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Running head: Correlation between cytokines, IgE, vitD and asthma

IL-35 and IL-37 are negatively correlated with high IgE production among children with asthma in Saudi Arabia

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Abstract

Asthma is one of the most prevalent inflammatory disorders among children in Saudi Arabia. This study aimed to determine the correlation between the serum levels of vitamin D, immunoglobulin E (IgE), and cytokine (interferon-gamma (IFN- γ), interleukin (IL)-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) in relation to the severity of disease in patients with asthma. This case-control study was carried out at King Abdullah Specialist Children's Hospital, Saudi Arabia, and included 48 patients with asthma and 47 matched controls, aged 6-14 years. A validated questionnaire was administered to the participants, after which each patient with asthma underwent pulmonary function tests. The serum levels of vitamin D, IgE, IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 of each participant were also measured. Patients with asthma demonstrated significantly higher IgE and cytokine (IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) levels compared to the control group (p-value < 0.001). The levels of IL-1 β , IL-4, IL-10, and IL-13 were consistently positively correlated with the serum levels of IgE among patients with asthma. However, the IgE levels in patients with asthma were consistently negatively correlated with IL-35 and IL-37. We found significantly higher levels of eosinophils, IgE, IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 in patients with asthma compared to the controls, but no relationship between vitamin D and asthma.

Keywords: asthma; vitamin D; IgE; cytokines; KSA

Introduction

Asthma is a chronic airway inflammatory disorder that affects children and adolescents, is commonly associated with atopy, and has a higher prevalence in males (1). Globally, it is a major cause of morbidity and a health-care burden (2). Several national studies have reported a dramatic increase in the prevalence of asthma in specific populations within Saudi Arabia (KSA) (3–6).

Inflammation is a pivotal founder effect required for the development of asthma. Patients with asthma typically present with a cough, recurrent episodic wheeze, and breathlessness. Immunoglobulin E (IgE) is essential for the pathogenesis of many allergic diseases and is the antibody that mediates type I hypersensitivity (7). In humans, IgE receptors are located on the cell membrane of basophils, eosinophils, mast cells, monocytes, macrophages, and platelets (7). In asthma, inflammation involves infiltration of the airway walls with different immune cells such as mast cells, eosinophils, neutrophils, and macrophages. Besides, variable inflammatory mediators exist, including interferon-gamma (IFN- γ), interleukin (IL)-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 (8, 9).

Furthermore, Vitamin D has been recently linked to several immune-related disorders, including asthma, allergy, and autoimmunity (10). In KSA, vitamin D deficiency is an emerging problem, and higher serum levels of vitamin D have been associated with reduced asthma attacks, improved lung function, enhanced glucocorticoid effects, and reduced airway hyperactivity (11, 12). Further studies need to be conducted that evaluate all possible factors responsible for uncontrolled asthma among children, particularly due to the evident involvement of cytokines and vitamin D in asthma at different levels. Therefore, this study aimed to determine the serum levels of vitamin D, IgE, and cytokines (IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) in children with asthma and correlate these levels with the severity of disease according to the Global Initiative for Asthma guidelines and pulmonary function tests.

Materials and methods

Study population

Ninety-five children at King Abdullah Specialist Children's Hospital were enrolled in this case-control study. These included 61 (64%) females and 34 (64%) males aged between six and 14 years. The patients were classified into two groups: Group I (healthy control; n = 47) and Group II (the patients with asthma; n = 48). Children with chronic pulmonary conditions, chronic diseases, and a history of developmental delay were excluded.

Questionnaires

The parents of each participating child completed a slightly modified version of the guidelines of the Global Initiative for Asthma, which can be retrieved online using this link: https://ginasthma.org/wp-content/uploads/2016/01/GINA_Pocket_2015.pdf

The questionnaire was designed to assess the presence or absence of asthma symptoms, use of bronchodilators and nutritional supplements, and activity limitations. Based on these criteria, in addition to the pulmonary function tests, asthma control was assessed and classified as well-controlled or uncontrolled.

Pulmonary function tests

Spirometry was performed only on children with asthma using Spirobank II[®] BLE (Medical International Research). The following parameters were measured in each child with asthma: the forced expiratory volume in the first second, the forced vital capacity, forced expiratory volume in the first second/forced vital capacity ratio, and the forced expiratory flow collected between 25% and 75% of the vital capacity.

Blood collection

Approximately 6 ml of venous blood was collected from each of the participants in two different vacutainers, one containing ethylenediaminetetraacetic acid (EDTA) and one non-additive. The blood samples from the EDTA vacutainers were used to ascertain the children's hematological profiles and baseline biochemical parameters and were processed at the King Abdullah Specialist Children's Hospital laboratory on the day of blood collection. The blood in the non-additive vacutainers was centrifuged to separate the serum, which was stored at -70 °C until used for the analysis of 25-hydroxyvitamin D, total IgE, IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 in the King Abdullah International Medical Research Center.

Measurement of serum vitamin D

The serum levels of vitamin D were determined using a DAsource 25OH Vitamin D total enzyme-linked immunosorbent assay (ELISA) kit (DAsource ImmunoAssays, Belgium, cat # KAP1971). Vitamin D levels were considered sufficient if they were above 30 ng/ml and deficient if they were below 30 ng/ml.

Total serum IgE estimation

The total serum IgE levels were determined using the Abnova IgE ELISA kit (IgE Human ELISA kit, cat # KA0216). IgE levels were considered normal if they were lower than 200 IU/ml (15). Both vitamin D and IgE were measured in duplicate, and the mean values were calculated.

Estimation of IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37

The serum levels of IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 were assessed using ELISA kits (Abcam, Cambridge, UK). Duplicate cytokine measurements were carried out in compliance with the company's protocols. The sensitivity value of the ELISA kits for the cytokines was 0.1 pg/ml, ranging from 0.1 to 3,500 pg/ml.

Ethical considerations

The parents of all the participants provided informed written consent. Individuals over eight years of age were directly asked to give their consent. King Abdullah International Medical Research Center approved and funded the study protocol (Institutional Review Board number: SP16/046).

Statistical analysis

Simple descriptive statistics for the quantitative values were identified and reported as means and standard deviations (SDs), while discrete variables were described as frequencies and percentages (n, %). A linear regression analysis model was used to assess the risk of cytokine concentration in patients with asthma. Linear regression models were used to predict each cytokine level fitted into a separated linear model within the unadjusted data to avoid any statistical influence on the result (Table 3). Unstandardized coefficient (B) was adjusted for each cytokine with sex and age (Table 3). A logistic regression analysis was used to assess the associations of the circulating levels of vitamin D, IgE, and cytokines (IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) in patients with uncontrolled atopic asthma compared to the group with well-controlled asthma (Table 4). Spearman's correlation analysis was performed to determine the relationship between serum IgE and cytokine levels among patients with asthma.

Results

Ninety-five patients that were enrolled in this study were divided into two groups: Group I (healthy controls; n = 47) and Group II (patients with asthma; n = 48), as shown in Table 1. The mean age of the control group was 9.09 ± 2.073 (mean age $\pm$ SD) years, which was comparable with the asthma group (9.35 ± 2.129 years). There was a higher prevalence of female patients with asthma than males to a statistically significant level (p-value = 0.008; Table 1). There was no statistically significant difference between the patients with asthma group and the controls

regarding weight, height, and total white blood cell count (p-value > 0.5; Table 1). However, the mean eosinophil count in the patients with asthma group was significantly higher compared to the control group (0.39 ± 0.20 vs. 0.27 ± 0.23 [$\times 10^{-9}$ L]; p-value = 0.01; Table 1). No significant difference was observed among the patients with asthma regarding calcium, phosphates, and magnesium levels compared to the control group (p-value > 0.05; Table 5).

Serum levels of vitamin D, IgE, and cytokines across the study groups

The patients with asthma displayed significantly higher IgE, IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 levels compared to the healthy control group (p-value < 0.001; Table 2).

On the other hand, there were no significant differences in the serum levels of vitamin D and IFN- γ among the patients with asthma compared to the controls (p-values 0.31 and 0.82, respectively; Table 2).

The linear regression analysis indicated significantly increased levels of circulating IgE, IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 among children with asthma compared to the controls. The unadjusted B (95% CI) for IgE was 5.86 (95% CI 5.49-6.24; p-value < 0.001), 41.85 (95% CI 41.85-42.3; p-value < 0.001) for IL-1 β , 140.47 (95% CI 138.77-142.18; p-value < 0.001) for IL-4, 116.45 (95% CI 110.82-122.07; p-value < 0.001) for IL-6, 120.59 (95% CI 117.89-123.27; p-value < 0.001) for IL-10, 125.37 (95% CI 123.89-127.54; p-value < 0.001) for IL-13, 22.68 (95% CI 19.78-25.58; p-value < 0.001) for IL-35, and 131.48 (95% CI 123.5-139.5; p-value < 0.001; Table 3) for IL-37. A similar statistical analysis indicated significantly increased levels of IgE, IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 among the patients with asthma compared to the controls where B was adjusted for sex and age.

The results showed a slight decrease in vitamin D levels in children with asthma compared to the control group, but this was not statistically significant (-0.05; 95% CI -0.16-0.05; p-value = 0.82) (Table 3). IFN- γ also showed an insignificant difference between the patients with asthma and controls (0.36; 95% CI -2.73-3.44; p-value = 0.33; Table 3).

Risk factors for increased circulating levels of vitamin D, IgE, and cytokines in patients with uncontrolled asthma compared to well-controlled asthma

The risk factors analysis of the increased circulating levels of vitamin D, IgE, and cytokines (IFN- γ , IL-1 β , IL-4, IL-6, IL-10, and IL-35) among patients with uncontrolled and well-controlled asthma showed no significant association (p-value > 0.05). Our results revealed a 1.2 unit significant increase in the levels of circulating IL-13 in patients with uncontrolled asthma compared to patients with well-controlled asthma (unadjusted OR = 1.22; 95% CI 1.02-1.46; p-value = 0.03 and adjusted OR = 1.22; 95% CI 1.01-1.47; p-value = 0.04; Table 4). However, patients with uncontrolled asthma showed significantly decreased levels of the cytokine, IL-37, compared to patients with well-controlled asthma (unadjusted OR = 0.98; 95% CI 0.95-1.01; p-value = 0.04 and adjusted OR = 0.98; 95% CI 0.95-1.02; p-value = 0.04; Table 4).

Correlations between the serum levels of IgE and the levels of cytokines (IL-1 β , IL-4, IL-10, IL-13, IL-35, and IL-37) in patients with asthma

The levels of cytokines (IL-1 β , IL-4, IL-10, and IL-13) were consistently positively correlated with the serum levels of IgE among the patients with asthma. However, the IgE levels were consistently negatively correlated with IL-35 and IL-37 (Figure 1).

Discussion

IL-4 and IL-13 have nearly similar functional properties and have been classically attributed to the pathogenesis of asthma and allergic conditions (13). Both IL-4 and IL-13 are implicated in the induction of the pathognomonic features associated with type II inflammation, such as IgE synthesis, airway hyper-responsiveness, smooth muscle contraction, mucus hypersecretion, and eosinophil cells recruitment (14). In this study, the IgE, IL-4, and IL-13 levels and eosinophils count among the patients with asthma were significantly higher than the controls. Moreover, the risk factors analysis revealed significantly higher levels of IL-13 in patients with uncontrolled asthma compared to patients with well-controlled asthma, reflecting the importance of IL-13 in the pathogenesis of asthma.

Recently, a number of studies have revealed the fundamental role of IL-6 in the pathogenesis of asthma and showed a significant correlation of IL-6 levels with compromised lung function in many patients with asthma (15). Our data revealed significantly higher levels of IL-6 in patients with asthma, and this finding can support the potential benefit of IL-6 in the treatment of asthma and other allergic disorders.

It has been reported that the immunosuppressive effect of IL-10 is mediated via the suppression of cytokine production and inhibition of cell-mediated immunity (16). Furthermore, IL-10 modulates IgE synthesis in asthma and allergic diseases and is involved in inhibiting cytokine production from Th2 cells, mast cells, and eosinophils (17). However, in several studies, the level of IL-10 in patients with asthma was lower than in the controls and inversely correlated with the incidence and severity of asthma and allergic diseases, denoting the crucial role of IL-10 in asthma control (18, 19). In contrast, another study reported that the levels of IL-10 in children with asthma that had no family history of asthma were comparable to the controls (20). However, our findings demonstrated higher levels of IL-10 in the patients with asthma compared to the

healthy controls. These results are in line with the findings from a viral-induced asthma study that reported high levels of IL-10 during the commencement of the disease (21). Furthermore, Zhang et al. reported an increase in the IL-10 levels in children with asthma and mouse models compared to controls, consistent with our findings (22).

High levels of IgE is a major immunological disorder in atopic diseases. IL-4 and IFN- γ reciprocally regulate IgE synthesis. That is, while IL-4 enhances IgE production, IFN- γ hinders this IL-4 effect. Moreover, IL-4 inhibits IFN- γ production at the transcription level. Therefore, the balance of these two cytokines appears to be essential in regulating the production of IgE (23). However, this study revealed no statistically significant difference in the levels of IFN- γ between the patients with asthma and healthy controls. IL-1 β is a pro-inflammatory cytokine reported to be increased in stable and exacerbating asthma (24). Accordingly, our data showed a significant increase in the level of IL-1 β among patients with asthma compared to the controls. Furthermore, IL-1 β was positively correlated with the IgE levels in these patients.

Our results suggest that high levels of IFN- γ are significantly associated with uncontrolled atopic asthma. This finding is not surprising, especially when patients with uncontrolled asthma are not under glucocorticoid therapy. Our finding is in line with a study by Naumov et al. (25), who indicated that the levels of IFN- γ were significantly increased in uncontrolled asthma compared with partially or fully controlled asthma.

Recent studies have reported the abnormal expression of anti-inflammatory cytokines, IL-35 and IL-37, in patients with asthma, advocating their crucial role in the pathogenesis of disease (26, 27). We also found a significantly higher plasma level of IL-35 in patients with asthma compared to the controls. Interestingly, we also noticed a significant negative correlation between the plasma levels of IL-35 and total IgE in the patients with asthma. Wong et al. (28) found that

patients with asthma had higher levels of IL-35 than the healthy controls, and these levels were positively correlated with the severity of asthma. In contrast, Wang et al. (29) observed decreased IL-35 levels in patients with asthma. Furthermore, Khoshkhui et al. (30) found no significant correlation between asthma and the level of IL-35 in children, although the level of IL-35 was higher in patients with asthma. The existence of different asthma phenotypes could explain this diversity of findings, and, as a result, IL-35 may contribute to asthma pathogenesis through different mechanisms depending on the phenotype of disease. However, further studies are required to understand the exact role of IL-35 in different asthma phenotypes, which could lead to a new approach to asthma therapy.

Several inflammatory cytokines have been identified in the pathogenesis of asthma and inflammatory disorders, and the equilibrium between the counteractions of these cytokines can affect the severity of asthma (35, 36). Previous studies have indicated that IL-37 has a suppressive immune response and shifts the cytokine balance aside from extreme inflammation (36). IL-37 has been reported to alleviate skin hypersensitivity in mice following antigen activation, as well as enhance the production of IL-10 and inhibition of IL-1 β , IL-6, and TNF- α (37). Following the treatment of mice with IL-37, the total count of eosinophil cells in a bronchoalveolar lavage fluid decreased significantly (38). However, Huang et al. observed that after IL-37 treatment, the number of macrophages, lymphocytes, and neutrophils in the bronchoalveolar lavage fluid was significantly reduced (39). In addition, Meng et al. found that mucus secretion was decreased in mice treated with IL-37 (31). In contrast, Lv et al. (32) indicated that IL-37 did not influence the production of mucus. Other studies have shown that IL-37 increased in different immune-mediated disorders (33, 34). Increased expression of IL-37 has also been positively correlated with the severity of adult-onset Still's disease, a rare type of

arthritis (35). However, our results revealed significantly higher IL-37 serum levels in the patients with asthma compared to the healthy controls. Interestingly, the risk factor analysis of the increased IL-37 levels showed that the patients with uncontrolled asthma had a significant decrease in IL-37 levels compared to patients with well-controlled asthma. Additionally, the IgE and IL-37 levels were negatively correlated in the patients with asthma group, which could be explained by the influence of IL-37 cytokine on the T-helper (Th) cell balance and suppression of the Th2 and Th17 reactions. This result is consistent with a recent study by Wang et al., which suggested that IL-37 cytokine therapy in the murine model reduces allergy symptoms and lowers IgE serum levels (36). In contrast, patients with no allergic asthma showed increased pro-inflammatory IL-37 (36). These differences may be due to variations in the affected tissues, experimental models, and measurement methods employed between studies.

In contrast to various studies that documented an association between asthma symptoms and vitamin D deficiency (37), no significant relationship between vitamin D and asthma was found in the current study. Children with asthma did not vary substantially from children without asthma in their plasma vitamin D concentrations. For patients with uncontrolled asthma, vitamin D concentrations also did not differ significantly from those of children with well-controlled asthma. These findings are consistent with the study by Menon et al. (38) in the United States, which reported no difference in vitamin D levels between patients with asthma and healthy controls. Besides, two more studies, one from India by Kavitha et al. (39) and another from Korea by Yang et al. (40), reported similar results. Furthermore, Albar et al. (41) in Saudi Arabia also demonstrated no significant correlation between vitamin D levels and asthma severity. The absence of an association between asthma and vitamin D does not exclude the role of vitamin D

in the pathogenesis of asthma. However, more multicenter studies are needed to acquire more accurate results and make recommendations on the influence of vitamin D on asthma.

The current study is limited in that it was only conducted in a single center. Therefore, more multicenter studies with a larger sample size are required to confirm our findings.

Conclusion

In conclusion, we found significantly higher levels of eosinophils, IgE, IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37 in patients with asthma compared to healthy controls. Nevertheless, we did not find a significant relationship between vitamin D and asthma. Among the patients with asthma, the levels of cytokines (IL-1 β , IL-4, IL-10, and IL-13) were consistently positively correlated with the serum levels of IgE. However, IL-35 and IL-37 were consistently negatively correlated with high levels of IgE among the patients with asthma.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Authors' contribution

AA, HA, MA, SBS, NM, and AN compiled the research hypothesis, formulated the research proposal, explored the data, designed the experimental work, while AA, HA, MA, SBS, and NM prepared the initial draft of the manuscript under AN guidance. AN, ASA, and MH conducted the ELISA and participated in the ELISA analysis. AN and AA carried out the data analysis and wrote the statistical parts of the study. AA, HA, MA, SBS, and NM contributed to the recruitment of patients and reviewed the manuscript.

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References

1. Dharmage SC, Perret J, Custovic A. Epidemiology of asthma in children and adults. *Frontiers in pediatrics*. 2019;7:246.
2. Anandan C, Nurmatov U, van Schayck OC, Sheikh A. Is the prevalence of asthma declining? Systematic review of epidemiological studies. *Allergy*. 2010;65(2):152-67.
3. Al Ghamdi B, Mahfouz A, Abdel Moneim I, Khan M, Daffallah A. Altitude and bronchial asthma in south-western Saudi Arabia. *EMHJ-Eastern Mediterranean Health Journal*, 14 (1), 17-23, 2008. 2008.
4. Musharrafieh U, Tamim H, Houry R, AlBuhairan F. A nationwide study of asthma correlates among adolescents in Saudi Arabia. *Asthma Research and Practice*. 2020;6(1):1-8.
5. Alahmadi TS, Banjari MA, Alharbi AS. The prevalence of childhood asthma in Saudi Arabia. *International Journal of Pediatrics and Adolescent Medicine*. 2019;6(2):74-7.
6. Al-Moamary MS, Alhaider SA, Alangari AA, Al Ghobain MO, Zeitouni MO, Idrees MM, et al. The Saudi Initiative for Asthma - 2019 Update: Guidelines for the diagnosis and management of asthma in adults and children. *Ann Thorac Med*. 2019;14(1):3-48.
7. Boyce JA. Mast cells: beyond IgE. *The Journal of allergy and clinical immunology*. 2003;111(1):24-32; quiz 3.
8. Hu D. Role of anti-inflammatory cytokines IL-35 and IL-37 in asthma. *Inflammation*. 2017;40(2):697-707.
9. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity*. 2019;50(4):975-91.
10. Wang M, Liu M, Wang C, Xiao Y, An T, Zou M, et al. Association between vitamin D status and asthma control: a meta-analysis of randomized trials. *Respiratory medicine*. 2019;150:85-94.
11. Aldubi HM, Alissa EM, Kamfar HZ, Gaber O, Marzouki ZM. Bronchial asthma and hypovitaminosis D in Saudi children. *Asia Pacific Allergy*. 2015;5(2):103-13.
12. Huang H, Porpodis K, Zarogoulidis P, Domvri K, Giouleka P, Papaiwannou A, et al. Vitamin D in asthma and future perspectives. *Drug design, development and therapy*. 2013;7:1003.
13. Corren J. Role of interleukin-13 in asthma. *Current allergy and asthma reports*. 2013;13(5):415-20.
14. Bao K, Reinhardt RL. The differential expression of IL-4 and IL-13 and its impact on type-2 immunity. *Cytokine*. 2015;75(1):25-37.
15. Jackson DJ, Bacharier LB, Calatroni A, Gill MA, Hu J, Liu AH, et al. Serum IL-6: A biomarker in childhood asthma? *Journal of Allergy and Clinical Immunology*. 2020.
16. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annual review of immunology*. 2001;19(1):683-765.
17. Hawrylowicz C, O'garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nature Reviews Immunology*. 2005;5(4):271-83.
18. Boonpiyathad T, Satitsuksanoa P, Akdis M, Akdis CA, editors. IL-10 producing T and B cells in allergy. *Seminars in Immunology*; 2019: Elsevier.
19. Heaton T, Rowe J, Turner S, Aalberse R, De Klerk N, Suriyaarachchi D, et al. An immunoepidemiological approach to asthma: identification of in-vitro T cell response patterns associated with different wheezing phenotypes in children. *The Lancet*. 2005;365(9454):142-9.
20. Pukelsheim K, Stoeger T, Kutschke D, Ganguly K, Wjst M. Cytokine profiles in asthma families depend on age and phenotype. *PloS one*. 2010;5(12):e14299.
21. Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, et al. Interleukin-10 gene expression in acute virus-induced asthma. *American journal of respiratory and critical care medicine*. 2005;172(4):433-9.
22. Zhang Y-L, Luan B, Wang X-F, Qiao J-Y, Song L, Lei R-R, et al. Peripheral blood MDSCs, IL-10 and IL-12 in children with asthma and their importance in asthma development. *PloS one*. 2013;8(5):e63775.

23. Tang M, Kemp A, Varigos G. IL-4 and interferon-gamma production in children with atopic disease. *Clinical & Experimental Immunology*. 1993;92(1):120-4.
24. Fu J-j, McDonald VM, Baines KJ, Gibson PG. Airway IL-1 β and systemic inflammation as predictors of future exacerbation risk in asthma and COPD. *Chest*. 2015;148(3):618-29.
25. Naumov D, Gassan D, Kotova O, Sheludko E, Afanaseva E, Perelman J, et al. Role of interferon-gamma as a marker of asthma severity and control. *Eur Respiratory Soc*; 2019.
26. Charrad R, Berraïes A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: Correlation with inflammatory cytokines TNF- α , IL- β , IL-6 and IL-17A. *Immunobiology*. 2016;221(2):182-7.
27. Lunding L, Webering S, Vock C, Schröder A, Raedler D, Schaub B, et al. Effect of IL-37 on allergic airway inflammation. *Annals of the American Thoracic Society*. 2016;13(Supplement 1):S95-S6.
28. Wong CK, Leung TF, Chu IMT, Dong J, Lam YYO, Lam CWK. Aberrant expression of regulatory cytokine IL-35 and pattern recognition receptor NOD2 in patients with allergic asthma. *Inflammation*. 2015;38(1):348-60.
29. Wang W, Li P, Yang J. Decreased circulating interleukin-35 levels are related to interleukin-4-producing CD8 $^{+}$ T cells in patients with allergic asthma. *Iranian Journal of Allergy, Asthma and Immunology*. 2015;14(4):379-85.
30. Khoshkhui M, Alyasin S, Sarvestani EK, Amin R, Ariaee N. Evaluation of serum interleukin-35 level in children with persistent asthma. *Asian Pac J Allergy Immunol*. 2017;35:91-5.
31. Meng P, Chen ZG, Zhang TT, Liang ZZ, Zou XL, Yang HL, et al. IL-37 alleviates house dust mite-induced chronic allergic asthma by targeting TSLP through the NF- κ B and ERK1/2 signaling pathways. *Immunology and cell biology*. 2019;97(4):403-15.
32. Lv J, Xiong Y, Li W, Cui X, Cheng X, Leng Q, et al. IL-37 inhibits IL-4/IL-13-induced CCL 11 production and lung eosinophilia in murine allergic asthma. *Allergy*. 2018;73(8):1642-52.
33. Chen B, Huang K, Ye L, Li Y, Zhang J, Zhang J, et al. Interleukin-37 is increased in ankylosing spondylitis patients and associated with disease activity. *Journal of translational medicine*. 2015;13(1):36.
34. Nold-Petry CA, Lo CY, Rudloff I, Elgass KD, Li S, Gantier MP, et al. IL-37 requires the receptors IL-18R α and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nature immunology*. 2015;16(4):354.
35. Chi H, Liu D, Sun Y, Hu Q, Liu H, Cheng X, et al. Interleukin-37 is increased in adult-onset Still's disease and associated with disease activity. *Arthritis research & therapy*. 2018;20(1):1-12.
36. Wang J, Shen Y, Li C, Liu C, Wang Z-H, Li Y-S, et al. IL-37 attenuates allergic process via STAT6/STAT3 pathways in murine allergic rhinitis. *International Immunopharmacology*. 2019;69:27-33.
37. Raedler D, Ballenberger N, Klucker E, Böck A, Otto R, Da Costa OP, et al. Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *Journal of Allergy and Clinical Immunology*. 2015;135(1):81-91.
38. Menon J, Maranda L, Nwosu BU. Serum 25-hydroxyvitamin D levels do not correlate with asthma severity in a case-controlled study of children and adolescents. *Journal of Pediatric Endocrinology and Metabolism*. 2012;25(7-8):673-9.
39. Kavitha T, Gupta N, Kabra SK, Lodha R. Association of serum vitamin D levels with level of control of childhood asthma. *Indian pediatrics*. 2017;54(1):29-32.
40. Yang H-k, Choi J, Kim WK, Lee S-Y, Park YM, Han MY, et al., editors. The association between hypovitaminosis D and pediatric allergic diseases: A Korean nationwide population-based study. *Allergy & Asthma Proceedings*; 2016.
41. Albar R, Malas M, Bafail M, Almatihmay Y, Alamoudi L, Saleh E. Asthma control not associated with vitamin D deficiency: A single-center retrospective study in Saudi Arabia. *Current Pediatric Research*. 2016.

Table 1: Description analysis for Sex, mean, and standard deviation of age, weight, height, and total white blood cell count among the study groups

	Controls N = 47	Asthma patients N = 48	p-value
<u>Sex</u>			
Female	31 (66%)	30 (62.5%)	0.008
Male	16 (34%)	18 (37.5%)	
<u>Mean ± Std. Deviation</u>			
Age (Years)	9.09 ± 2.073	9.35 ± 2.129	0.53
Weight (Kg)	35.28 ± 13.87	38.35 ± 15.21	0.31
Height (cm)	135.68 ± 13.39	138.09 ± 13.48	0.38
TWBCs ($\times 10^{-9}$ L)	7.93 ± 2.12	8.054 ± 2.29	0.78
Eosinophil ($\times 10^{-9}$ L)	0.27 ± 0.23	0.39 ± 0.20	0.01
Lymphocyte ($\times 10^{-9}$ L)	3.03 ± 0.94	2.96 ± 0.85	0.7
Calcium (mmol/L)	2.42 ± 0.16	2.44 ± 0.08	0.64
Phosphate (mmol/L)	1.64 ± 0.15	1.58 ± 0.19	0.08
Magnesium (mmol/L)	0.85 ± 0.05	0.86 ± 0.09	0.38

Table 2: Description of mean and standard deviation (SD) of vitamin D, IgE, and cytokines (INF- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) levels (pg/ml) in the different study groups

Groups	Controls (Mean $\pm$ SD)	Asthma patients (Mean $\pm$ SD)	t-value (95% CI)	p-value$\ddagger$
Vitamin D ($\times 10^3$) pg/ml	(2.7 $\pm$ 0.23)	(2.6 $\pm$ 0.3)	0.99 (-0.05 to 0.16)	0.33
IgE pg/ml	(0.97 $\pm$ 0.91)	(6.83 $\pm$ 0.93)	-31.03 (-6.24 to -5.49)	<0.001
IFN-γ pg/ml	(10.94 $\pm$ 7.24)	(11.3 $\pm$ 7.9)	-0.23 (-3.44 to 2.73)	0.82
IL-1β pg/ml	(4.92 $\pm$ 0.64)	(46.78 $\pm$ 1.4)	-33.73 (-42.50 to -37.77)	<0.001
IL-4 pg/ml	(18.85 $\pm$ 2.02)	(159.3 $\pm$ 5.6)	-47.0 (-143.30 to -131.69)	<0.001
IL-6 pg/ml	(21.20 $\pm$ 7.60)	(137.65 $\pm$ 17.9)	-31.67 (-121.26 to -106.95)	<0.001
IL-10 pg/ml	(26.96 $\pm$ 4.99)	(147.6 $\pm$ 7.9)	-43.8 (-124.07 to -113.31)	<0.001
IL-13 pg/ml	(20.86 $\pm$ 6.70)	(146.2 $\pm$ 3.5)	-11.17 (-127.16 to -122.69)	<0.001
IL-35 pg/ml	(5.38 $\pm$ 5.54)	(28.06 $\pm$ 8.39)	-15.5 (-25.58 to -19.77)	<0.001
IL-37 pg/ml	(2.69 $\pm$ 0.88)	(134.17 $\pm$ 27.54)	-32.7 (-139.46 to -123.5)	<0.001

Table 3: Linear regression analysis of circulating vitamin D, IgE, and cytokines (IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) levels (pg/ml) in patients with asthma compared to the control group

Unadjusted*			Adjusted [†]	
Variables	B* (95% CI for B)	p-value	B (95% CI for B)	p-value
Vitamin D	-0.05 (-0.16–0.05)	0.33	-0.02 (-0.13–0.09)	0.67
IgE	5.86 (5.49–6.24)	< 0.001	5.83 (5.44–6.22)	< 0.001
IFN- γ	0.36 (-2.73–3.44)	0.82	-0.18 (-3.41–3.05)	0.91
IL-1 β	41.85 (41.4–42.3)	< 0.001	41.80 (41.33–42.26)	< 0.001
IL-4	140.47 (138.77–142.18)	< 0.001	140.27 (138.49–142.04)	< 0.001
IL-6	116.45 (110.82–122.07)	< 0.001	115.92 (110.04–121.8)	< 0.001
IL-10	120.59 (117.89–123.27)	< 0.001	120.21 (117.42–122.99)	< 0.001
IL-13	125.37 (123.21–127.54)	< 0.001	125.16 (122.89–127.43)	< 0.001
IL-35	22.68 (19.78–25.58)	< 0.001	23.14 (20.12–26.17)	< 0.001
IL-37	131.48 (123.5–139.5)	< 0.001	132.14 (123.9–140.35)	< 0.001

*B represents unstandardized coefficients

[†]B (95%CI) adjusted with sex and age.

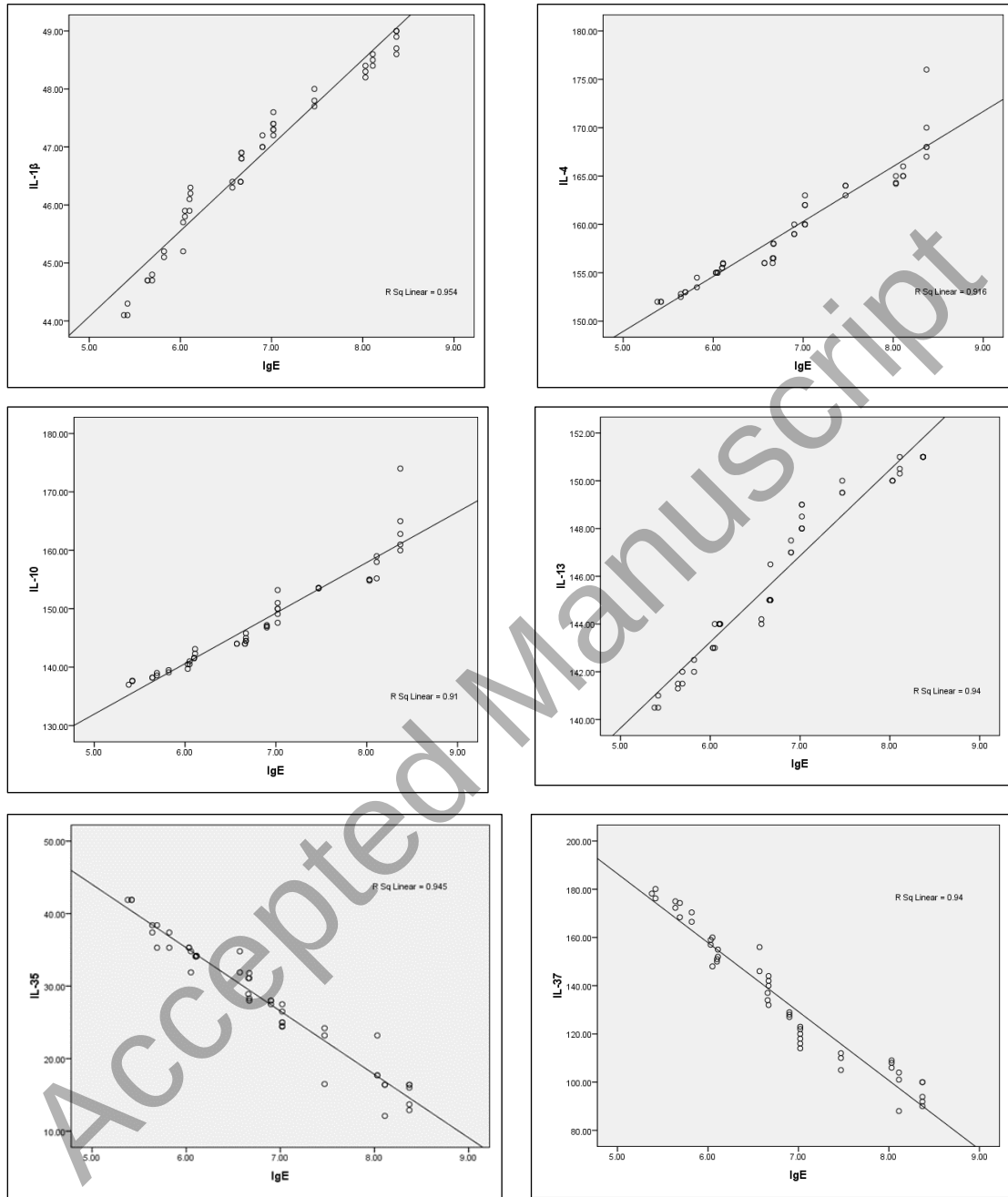
Table 4: Logistic regression analysis of circulating vitamin D, IgE, and cytokines (IFN- γ , IL-1 β , IL-4, IL-6, IL-10, IL-13, IL-35, and IL-37) level/ μ in patients with uncontrolled atopic asthma compared to the patients with well-controlled asthma group

Unadjusted*			Adjusted [†]	
Variables*	OR (95% CI for OR)	p-value	OR (95% CI for OR)	p-value
Vitamin D	0.88 (0.12–6.42)	0.90	0.72 (0.8–6.2)	0.77
IgE	1.79 (0.93–3.47)	0.08	1.78 (0.90–3.55)	0.10
IFN- γ	1.47 (0.91–2.37)	0.12	1.59 (0.96–2.66)	0.07
IL-1 β	1.54 (0.99–2.39)	0.06	1.55 (0.97–2.46)	0.07
IL-4	1.09 (0.97–1.22)	0.13	1.09 (0.96–1.23)	0.17
IL-6	0.99 (0.96–1.02)	0.52	0.99 (0.96–1.02)	0.43
IL-10	1.05 (0.96–1.13)	0.21	1.05 (0.97–1.13)	0.27
IL-13	1.22 (1.02–1.46)	0.03	1.22 (1.01–1.47)	0.04
IL-35	0.93 (0.87–1.01)	0.07	0.93 (0.86–1.01)	0.08
IL-37	0.98 (0.95–1.01)	0.04	0.98 (0.95–1.02)	0.04

*OR represents odds ratio

[†]Adjustment of OR (95%CI) were performed for sex and age.

Figure 1: Correlations between the serum levels of IgE and cytokines (IL-1 β , IL-4, IL-10, IL-13, IL-35, and IL-37) among the patients with asthma group only



* Spearman's rho correlation is significant at the 0.01 level (2-tailed).