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Interleukin-4 Gene Expression and Serum Levels as Biomarkers of Allergic Asthma in Iraqi Patients

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Abstract

Background. Allergic asthma is a chronic inflammatory disease of the airway in which exposure to allergens leads to a complex interaction between genetic and environmental factors, while many cytokines are involved in the immune mechanism of the disease including cytokines such as interleukin-4 (IL-4), IL-4 plays a critical role in the inflammatory process. **Aim.** This study aimed to investigate the relationship between gene expression and serum levels of IL-4 with Iraqi allergic asthmatic patients. **Methods.** A total of 100 patients and 50 healthy controls participated in reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used for gene expressions and IL-4 serum levels determined through ELISA. **Results.** The Results demonstrated a significant increase in *IL-4* gene expression in asthma patients compared to control, (Average fold change of 6.885). Similarly, serum IL-4 concentrations were significantly elevated in patients (mean fold change of 383.6516), correlating moderately with gene expression changes (ρ = 0.678). **Conclusion.** The study underscores the importance of IL-4 in allergic asthma and suggests that *IL-4* expression and serum levels could aid in monitoring asthma activity.

Keywords: Allergic asthma, IL-4, Gene expression, RT-qPCR, ELISA.

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Introduction

Asthma is a prevalent multifactorial inflammatory condition affected by genetic and environmental variables, marked by airway inflammation, blockage, coughing, and wheezing triggered by allergens or pollution. It is a chronic respiratory disease with reversible airway contraction and excessive mucus production. **(1**,2). According to the World Health Organization (WHO), asthma resulted in 455,000 fatalities globally in 2019 and affected more than 262 million individuals (3). The incidence of asthma and allergies has significantly risen in recent decades, attributable to various factors including rapid urbanization, industry leading to air pollution, smoking, mold exposure, dietary habits, and obesity (4).

Allergic asthma is the predominant asthma phenotype, characterized by IgE sensitivity to airborne allergens and the manifestation of classic asthmatic symptoms upon exposure (5). It is marked by heightened T helper (Th) type 2 immune responses to airborne allergens(6).

Cytokines regulate allergic asthma by recruiting, activating, and promoting inflammatory cells in the respiratory tract, with numerous identified in asthma, playing a crucial role in the complex airway disease pathophysiology (7). Interleukin 4 (IL-4) is a cytokine associated with the cause of asthma and

several allergic diseases (8). Exposure of a susceptible individual to aeroallergens causes activation of the epithelium in the airways, which then leads to the release of epithelial-derived cytokines that are referred to as "alarm signs" or "alarmins (9). Antigen-presenting cells (APC) are responsible for presenting allergens to naïve T lymphocytes in the lymph nodes of the local area. These cells, in conjunction with co-stimulatory molecules, undergo differentiation into Th2 cells, which are responsible for the secretion of significant quantities of type 2 cytokines, including IL-4, IL-5, and IL-13 (10). IL-4 drives the IgE isotype switch change in B cells and the subsequent production of IgE allergen-specific antibodies in response to allergen stimulation (11).

The *IL-4* gene is located on chromosome 5q31, a region known to harbor multiple cytokine genes, including *IL-13* and *IL-5*, which are closely linked to the Th2 immune response (12,13). Recent studies suggest that *IL-4* gene expression in peripheral blood could serve as a potential biomarker for allergic asthma, as it correlates with asthma severity in blood cells (14).

In Iraq, various studies have investigated factors contributing to allergic asthma. Al-Qadhi and Al-Saadi **(15)** examined the influence of smoking, family history, disease severity, allergens, and IL-4R levels in allergic asthma patients. Furthermore, associations between cytokines and allergic asthma have been extensively studied. Al-Thwani *et al*. **(16)** reported elevated serum interleukin-1β (IL-1β) levels in 50 asthma patients, while Abdulkareem and Al-Saadi **(17)** evaluated interleukin-17 (IL-17) gene expression in a cohort of 75 asthma patients in Baghdad.

This study aimed to investigate the relationship between allergic asthma and the up-regulation of *IL-4* gene expression levels in allergic asthma patients compared to the control through measuring mRNA levels by qPCR technique and confirming the results by measuring the protein levels using ELISA.

Materials and Methods

Patients and control

One hundred patients were enrolled in this study, including 62 females and 38 males (patients group) diagnosed with allergic asthma, within an age range of 7 to 76 year. Additionally, 50 apparently healthy individuals, comprising 31 females and 19 males (control group), aged between 10 and 50 years, were included. The participants were attended the Al-Zahra Center for Allergy and Asthma in Baghdad/Al-Karkh/Iraq and the Specialized Centre for Allergy in Baghdad/Al-Rusafa between October 2023 and June 2024. The study obtained approval from the Ministry of Health (MOH) and obtained informed consent from all participants.

Blood samples

Blood samples were obtained through venipuncture utilizing disposable syringes in accordance with aseptic protocols. Five milliliters of venous blood were collected from each participant into sterile tubes. Each blood sample was subsequently partitioned into two aliquots: two milliliters placed into a sterile tube containing ethylene diamine tetraacetic acid (EDTA) and then converted to 250 µL of EDTA blood in Eppendorf tubes that contain 750 µL Trizol and freezed at -20°C to be a source for RNA extraction and gene expression analysis by using Real time-PCR technique, and three

milliliters transferred into a plain tube for serum separation intended for enzyme-linked immunosorbent assay (ELISA).

Gene expression of *IL4*

The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) technique applied for the *IL4 gene* expression Briefly, using Luna Universal qPCR MasterMix System kit (NEB, USA) to assess the expression with the forward and reverse primers as following:

F- CTGCTTCCCCCTCTGTTCTTC

R- TCTGTTACGGTCAACTCGGTG

Also *GAPDH* (reference gene: *glyceraldehyde-3- phosphate dehydrogenase*) using as housekeeping gene with primer as following:

F- 5'- GTCTCCTCTGACTTCAA-3′ R- 5'- ACCACCCTGTTGCTGTA-3′

The assessment was carried out in a 20 µl reaction volume, which consisted of 10 µl master mix, 0.5 µl Forward primer, 0.5 Reverse primer, 5 µl Template DNA and 4 µl nuclease-free water.

The PCR cycling conditions were performed according to the manufacturer's instructions (NEB, USA). cDNA synthesis was carried out at 37°C for 15 minutes (1

cycle), followed by an initial denaturation at 95°C for 1 minute (1 cycle). Subsequently, 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds were conducted. The *IL4* gene expression was evaluated using the double delta Ct method, with reference *housekeeping gene* (Figure, 1). The results were presented as a fold change in gene expression (2 -ΔΔCt), normalized to the endogenous control and relative to the calibrator, which represented the target gene in control subjects (18).

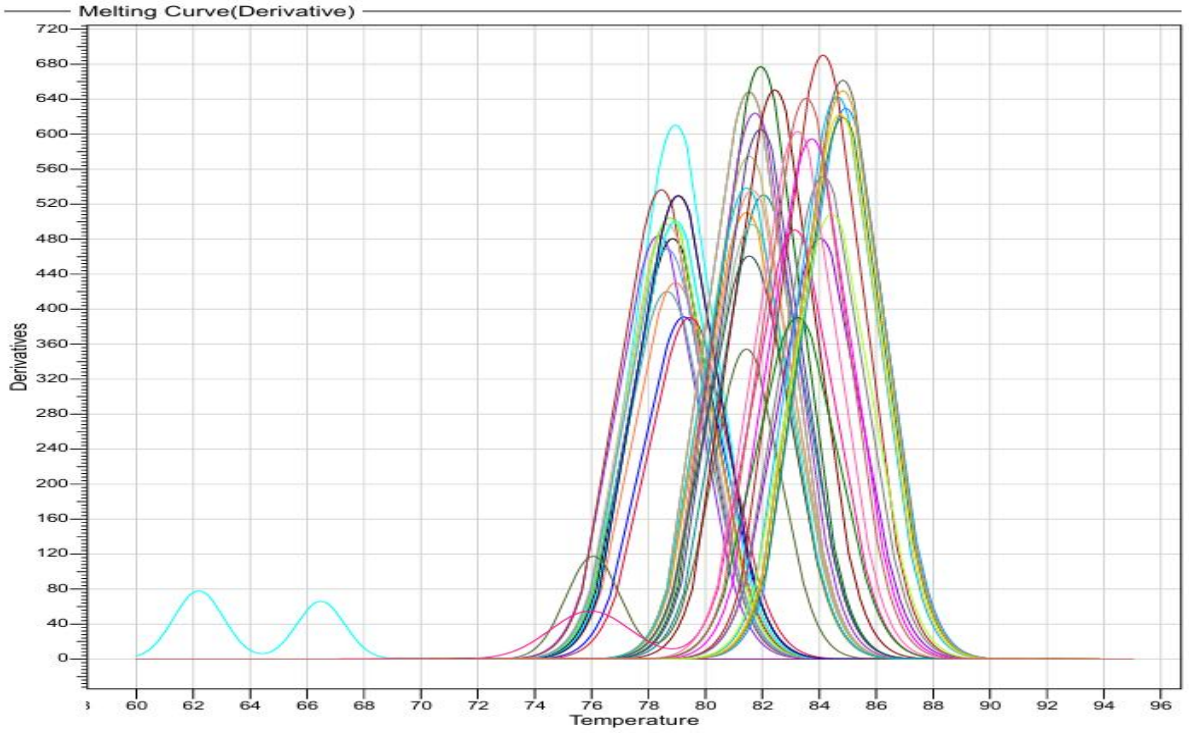

Figure (1): The melting curve of primer which explain the high specificity for the primer that have been used in this study.

Determination the IL-4 serum level by ELISA

A sample of 3 mL of peripheral blood was collected from asthma patients and healthy control. The blood sample (3ml) which clotted for 15 minutes at room temperature and centrifuged for 15 minutes. The serum was frozen at -20°C, the used to assess IL-4 serum levels by Sandwich ELISA kits (Elabscience, U.S.A.). The optical density (OD) was measured spectrophotometrically at 450 nm ± 2 nm, and the concentration of human IL-4 was calculated by comparing the OD to the standard curve.

Result and discussion

The main objective of this study is to investigate the connection between allergic asthma and IL-4, by assessing its serum levels and gene expression in allergic asthma patients.

Gene expression of *IL4*

The study assessed the log fold change in *IL4* gene expression in sample of Iraqi patients with allergic asthma. The results demonstrated a significant alteration in gene expression within the patients group, with the fold change in gene expression in the asthmatic patients group being 6.885 times higher than in the control group. In contrast, the fold change in the control group was 1.00, as shown in Table 1.

Table (1): Fold of IL-4 gene expression

Group	Mean Ct IL-4	Mean Ct Housekeeping Gene	Δ CT	ΔΔ CT	2^-(ΔΔ CT)	Average Fold Change
Patients	29.86	33.92	-4.06	-2.75	6.885	6.885
control	30.075	31.39	-1.31	0	1.00	1.00

The substantial increase in *IL-4* expression observed in this study corroborates its established function as a central player in the allergic inflammatory cascade. This finding is consistent with Vasudevan *et al.* (19), who reported higher levels of *IL-4* expression in individuals with asthma compared to healthy control , emphasizing its involvement in immune dysregulation during allergic reactions. Additionally, Tavakol *et al.* (20) in Iran, who heightened *IL-4* expression was observed in a cohort of twenty allergic asthmatic individuals. Moreover, Al-Daghri *et al*. (21) found that *IL-4 mRNA* expression was significantly upregulated in pediatric asthma patients and highlighting its fundamental role in asthma pathogenesis, in a study, involving 85 patients and an equal number of healthy individuals from Saudi Arabia. Recent investigations have focused on the gene expression of *IL-4* in peripheral blood as a potential biomarker for allergic asthma, when levels of *IL-4* mRNA expression in blood cells have been shown to correlate with asthma severity, suggesting that *IL-4* gene expression may serve as a valuable indicator of asthma activity (22). Biological Explanations for *IL-4* Dysregulation can be attributed to genetic predisposition and environmental triggers, such as pollution, smoking, and exposure to indoor allergens like fungi and dust mites. These factors stimulate epithelial-derived cytokines, known as "alarmins," which activate T-helper 2 (Th2) cells. These cells produce IL-4, which facilitates the isotype switching of B cells to produce

allergen-specific IgE antibodies, perpetuating the allergic response (9).

Determination of IL-4 serum level in studied groups

Meanwhile, to study the influence of allergic asthma on the IL4 serum concentration, an ELISA test was performed on the sera of both patients and control. The results revealed a significant increase in the serum level of the patients group (383.6516) compared to the control group (88.9669), as shown in Figure (2).

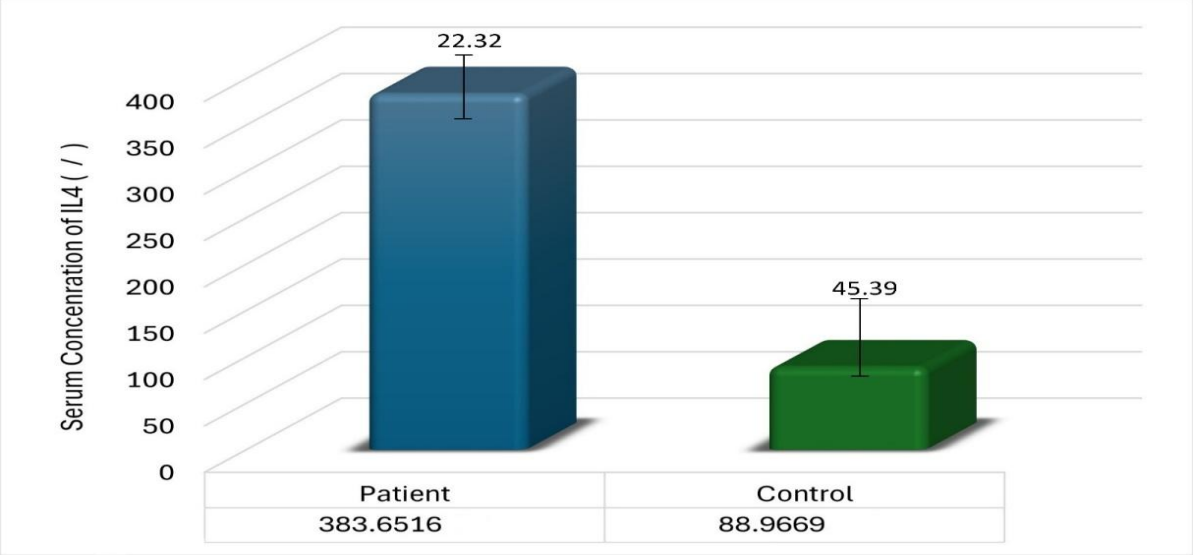

Figure (2): The variation in the sera level concentration of the IL4 between the patients and control groups.

The study conducted a two-tailed independent samples t-test to confirm the change in IL4 fold serum concentration between the patients and control groups. The Table (2) presents the results of a two-tailed independent samples t-test, which demonstrated a statistically significant difference ($P < 0.00001$) in IL-4 serum levels between the patients and the control groups. The mean serum IL-4 level in the patients group was significantly higher ($M = 393.41$) compared to the control group ($M = 99.36$). This highly significant difference underscores the potential role of IL-4 as a biomarker for distinguishing between the two groups.

Table (2): The IL4 serum levels of patients and control groups.

Parameter	Patients	Control
Mean	393.41	99.36
Observations	100	50
t-test Value	40.13	
P value two-tail	0.00001	highly significance

The results shown in Table (2) demonstrate a significant elevation in IL-4 serum levels among individuals with allergic asthma compared to the control group. This finding is consistent with the research conducted by Naif and Ibraheem (23), who investigated 50 Iraqi asthmatic patients and an equal number of healthy control from the Babylon Governorate, their study similarly reported that serum IL-4 concentrations were markedly higher in asthma patients than in the healthy control group. Also in a recent systematic review and meta-analysis conducted by Nepolo *et al*. (24) showed elevated IL-4 levels in patients with allergies, highlighting its role in disease prevalence. Factors such as rapid urbanization, dietary shifts, and heightened exposure to industrial pollutants significantly contribute to the increasing prevalence of allergic asthma.

Increased *IL-4* activity not only amplifies inflammation but also disrupts airway epithelial integrity, leading to enhanced susceptibility to environmental allergens. This cascade exacerbates asthma symptoms, including airway narrowing, excessive mucus production, and chronic inflammation, as corroborated by Robinson *et al*. (7).

Spearman's rank correlation analysis was performed to assess the relationship between serum IL-4 concentration and the Log_2 fold change of IL-4 gene expression in the study groups. The analysis revealed a moderate positive correlation ($\rho = 0.678$), indicating an association between serum IL-4 levels and changes in its gene expression. Similar findings were reported by Tavakol et al. (20), who demonstrated a correlation between IL-4 gene expression and its corresponding serum levels.

Conclusion

This study highlights the role of IL-4 in allergic asthma, revealing elevated gene expression and sera level in Iraqi patients. The correlation between *IL-4* gene expression and serum concentrations suggests its potential as a biomarker for monitoring asthma severity and activity. The results underscore IL-4's importance in asthma pathogenesis and its role in diagnostic and therapeutic approaches.

References

1. Goodi, G., & AL-Saadi, B. Q. (2018). Polymorphism of FOXO3a Gene and Its Association with Incidence of Asthma in Iraqi Patients. Iraqi Journal of Biotechnology, 17(3).
2. Kowalewicz-Kulbat, M., & Locht, C. (2021). BCG for the prevention and treatment of allergic asthma. Vaccine, 39(50), 7341–7352.
3. Shahid, S., Jaan, G., Nadeem, A., Nadeem, J., Fatima, K., Sajjad, A., Javed, F., Mazhar, M., & Iqbal, M. Z. (2024). Effect of educational intervention on quality of life of asthma patients: A systematic review. Medical Science, 28, e9ms3300.
4. Baldacci, S., Maio, S., Cerrai, S., Sarno, G., Baïz, N., Simoni, M., Annesi-Maesano, I., Viegi, G., & Study, H. (2015). Allergy and asthma: effects of the exposure to particulate matter and biological allergens. Respiratory Medicine, 109(9), 1089–1104.
5. Papapostolou, N., & Makris, M. (2022). Allergic asthma in the era of personalized medicine. Journal of Personalized Medicine, 12(7), 1162.
6. Morianos, I., & Semitekolou, M. (2020). Dendritic cells: critical regulators of allergic asthma. International Journal of Molecular Sciences, 21(21), 7930.
7. Robinson, D., Humbert, M., Buhl, R., Cruz, A. A., Inoue, H., Korom, S., Hanania, N. A., & Nair, P. (2017). Revisiting T ype 2-high and T ype 2-low airway inflammation in asthma: current knowledge and therapeutic implications. Clinical & Experimental Allergy, 47(2), 161–175.

8. Hameed, R. M., Najim Abood, H., & Al-Hasnawy, H. H. (2023). Association of Interleukin-4 in Immunoglobulin-E Mediated Asthma: A Cross Sectional Study. Journal of Medicine, 24(2).
9. Mitchell, P. D., & O'Byrne, P. M. (2017). Epithelial-derived cytokines in asthma. Chest, 151(6), 1338–1344.
10. Kuruvilla, M. E., Lee, F., & Lee, G. B. (2019). Understanding asthma phenotypes, endotypes, and mechanisms of disease. Massey O, Suphioglu C. Recent Advances in the Inhibition of the IL-4 Cytokine Pathway for the Treatment of Allergen-Induced Asthma. Int J Mol Sci. 2021;22(24):13655., 56, 219–233.
11. Gause, W. C., Wynn, T. A., & Allen, J. E. (2013). Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nature Reviews Immunology, 13(8), 607–614.
12. Sevak, V., Chinniah, R., Pandi, S., Sampathkumar, K., Dinakaran, T., & Karuppiah, B. (2024). Association of IL-4 (− 590 C/T) and IL-6 (− 174 G/C) gene polymorphism in South Indian CKD patients. Egyptian Journal of Medical Human Genetics, 25(1), 17.
13. Seyfizadeh, N., Seyfizadeh, N., Gharibi, T., & Babaloo, Z. (2015). Interleukin-13 as an important cytokine: A review on its roles in some human diseases. Acta Microbiologica et Immunologica Hungarica, 62(4), 341–378.
14. Kousha, A., Mahdavi Gorabi, A., Forouzesh, M., Hosseini, M., Alexander, M., Imani, D., Razi, B., Mousavi, M. J., Aslani, S., & Mikaeili, H. (2020). Interleukin 4 gene polymorphism (− 589C/T) and the risk of asthma: a meta-analysis and met-regression based on 55 studies. BMC Immunology, 21, 1–16.
15. AL-Qadhi, I. Y., & AL-Saadi, B. Q. (2022). Impact of IL-4R (rs1805011) Gene Polymorphism on IL-4 Serum Level in Iraqi Allergic Asthma Patients. Iraqi Journal of Biotechnology, 21(2).
16. Al-Thwani, A., Janabi, yehya, & Tiaee, K. (2021). Evaluation the Role of IL-1βand TNFα with Asthma. Annals of Tropical Medicine and Public Health, 24, 1–6. https://doi.org/10.36295/ASRO.2021.24405
17. Abdulkareem, Z. T., & AL-Saadi, B. Q. H. (2020). Determine gene expression of IL-17 in Iraqi Child Asthmatic Patients. Iraqi Journal of Biotechnology, 3(19).
18. Stephenson, F. H. (2016). Chapter 9–Real-Time PCR. Calculations for Molecular Biology and Biotechnology, 215–320.
19. Vasudevan, R., Norhasniza, M. N., & Patimah, I. (2011). Association of variable number of tandem repeats polymorphism in the IL-4 gene with end-stage renal disease in Malaysian patients. Genet Mol Res, 10(2), 943–947.
20. Tavakol, A. J., Hosseini, F. S., Khoshnavazi, R., Ghayour, K. E., Ghassemi, G., Farid, H. R., Heydarian, F., Boskabadi, M. H., & Razavi, A. R. (2007). Association of the expression of IL-4 and IL-13 genes, IL-4 and IgE serum levels with allergic asthma.
21. Al-Daghri, N. M., Abd-Alrahman, S., Draz, H., Alkharfy, K., Mohammed, A. K., Clerici, M. S., & Alokail, M. S. (2014). Increased IL-4 mRNA expression and poly-aromatic hydrocarbon concentrations from children with asthma. BMC Pediatrics, 14, 1–8.
22. Kousha, A., Mahdavi Gorabi, A., Forouzesh, M., Hosseini, M., Alexander, M., Imani, D., Razi, B., Mousavi, M. J., Aslani, S., & Mikaeili, H. (2020). Interleukin 4 gene polymorphism (− 589C/T) and the risk of asthma: a meta-analysis and met-regression based on 55 studies. BMC Immunology, 21, 1–16.
23. Naif, A. J., & Ibraheam, I. A. (2024). Cytokine Genetic Polymorphism of Interleukin-4 and Risk of Asthma in Some Iraqi Patients. Medical Journal of Babylon, 21(2), 359–363. htps/doi.org/10.4103/MJBL.MJBL_816_23
24. Nepolo, E. P., Nkambule, B. B., Dludla, P. V, Ndevahoma, F., & Nyambuya, T. M. (2022). Association between the type of allergen and T-helper 2 mediated inflammation in allergic reactions: a systematic review and a meta-analysis. Allergologia et Immunopathologia, 50(1), 37–50.