Medical Science Journal for Advance Research

Vol. 5, No. 3, September 2024
e-ISSN: 2774-4892

Evaluating of IL-34 as a Biomarker for Asthma in Children: Findings from Dhi Qar Province, Iraq

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ABSTRACT

Objective: *To investigate the possible role of interleukin-34 in development of asthma in children and determine its effectiveness as a biomarker for monitoring disease severity.*

Methods: *the current study focused on asthmatic children who were admitted to the two hospitals in Dhi-Qar province: Muhammad Al-Moussawi Children's Hospital, Bint Al-Huda Teaching Hospital for Women and Children. The period of study was placed between December 2023 to March 2024. Specifically included (85) asthmatic children's patients, their ages ranged from 6 months to 6 years old. To compare the results with healthy control subjects (85) children of the same ages and sex as the patients were randomly selected from the local community and hospital. The concentration level of serum interleukin-34 was measured by Enzyme-linked immunosorbent assay (ELISA).*

Results: *the present study revealed a striking difference in IL-34 concentrations between asthma patients and controls. Asthma patients exhibited significantly elevated IL-34 levels (409.4±114.7 pg/ml) compared to their healthy counterparts (307.6±56.43 pg/ml), with a p-value of <0.001.*

Keywords: Asthma, Interleukin-34, Respiratory Disorder, Risk Factors, Immune Biomarker.

Article Information

Received: June 23, 2024; Revised: August 28, 2024; Online: September, 2024

1.INTRODUCTION

Bronchial asthma is one of the most widespread chronic inflammatory disorder in childhood, affecting the respiratory system.[1] It cause symptoms like chronic airway, wheezing, difficulty breathing, chest tightness, and coughing due to airflow inflammation, hyperresponsivenes,s and bronchoconstriction.[2] In recent years a prevalent chronic respiratory condition has shown an increase, affecting more than 300 million individuals global, typically manifesting in adolescence and children that presents a significant health challenge. [3,4] In Iraq, the prevalence of asthma was 16.22% in younger children [5] Hence, asthma till remains a remarkable and challenging disorder.

Interleukine-34 is a pleiotropic pro-inflammatory cytokine was discovered in 2008, that ligand to four distinct receptors, namely (CSF-1R), Syndecan-1, PTP-ζ and TREM2.[6] Play as key in regulate of proliferation, survival, homeostasis and diffraction of myeloid lineage cells including monocytes, macrophages and osteoclasts due to its inflammatory and immunosuppressive properties, which is implicated in various autoimmune diseases. [7,S] several studies found this interleukin widely in kidney, heart, brain, spleen, lung, liver, thymus, testes, ovary, small intestine, prostate and colon. [6] The goal of this investigation was to determine whether was any relationship between the levels of serum IL34 in developing of asthma severity in children, also to detect its

https://doi.org/10.46966/msjar.v5i3.227

effectiveness as a biomarker for monitoring the disease activity.

2.METHODS AND STUDY DESIGN

From December 2023 to March 2024, a case-control study was conducted involving (85) pediatric patients diagnosed with asthma, comprising [53 males (62.3%) and 32 females (37.7%)]. This research was undertaken at two medical institutions located in Dhi-Qar province: Muhammad Al-Moussawi Children's Hospital and Bint Al-Huda Teaching Hospital for Women and Children. The study focused on newly diagnosed asthmatic children, whose diagnoses were established based on clinical symptoms observed during routine clinic visits. The age range of the participants was between [6 months and 6 years]. To enable a robust comparative analysis, a control group of (85) children, consisting of [46 males (54.1%) and 39 females (45.9%)], was randomly selected from the local community and affiliated hospitals, ensuring matching by age and gender. All asthma children and control subjects underwent testing for anti-COVID-19 IgM and IgG antibodies utilizing the Rapid Test Cassette (Bio germ, Shanghai, China), with all results indicating negative findings. Also Any patient presented with Allergic Rhinitis, Allergic Sinusitis that was exclusion from this study .The practical aspects of this investigation were executed in the laboratories of Muhammad Al-Moussawi Children's Hospital.

2.1. Ethical and Scientific Approval:

1.Ethical approval was obtained from the ethical committee in Kufa medical college and ethical committee in the health directorate of Dhi-Qar province.

2.Health measures and safety have been taken when sampling.

2.2. Data Collection

Demographic and clinical information was gathered by interviewing the parents of the children using a questionnaire tailored for each patient. An information sheet was completed, and written consent was secured. The socio-demographic and observed data included factors such as gender, age, family history of asthma, exposure to tobacco smoke, presence of pets or other domestic animals in the household, and personal history of sign and symptom like cough, dust and asthma triggers.

2.3. Collection of blood samples

Three ml (3 mL) of venous blood were drawn from each participant of venous blood samples were obtained from both asthma patients as well as Apparently healthy. (1 ml) preserved in EDTA tubes for complete blood count (CBC) test and (2ml) centrifuged for 15 minutes at 3000 rpm, then serum was separated and transferred to three Eppendorf tubes and stored in deep freeze (-20°c) to be used for immunological assay**.**

2.4. Method of measurement

The serum of Interleukin-34 levels was demonstrated by using enzyme linked immunosorbent assay (ELISA) KITS (BT LAB / China). The operation processes were performed accordioning the manufacturer’s protocol of kits.

2.5. Data Analysis

Statistical analysis of the data was performed R version 4.1.0 (R Foundation for statistical computing ,Vienna, Austria) with various packages including ggplot2, dplyr and pROC for data management and visualization [9-11]. A comprehensive analysis was done to examine the relationships between various biomarkers, demographic factors, and asthma status. Descriptive statistics were calculated for all variables, including means and standard

deviations for normally distributed continuous variables (or median with interquartile range, IQR, for non-normally distributed ones), Normality of variables was assessed using Shapiro-Wilk tests. For comparisons between asthma and control groups, independent t-tests were used for normally distributed continuous variables, while Mann-Whitney U tests were employed for non-normally distributed data. Chi-square tests or Fisher's exact tests was utilized for categorical variables. Correlations between continuous variables were evaluated using Spearman's rank correlation coefficient for continuous variables with odds ratios and 95% confidence intervals reported. Receiver Operating Characteristic (ROC) curve analysis was performed to assess the diagnostic accuracy of key biomarkers (IL-34), with Area Under the Curve (AUC), sensitivity, specificity, and optimal cut-off values determined. All statistical tests were two-tailed, with a significance level set at $p<0.05$ (or at $p<0.01$ for high statistical significance and <0.001 for very high significance).

3. RESULTS

3.1. Basic and clinical characteristics

The comparative analysis of 85 asthma patients and 85 control subjects revealed several significant differences between the groups. While age and sex distribution were similar, family history of asthma was markedly more prevalent in the asthma group (52.9%) compared to the control group (7.1%, $p<0.001$). Symptoms also differed significantly, with cough being more common in asthma patients (95.3% vs 72.9% in controls, $p<0.001$). Interestingly, allergies such as dust and smoking sensitivity were slightly more frequent in the asthma group, though not statistically significant (Table 1). However, hematological parameters revealed some notable differences. While total white blood cell count and neutrophil levels were comparable, lymphocyte and monocyte counts were significantly lower in the asthma group ($p=0.042$ and $p=0.004$, respectively).

Table 1. Comparison of characteristics between asthma and control groups.

Characteristic	Control group (N= 85)	Asthma group (N= 85)	P-value
Age (years)	**3.00 [1.00, 5.00]**	**2.50 [0.80, 4.00]**	**0.085**
Sex			
Male	**46 (54.1%)**	**53 (62.3%)**	**0.834**
Female	**39 (45.9%)**	**32 (37.7%)**	
Family history			
Yes	**6 (7.1%)**	**45 (52.9%)**	**<0.001***
No	**79 (92.9%)**	**40 (47.1%)**	
Symptoms			
Cough	**62 (72.9%)**	**81 (95.3%)**	**<0.001***
Wheezing	**1 (1.2%)**	**2 (2.4%)**	

Allergies			
Dust sensitivity	**39 (45.9%)**	**47 (55.3%)**	**0.283**
Smoking sensitivity	**38 (44.7%)**	**47 (55.3%)**	**0.220**
Others	**1 (1.2%)**	**3 (3.5%)**	**0.621**
WBCs (10^9/L)	**10.20 [6.19, 15.73]**	**7.56 [5.59, 10.20]**	**0.081**
Neutrophils (10^9/L)	**5.42 [0.87, 10.17]**	**3.87 [1.87, 5.54]**	**0.509**
Lymphocytes (10^9/L)	**3.66 [2.83, 5.59]**	**3.05 [1.31, 3.66]**	**0.042***
Monocytes (10^9/L)	**0.86 [0.80, 1.85]**	**0.76 [0.40, 0.82]**	**0.004***
Eosinophiles (10^9/L)	**0.12 [0.05, 0.29]**	**0.02 [0.00, 0.24]**	**0.107**
Basophils (10^9/L)	**0.01 [0.01, 0.04]**	**0.01 [0.01, 0.01]**	**0.550**

2.3. Association between serum IL34 levels in patient with asthma and control:

The levels of interlukine-34 were also investigated in asthma patients and healthy controls (Figure 1), as well as its distribution across various patient subgroups (Figure 2). The findings revealed a striking difference in IL-34 concentrations between asthma patients and controls. Asthma patients exhibited significantly elevated IL-34 levels (409.4±114.7 pg/ml) compared to their healthy counterparts (307.6±56.43 pg/ml), with a *p*-value of <0.001. Further analyses within the asthma cohort explored the relationship between IL-34 levels and various clinical and demographic factors. Age-stratified analysis, comparing patients ≤2.5 years (405.4±110.6 pg/ml) to those >2.5 years (415.8±122.3 pg/ml), revealed no significant difference (*p*=0.684). Similarly, sex-based comparison showed slightly higher IL-34 levels in male patients (417.9±126.4 pg/ml) compared to females (395.5±92.34 pg/ml), but this difference did not reach statistical significance (*p*=0.387). Intriguingly, patients with a family history of asthma demonstrated marginally higher IL-34 levels (421.6±121.2 pg/ml) compared to those without (395.8±106.7 pg/ml), although this difference was not statistically significant (*p*=0.185). The presence of cough as a symptom was associated with slightly lower IL-34 levels (407.6±116.1 pg/ml) compared to its absence (446.2±84.27 pg/ml), but again, this difference lacked statistical significance (p=0.515). Examination of allergic sensitivities revealed minimal impact on IL-34 levels. Patients with dust allergy showed comparable IL-34 concentrations (399.5±134.3 pg/ml) to those without (409.3±80.61 pg/ml, *p*=0.933). Similarly, smoking sensitivity appeared to have little influence on IL-34 levels (328.3±37.92 pg/ml vs. 329.2±51.28 pg/ml, *p*=0.461).

3.3. Receiver operating characteristic (ROC) analysis results of the studied biomarkers .

The ROC analysis was performed to evaluate the diagnostic potential of IL-34 as biomarkers for asthma. The results are summarized in Table 2 and illustrated in Figures 3. The ROC analysis reveals that IL-34 biomarker demonstrate statistically significant diagnostic potential for asthma, as evidenced by their *p*-values (*p*<0.05). IL-34 shows the highest diagnostic accuracy with an

AUC of 0.816. the IL-34 demonstrate good balance between sensitivity and specificity. IL-34 appears to be the most promising biomarker, offering high sensitivity (88.24%) and moderate specificity (68.84%) at the optimal cut-off value.

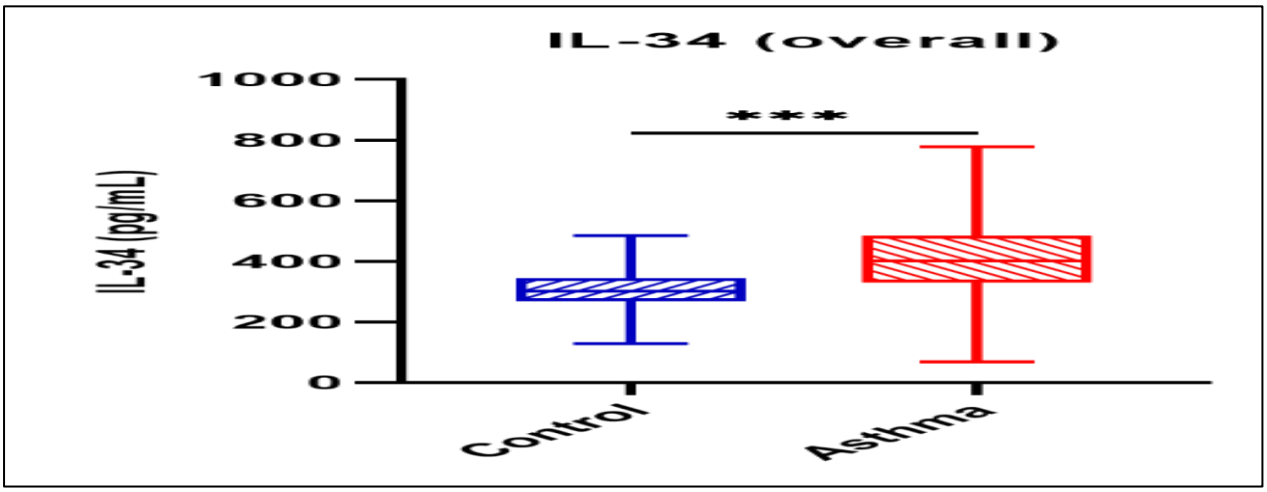

Figure 1. Serum IL-34 levels in control and asthma patient groups. Data are presented as box plots, where the line within each box represents the median. The asterisks (***) indicate a statistically significant difference (p<0.001) between the groups.

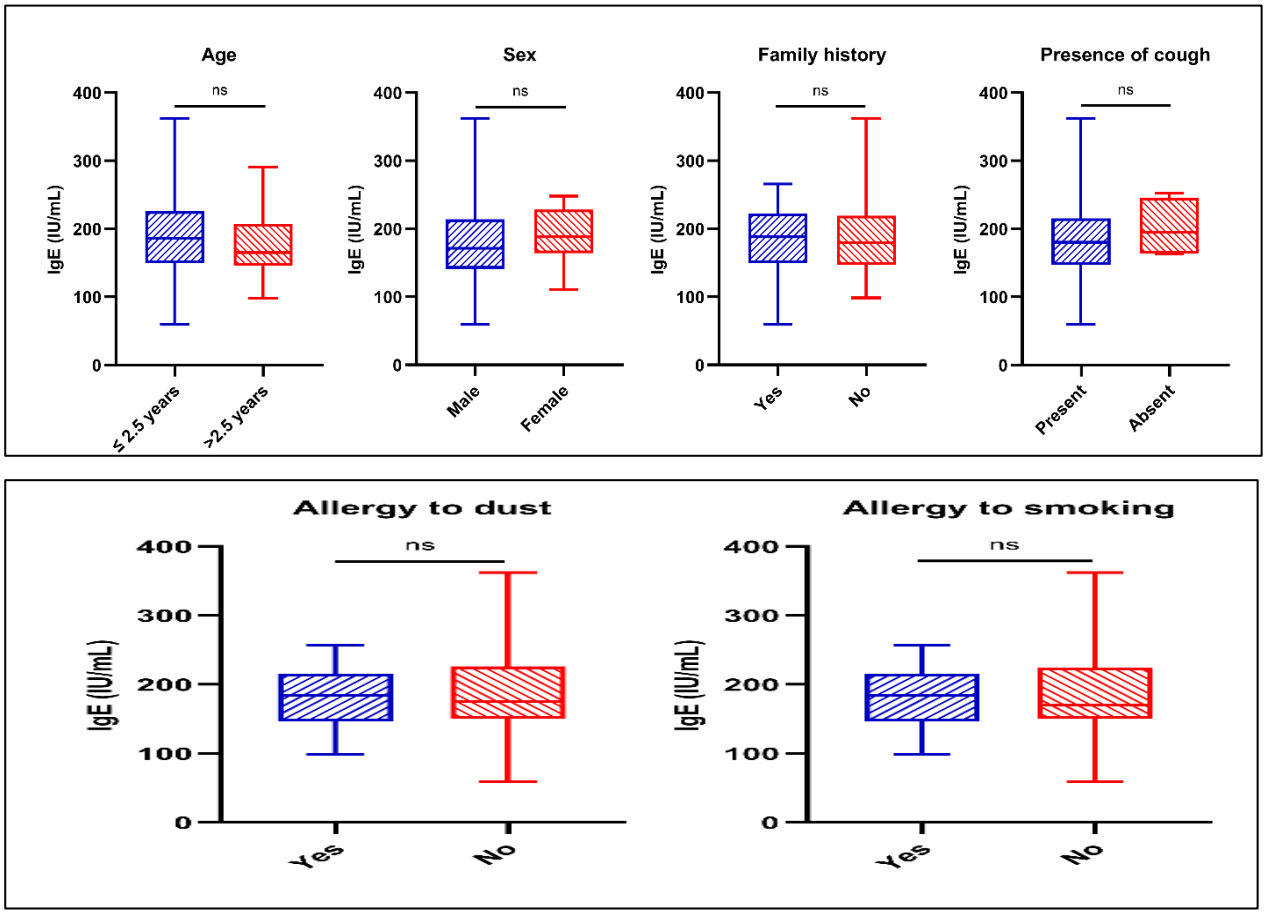

Figure 2. Subgroup analyses of serum IL-34 levels within the asthma patient group. All plots are presented as box plots, with the line in each box representing the median.

Table 2. Receiver operating characteristic (ROC) analysis results of the studied biomarkers.

	Cut-off	AUC	Sensitivity (95% CI)	Specificity (95% CI)	*P*-value
IL-34	≤373.4	0.816	88.24 (79.4 – 94.2)	68.84 (53.6 - 79.2)	<0.001

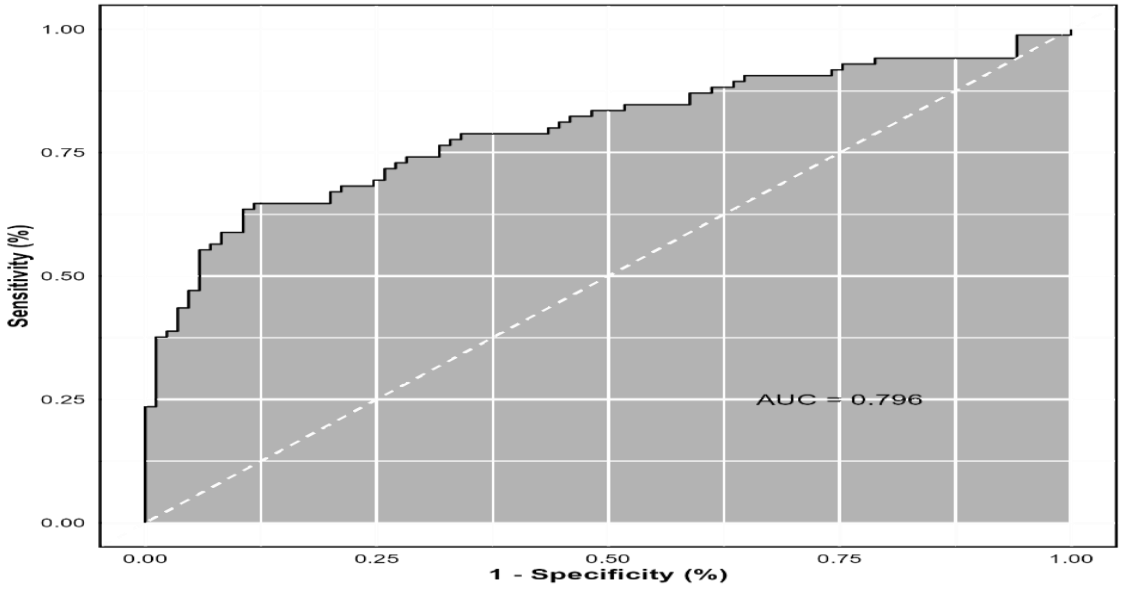

Figure 3. ROC curve for IL-34 as a diagnostic marker for asthma. The AUC is 0.816, indicating excellent diagnostic accuracy. The optimal cut-off value is ≤373.4, with a sensitivity of 88.24% (95% CI: 79.4% – 94.2%) and specificity of 68.84% (95% CI: 53.6% - 79.2%).

4. Discussion

To the best of our Acknowledge, this is the first study to establish an association between interleukin-34 (IL-34) and asthma among children under six years of age in Iraq. The demographic and clinical information is particularly vulnerable to respiratory diseases, but the results in (table 1) suggest that family history strongly significant associated with asthma in this population of study, and the specific symptoms that increased risk among children like cough and others also shown linked with asthmatic patient. This finding was consistent with many asthma family history studies, which confirmed that Asthma exhibited a strong correlation with a familial history of the condition, which is considered a significant risk factor. [12,13] In addition, (table1) also observed that Eosinophil and basophil counts, often associated with allergic responses, did not differ significantly between the groups. But there was found differences in lymphocyte and monocyte counts that play role in inflammatory response or may indicate alterations in immune function in asthma patients, potentially contributing to the disease pathogenesis or resulting from it, that was May effected by age group and gender kind. Many Previous studies in other disease noted that monocyte stimulated and chemoattractant due to present (IL34) chemokines and cytokines like monocyte chemoattractant indicate that immune dysregulation may contribute to asthma severity and increase risk in children. Other study showed the level of WBCs count

effected by age, life style and gender of patient. [14-16]

Moreover, found the significant outcomes of the study is the Interleukin-34 high elevated in the serum concentrations of asthmatic children compared to healthy controls. Additionally, From the ROC analysis reveals that IL-34 biomarker demonstrate statistically significant diagnostic potential for asthma in children, this finding is particularly important and reveals a positive correlation. also, it could lead to the development of new diagnostic and monitoring test for pediatric asthma. Our results of the study were agreement with previous studies in different disease [17-20] very little study is known about the actual function of interlukine-34 in pediatric asthma, so suggest to need for more study to understand the pathophysiology of this cytokine in this disease.

CONCLUSION

Serum interleukin-34 (IL34) is an important Biomarker in children asthma as its levels were elevated in asthma patients and correlated with disease activity.

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