureus is much higher in individuals with the condition (up to 90% compared to healthy people Nowicka et al. [50]; Weidinger et al., [51]. Several investigations have established a connection between changes in the airway microbiota and the development of atopy and asthma phenotype [52]. Patients with airway allergies frequently exhibit IgE reaction to antigens derived from microorganisms. HDM has been documented as carriers of bacteria and microbial antigens, which can induce IgE sensitization [53].

REFERENCES

1. Sockrider M, Fussner L. What is asthma? Am *J Respir Crit Care Med.* 2020 Nov 1;202(9):P25-6. http://doi.org/10.1164/rccm.2029P25

2. Turrin M, Rizzo M, Bonato M, Bazzan E, Cosio MG, Semenzato U, et al. Differences between early-and late-onset asthma: role of comorbidities in symptom control. *J Allergy Clin Immunol Pract.* 2022;10(12):3196-203. http://doi.org/10.1016/j.jaip.2022.08.007

3. Jartti T, Bønnelykke K, Elenius V, Feleszko W. Role of viruses in asthma. InSeminars in immunopathology 2020 Feb (Vol. 42, No. 1, pp. 61-74). Berlin/Heidelberg: Springer Berlin Heidelberg.

4. Cunha F, Amaral R, Jacinto T, Sousa-Pinto B, Fonseca JA. A systematic review of asthma phenotypes derived by data-driven methods. *Diagnostics.* 2021;11(4):644. https://doi.org/10.3390/diagnostics11040644

5. Chatkin J, Correa L, Santos U. External environmental pollution as a risk factor for asthma. *Clin Rev Allergy Immunol.* 2022;62(1):72-89. https://doi.org/10.1007/s12016-020-08830-5

6. Habib N, Pasha MA, Tang DD. Current understanding of asthma pathogenesis and biomarkers. *Cells.* 2022;11(17):2764. https://doi.org/10.3390/cells11172764

7. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity.* 2019;50(4):975-91. http://doi.org/10.1016/j.immuni.2019.03.018

8. Shankar A, McAlees JW, Lewkowich IP. Modulation of IL-4/IL-13 cytokine signaling in the context of allergic disease. *J Allergy Clin Immunol.* 2022;150(2):266-76. http://doi.org/10.1016/j.jaci.2022.06.012

9. Kabata H, Moro K, Koyasu S. The group 2 innate lymphoid cell (ILC 2) regulatory network and its underlying mechanisms. *Immunol Rev.* 2018;286(1):37-52. https://doi.org/10.1111/imr.12706

10. Kim EY, Park H, Kim EJ, Lee SH, Choi JW, Kim J, et al. Efficacy of Trigonella foenum-graecum Linné in an animal model of particulate matter-induced asthma exacerbation. *J Ethnopharmacol.* 2024;319:117228. https://doi.org/10.1016/j.jep.2023.117228

11. Valatas V, Kolios G, Bamias G. TL1A (TNFSF15) and DR3 (TNFRSF25): a co-stimulatory system of cytokines with diverse functions in gut mucosal immunity. *Front Immunol.* 2019;10:421466. https://doi.org/10.3389/fimmu.2019.00583

12. Yu Y, Jiang P, Sun P, Su N, Lin F. Analysis of therapeutic potential of preclinical models based on DR3/TL1A pathway modulation. *Exp Ther Med.* 2021;22(1):1-9. https://doi.org/10.3892/etm.2021.10125

13. Bouros E, Filidou E, Arvanitidis K, Mikroulis D, Steiropoulos P, Bamias G. Lung fibrosis-associated soluble mediators and bronchoalveolar lavage from idiopathic pulmonary fibrosis patients promote the expression of fibrogenic factors in subepithelial lung myofibroblasts. *Pulm Pharmacol Ther.* 2017;46:78-87. https://doi.org/10.1016/j.pupt.2017.08.012

14. Bessa SS, El-Magd GH, Mabrouk MM. Serum chemokines RANTES and monocyte chemoattractant protein-1 in Egyptian patients with atopic asthma: relationship to disease severity. Archives of medical research. 2012 Jan 1;43(1):36-41.

15. Lun SW, Wong CK, Ko FW, Ip WK, Hui DS, Lam CW. Aberrant expression of CC and CXC chemokines and their receptors in patients with asthma. *J Clin Immunol.* 2006;26:145-52. https://doi.org/10.1007/s10875-006-9003-9

16. Dimitrova D, Youroukova V, Ivanova-Todorova E, Tumangelova-Yuzeir K, Velikova T. Serum levels of IL-5, IL-6, IL-8, IL-13 and IL-17A in pre-defined groups of adult patients with moderate and severe bronchial asthma. *Respir Med.* 2019;154:144-54. http://doi.org/10.1016/j.rmed.2019.06.024

17. Alturaiki WH. Evaluation of CC chemokine ligand 5 (CCL5) chemokine, interleukin 5 (IL-5) cytokine, and eosinophil counts as potential biomarkers in Saudi patients with chronic asthma during sandstorms. *Cureus.* 2020;12(4). http://doi.org/10.7759/cureus.7809

18. John M, Oltmanns U, Binder C, Meiners S, Gellert K, Chung KF, Witt C. Inhibition of chemokine production from human airway smooth muscle cells by fluticasone, budesonide and beclomethasone. *Pulm Pharmacol Therap.* 2004 Feb 1;17(1):41-7. https://doi.org/10.1016/j.pupt.2003.09.002

19. Schuliga M. NF-kappaB signaling in chronic inflammatory airway disease. *Biomolecules.* 2015 Jun 26;5(3):1266-83. https://doi.org/10.3390/biom5031266

20. Porsbjerg CM, Sverrild A, Lloyd CM, Menzies-Gow AN, Bel EH. Anti-alarmins in asthma: targeting the airway epithelium with next-generation biologics. *Eur Resp J.* 2020;56(5). http://doi.org/10.1183/13993003.00260-2020

21. Schleimer RP, Kato A, Kern R, Kuperman D, Avila PC. Epithelium: at the interface of innate and adaptive immune responses. *J Allergy Clin Immunol.* 2007;120(6):1279-84. http://doi.org/10.1016/j.jaci.2007.08.046

22. Ramos-Martínez E, López-Vancell MR, de Córdova-Aguirre JF, Rojas-Serrano J, Chavarría A, Velasco-Medina A, Velázquez-Sámano G. Reduction of respiratory infections in asthma patients supplemented with vitamin D is related to increased serum IL-10 and IFNγ levels and cathelicidin expression. *Cytokine.* 2018;108:239-46. https://doi.org/10.1016/j.cyto.2018.01.001

23. Son JH, Kim JH, Chang HS, Park JS, Park CS. Relationship of microbial profile with airway immune response in eosinophilic or neutrophilic inflammation of asthmatics. *Allergy, Asthma Immunol Res.* 2020;12(3):412. https://doi.org/10.4168/aair.2020.12.3.412

24. Jakubovics NS, Strömberg N, Van Dolleweerd CJ, Kelly CG, Jenkinson HF. Differential binding specificities of oral streptococcal antigen I/II family adhesins for human or bacterial ligands. *Mol Microbiol.* 2005;55(5):1591-605. https://doi.org/10.1111/j.1365-2958.2005.04495.x

25. Kazaal MA, Habeeb AA, Hasan HN. Evaluation Role of IL-13 and Eosinophils in Adult Asthmatic Patients. *J Biomed Biochem.* 2023;2(2):17-24. https://doi.org/10.57238/jbb.2023.6940.1033

26. Cai F, Hornauer H, Peng K, Schofield CA, Scheerens H, Morimoto AM. Bioanalytical challenges and improved detection of circulating levels of IL-13. *Bioanalysis.* 2016;8(4):323-32.

27. Moran A, Pavord ID. Anti-IL-4/IL-13 for the treatment of asthma: The story so far. Expert Opin Biol Ther. 2020;20(3):283-94. https://doi.org/10.1080/14712598.2020.1714027

28. Sahoo A, Wali S, Nurieva R. T helper 2 and T follicular helper cells: Regulation and function of interleukin 4. *Cytokine Growth Factor Rev.* 2016;30:29-37. http://doi.org/10.1016/j.cytogfr.2016.03.011

29. Okab HF, Salih MB, Jarulla BA. Immunopathy of COVID-19 Patients without Chronic Disease: Proinflammatory and Anti-Inflammatory Cytokines Attributable to Disease Severity. *Laboratory Research in Clinical Practice.* 2024;13(1):47-59. http://doi.org/10.34883/PI.2024.13.1.004

30. Hams E, Fallon PG. Innate type 2 cells and asthma. *Curr Opin Pharmacol.* 2012;12(4):503-9. https://doi.org/10.1016/j.coph.2012.06.001

31. Doherty TA, Broide DH. Airway innate lymphoid cells in the induction and regulation of allergy. *Allergol Int.* 2019;68(1):9-16. https://doi.org/10.1016/j.alit.2018.11.001

32. Kubysheva N, Boldina M, Eliseeva T, Soodaeva S, Klimanov I, Khaletskaya A, et al. Relationship of Serum Levels of IL-17, IL-18, TNF-α, and Lung Function Parameters in Patients with COPD, Asthma-COPD Overlap, and Bronchial Asthma. *Mediators Inflamm.* 2020;2020(1):4652898. https://doi.org/10.1155/2020/4652898

33. Bazan-Socha S, Mastalerz L, Cybulska A, Zareba L, Kremers R, Zabczyk M, et al. Prothrombotic state in asthma is related to increased levels of inflammatory cytokines, IL-6 and TNFα, in peripheral blood. *Inflammation.* 2017;40:1225-35. http://doi.org/10.1007/s10753-017-0565-x

34. Kang MJ, Choi JM, Kim BH, Lee CM, Cho WK, Choe G, et al. IL-18 induces emphysema and airway and vascular remodeling via IFN-γ, IL-17A, and IL-13. *Am J Resp Crit Care Med.* 2012;185(11):1205-17. https://doi.org/10.1164/rccm.201108-1545OC

35. Dinarello CA. IL-18: A TH1-inducing, proinflammatory cytokine and new member of the IL-1 family. *J Allergy Clin Immunol.*

1999;103(1):11-24. http://doi.org/10.1016/S0091-6749(99)70518-X

36. Wardzyńska A, Pawełczyk M, Rywaniak J, Makowska J, Jamroz-Brzeska J, Kowalski ML. Circulating miRNA expression in asthmatics is age-related and associated with clinical asthma parameters, respiratory function and systemic inflammation. *Resp Res.* 2021;22(1):177. http://doi.org/10.1186/s12931-021-01769-x

37. Virchow Jr JC, Kroegel C, Walker C, Matthys H. Inflammatory determinants of asthma severity: mediator and cellular changes in bronchoalveolar lavage fluid of patients with severe asthma. *J Allergy Clin Immunol.* 1996;98(5):S27-40.

38. Giuffrida MJ, Valero N, Mosquera J, Duran A, Arocha F, Chacín B, et al. Increased Systemic Cytokine/Chemokine Expression in Asthmatic and Non-asthmatic Patients with Bacterial, Viral or Mixed Lung Infection. *Scand J Immunol.* 2017;85(4):280-90. http://doi.org/10.1111/sji.12532

39. Costa EL, Schettino IA, Schettino GP. The lung in sepsis: guilty or innocent? *Endocr Metab Immune Disord Drug Targets*. 2006;6(2):213-6. http://doi.org/10.2174/187153006777442413

40. Tirado R, Ortega A, Sarmiento RE, Gómez B. Interleukin-8 mRNA synthesis and protein secretion are continuously up-regulated by respiratory syncytial virus persistently infected cells. *Cell Immunol.* 2005;233(1):61-71. https://doi.org/10.1016/j.cellimm.2005.04.003

41. Fujitsuka A, Tsukagoshi H, Arakawa M, Goto-Sugai K, Ryo A, Okayama Y, et al. A molecular epidemiological study of respiratory viruses detected in Japanese children with acute wheezing illness. *BMC Infect Dis.* 2011 Dec;11:1-0. https://doi.org/10.1186/1471-2334-11-168

42. Chung KF. Cytokines in chronic obstructive pulmonary disease. *Eur Resp J Suppl.* 2001;18(34 suppl):50s-9s.

43. Önell A, Whiteman A, Nordlund B, Baldracchini F, Mazzoleni G, Hedlin G, et al. Allergy testing in children with persistent asthma: comparison of four diagnostic methods. *Allergy.* 2017;72(4):590-7. https://doi.org/10.1111/all.13047

44. Huang HJ, Campana R, Akinfenwa O, Curin M, Sarzsinszky E, Karsonova A, et al. Microarray-based allergy diagnosis: quo vadis? *Front Immunol.* 2021;11:594978. https://doi.org/10.3389/fimmu.2020.594978

45. Naumova V, Beltyukov E, Niespodziana K, Errhalt P, Valenta R, Karaulov A. Cumulative IgE-levels specific for respiratory allergens as biomarker to predict efficacy of anti-IgE-based treatment of severe asthma. *Front Immunology.* 2022;13:941492. https://doi.org/10.3389/fimmu.2022.941492

46. Crespo-Lessmann A, Curto E, Mateus E, Soto L, García-Moral A, Torrejón M. Total and specific immunoglobulin E in induced sputum in allergic and non-allergic asthma. *PloS one.* 2020;15(1):e0228045. https://doi.org/10.1371/journal.pone.0228045

47. Rondón C, Bogas G, Barrionuevo E, Blanca M, Torres MJ, Campo P. Nonallergic rhinitis and lower airway disease. *Allergy.* 2017;72(1):24-34. https://doi.org/10.1111/all.12988

48. Borriello EM, Vatrella A. Does non-allergic asthma still exist? Shortness of breath. 2013;2:55-60.

49. Humbert M, Grant JA, Taborda-Barata L, Durham SR, Pfister R, Menz G, et al. High-affinity IgE receptor (FcepsilonRI)-bearing cells in bronchial biopsies from atopic and nonatopic asthma. *Am J Respir Crit Care Med.* 1996;153(6):1931-7. https://doi.org/10.1164/ajrccm.153.6.8665058

50. Nowicka D, Grywalska E. The role of immune defects and colonization of Staphylococcus aureus in the pathogenesis of atopic dermatitis. *Anal Cell Pathol.* 2018;2018(1):1956403. https://doi.org/10.1155/2018/1956403

51. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic Dermatitis. *Nat Rev Dis Primers.* 2018;4(1):1. http://doi.org/10.1038/s41572-018-0001-z

52. Durack J, Lynch SV, Nariya S, Bhakta NR, Beigelman A, Castro M, et al. Features of the bronchial bacterial microbiome associated with atopy, asthma, and responsiveness to inhaled corticosteroid treatment. *J Allergy Clin Immunol.* 2017;140(1):63-75. http://doi.org/10.1016/j.jaci.2016.08.055

53. Heimann E, Nyman M, Pålbrink AK, Lindkvist-Petersson K, Degerman E. Branched short-chain fatty acids modulate glucose and lipid metabolism in primary adipocytes. *Adipocyte.* 2016;5(4):359-68. https://doi.org/10.1080/21623945.2016.1252011