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REVIEW ARTICLE

The validity of IL12 and miRNA-140-5p as potential bio-markers of asthma

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Abstract:

Background: Asthma is a chronic respiratory disorder characterized by airway inflammation, hyperresponsiveness, and variable airflow limitation. It affects millions worldwide and results from complex interactions between genetic, environmental, and immunological factors.

Aim: To evaluate serum IL-12 levels and miRNA-140-5p expression in asthma patients compared to healthy controls and to explore their potential as diagnostic and follow-up biomarkers.

Methods: The study included 60 patients diagnosed with asthma and 40 apparently healthy controls. Venous blood samples (5 mL) were collected under aseptic conditions from each participant. Serum IL-12 concentrations were measured using ELISA, and miRNA-140-5p expression was quantified by RT-qPCR following standard protocols.

Results: Analysis revealed a significant elevation in serum IL-12 concentrations among asthma patients (mean ± SD: 1432.5 pg/mL) compared with healthy controls (560.5 pg/mL; p = 0.001), demonstrating perfect discriminatory power (AUC = 1.0). Conversely, miRNA-140-5p expression levels were markedly downregulated in the patient group, with a mean fold change of 1.46 relative to 10.11 in controls (p < 0.001).

Conclusion: Elevated IL-12 and reduced miRNA-140-5p may serve as simple, noninvasive biomarkers reflecting airway inflammation and alteration in asthma.

Key words: Asthma; Interleukin-12; miRNA-140-5p; Biomarkers; Airway inflammation; RT-qPCR; ELISA.

Introduction

Acin Asthma is a chronic inflammatory disorder of the airways that causes symptoms like coughing, wheezing, chest tightness, and shortness of breath. It affects nearly 300 million individuals worldwide, with its burden projected to rise significantly [1-2]. The disease results from complex interactions between genetic susceptibility, environmental triggers, and host factors, leading to persistent airway inflammation and immune dysregulation [3-4-5].

Interleukin-12 (IL-12) is a crucial immunoregulatory cytokine that plays a crucial character in shaping the adaptive immune response, particularly by promoting T helper 1 (Th1) cell differentiation and suppressing the T helper 2 (Th2) pathway, thus contributing to the Th1/Th2 balance [6]. It is concealed primarily by antigen-presenting cells (APCs), comprising dendritic cells, monocytes/macrophages, and lymphocytes [7]. Among these, dendritic cells located in the airway epithelium are considered the main APCs involved in aeroallergen sensitization, although they exhibit high antigen uptake capacity but limited antigen-presenting efficiency [8].

IL-12 also serves as a potent inducer of interferon-gamma (IFN-γ) construction in latent peripheral blood mononuclear cells [9], and many of its downstream effects are mediated via IFN-γ signaling. In a recent antigen-challenge study on patients with mild asthma, systemic administration of IL-12 in gradually increasing doses directed to a noticeable decrease in circulating eosinophil levels, although it did not significantly alter the early or late asthmatic responses triggered by allergen exposure [10].

Through their interactions with their target messenger RNA, miRNAs—small, non-coding, single-stranded RNAs—play a critical role in controlling gene expression by either suppressing protein synthesis or degrading RNA. [11].

Recent evidence highlights the regulatory function of microRNAs (miRNAs) in asthma, with miRNA-140-5p emerging as a key modulator. miRNA-140-5p contributes to the regulation of airway inflammation by targeting pro-inflammatory cytokines, for instance, IL-6 and TNF-α, modulating pathways like TLR4 and NF-κB, and maintaining immune homeostasis through its effects on Th2 cells [12]. Altered expression of miRNA-140-5p has been linked to increased airway inflammation and asthma

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severity, underscoring its possibility as a biomarker and medicinal object [13-14-15].

Materials and Methods:

Between November 2024 and January 2025, a total of 60 patients (24 females and 36 males) diagnosed with asthma based on clinical evaluation were recruited from Al-Diwaniya Teaching Hospital. Additionally, a control group consisting of 40 apparently healthy individuals (19 females and 21 males) with no history of systemic sicknesses was included for comparison.

For each participant, five milliliters of venous blood were composed under aseptic conditions and transferred into gel tubes. After allowing the samples to clot, serum was separated by centrifugation. Three milliliters of the separated serum were aliquoted into Eppendorf tubes and kept at –20°C for subsequent measurement of interleukin-12 (IL-12) levels using a commercially available (ELISA) kit. The analysis was achieved according to the producer's instructions, employing plates pre-coated with specific antibodies, and the colorimetric reaction obtained was directly proportional to the IL-12 concentration in the samples.

The remaining two milliliters of serum were preserved at 4°C for total RNA extraction.

STEM-LOOP RT-qPCR:

The expression levels of miRNA in serum samples from both asthma patients and healthy control groups were quantified using the stem-loop RT-qPCR technique. Total RNA was extracted from serum using the TRIzol® reagent kit, following the manufacturer's protocol. The concentration and purity of the isolated RNA were determined by measuring the absorbance at 260/280 nm with a NanoDrop spectrophotometer (THERMO, USA). To eliminate any residual genomic DNA contamination, the extracted RNA was treated with DNase I enzyme (Promega, USA).

The specific miRNA sequence (miRNA-140-5p) was selected based on the Sanger miRBase database. Primers were designed using the miRNA primer design tool, as shown in Table (1). Complementary DNA (cDNA) was created as of DNase I-treated RNA testers by means of the M-MLV Reverse Transcriptase kit, succeeding the producer's strategies. (RT-qPCR) stayed, then directed to assess the relative expression of miRNA-140-5p, with GAPDH engaged as the housekeeping gene for standardization.

Table (1): Primer Sequence

SEQUENCE (5'-3')	PRIMER	
CAGTGCAGGGTCCGAGGTCAGAGCCACCTGGGCAATTTTTTTTTTTTCAACA	miRNA universal RT primers	
AACACGCCAGTGGTTTTACCC	F	miR-140 qPCR primer
CAG TGCAGGGTCCGAGGT	R	
AAAATCAAGTGGGGCGATGC	F	GAPDH qPCR primer
TTCTCCATGGTGGTGAAGACG	R	

Statistical Investigation

Microsoft Excel 2010 and the Statistical Package for the Social Sciences (SPSS) version 26 were used to gather, compile, and analyze the data. The Kolmogorov-Smirnov test was used to determine whether numerical variables were normal, and the results were expressed as mean ± standard deviation. For normally distributed variables, the independent samples t-test was used to compare two groups, and the chi-square test was used to evaluate relationships between categorical variables. To find the best cutoff values, a receiver operating characteristic (ROC) curve analysis was performed; the results were presented as area under the curve (AUC), sensitivity, specificity, accuracy, and significance level. To assess the relationships between numerical variables, Pearson's correlation coefficient (r) was computed. $P \leq 0.01$ was regarded as highly significant, and $P < 0.05$ was the threshold for statistical significance.

Results and Discussion:

Results:

Demographic and Clinical Characteristics

The age range of the asthmatic patients and controls was comparable. According to the current training, the control group consisted of 10 (25.0 percent) less than 30 years, 8 (20.0 percent) between 30 and 39 years, 6 (15.0 percent) between 40 and 49 years, and 16 (40.0 percent) more than 50 years. The asthmatic patients involved 13 (21.7 percent) less than 30 years, 14 (23.3 percent) between 30 and 39 years, 15 (25.0 percent) between 40 and 49 years, and 18 (30.0 percent) more than 50 years. The occurrence dissemination of sick and control issues by age groups did not differ considerably (P = 0.555).

Table (2) Comparison by age group of the sick and controlling groups.

Age	Asthmatic patients	Well control	Total	p-value
Mean ± SD	43.17± 11.4	42.00 ± 11.6		0.718 † NS
< 30 years, n	13 (21.7%)	10 (25.0%)	23 (23.0%)	0.555 ¥ NS
30-39 years, n	14 (23.3%)	8 (20.0%)	22 (22.0%)	
40-49 years, n	15 (25.0%)	6 (15.0%)	21 (21.0%)	
≥ 50 years	18 (30.0%)	16 (40.0%)	34 (34.0%)	

To evaluate the gender distribution between the asthmatic patients and the healthy control groups, a comparison was made. The findings, which are summed together in Table (3), show that the proportion of men and females in the two groups did not differ statistically significantly (p = 0.620).

Table (3): Sex-based comparison of the sick and controlling groups.

Groups	Gender		Entire	p-value
	Man	Woman		
Asthmatic patients	36 (60.0%)	24 (40.0%)	60	0.620 ¥ NS
Control	22 (55.0%)	18 (45.0%)	40	
Total	58 (58.0%)	42 (42.0%)	100	

The blood levels of Interleukin-1β (IL-1β) were statistically significantly higher in asthmatic patients (997.8–1767.8 pg/mL)

than in healthy controls (225.2–908.1 pg/mL), with a p-value of 0.001, as shown in Table (4). This shows that IL-12 levels are much greater in asthmatic patients, which may imply that this pro-inflammatory cytokine plays a part in the pathogenesis of the condition. It is confirmed by the low p-value ($p < 0.01$)

Table (4): Interleukin-12 (IL-12) level in patients and healthy control.

Groups		Interleukin-12 (pg/ml) level
Asthmatic disease	Mean ± SD	1432.5 ± 93.3
	Range	997.8-1767.8
Healthy control	Mean ± SD	560.5 ± 58.9
	Range	225.2-908.18
p-value		0.001** †

Diagnostic Accuracy of Interleukin-12 (IL-12):

ROC curve analysis demonstrated excellent diagnostic performance of IL-12 in differentiating asthma patients from healthy individuals. A cutoff value above 953.0 pg/mL yielded 100% sensitivity and 100% specificity, with an AUC of 1.000 (95% CI: 1.000–1.000).

Table (5): Sensitivity and specificity of IL-12 level (> 953.0 pg/mL -fold) in asthmatic disease

IL-12 level	patients n = 60	Well control n = 40
> 953.0	60 (%)	0 (%)
> 953.0	0 (%)	40 (%)
Specificity %	100.0 %	
NPV %	100.0%	
Sensitivity %	100.0 %	
PPV %	100.0 %	
AUC (95% CI)	1.000 (1.000- 1.000)	

Quantification of miRNA-140-5p Expression by RT- PCR

qRT-PCR analysis showed that the mean Ct value for miRNA-140-5p was higher in asthma patients (29.15) compared with healthy controls (27.27), indicating reduced expression in the patient group. The mean ΔCt values were –0.59 for patients and –3.28 for controls, corresponding to mean 2^–ΔCt values of –0.008 and –2.69, respectively. This difference resulted in a fold change of 1.46 in patients versus 10.11 in controls, demonstrating a significant downregulation of miRNA-140-5p in asthma ($p < 0.001$). (Table 6)

Table (6): Comparison of (Ct, $2^{-}\Delta ct$ and Folding) between asthmatic patients and healthy controls

Groups	Means Ct of miRNA140-5p	Means Ct of GAPDH	$^{\Delta}$Ct (Means Ct of miRNA140-5p)	$2^{-\Delta Ct}$	Fold of gene expression
Asthmatic	29.15	29.74	-0.59	-0008	1.46
Control	27.27	30.55	-3.28	-2.69	10.11

The mean expression level of miRNA-140-5p was significantly lower in asthma patients (1.46 ± 0.64, SE = 0.14) compared with healthy controls (10.11 ± 3.20, SE = 1.02). Statistical analysis revealed a highly significant difference between the two groups ($p = 0.001$), indicating a marked downregulation of miRNA-140-5p in the asthma group.

Table (7): Comparison of mean of miRNA140-5p gene expression between asthmatic patients and healthy controls

Groups	Mean	SD	SE	p-value
Asthmatic patients	1.46	0.64	0.14	0.001**
Control	10.11	3.2	1.02	

Discussion

The findings of the present study revealed that there were no statistically significant differences in the mean age or age distribution between asthma patients and healthy controls. These results are consistent with previous studies [16,17,18], which reported similar age distributions across study groups. However, in contrast, other investigations [19, 20] documented significantly higher mean ages among asthma patients, attributing these differences to increased disease prevalence in older populations as a result of cumulative environmental exposures and age-related physiological changes. [21] supports this association by demonstrating a link between aging, reduced bronchial elasticity, and diminished lung capacity. Furthermore, [22] emphasized that the prevalence of asthma phenotypes varies with age, with allergic asthma being more common in younger individuals and non-allergic asthma dominating in older patients.

Regarding gender distribution, no significant difference was observed between asthma patients and healthy controls, aligning with previous reports. Evidence indicates that asthma prevalence exhibits a sex-specific pattern across the lifespan: males are more frequently affected during childhood, whereas females have a higher prevalence after puberty, a trend influenced by hormonal changes. Sex hormones are known to modulate immune responses, thereby affecting asthma severity and treatment outcomes, particularly in adult females with non-allergic asthma [23]. This pattern was also confirmed by [24], who observed increased asthma severity in postmenopausal women.

The present study demonstrated a marked increase in serum IL-12 levels among asthma patients compared with healthy controls, with values showing excellent diagnostic accuracy (AUC = 1.0). Elevated IL-12 concentrations likely reflect heightened airway inflammation mediated by activated dendritic cells and macrophages, the primary sources of this cytokine [25]. IL-12 plays a crucial role in directing naïve T cells toward a Th1 phenotype and stimulating interferon-gamma (IFN-γ) production, thereby enhancing cellular immune responses within the airways [26]. The observed variability in IL-12 concentrations among asthma patients may be attributed to differences in inflammatory activity, disease severity, immunological phenotypes (allergic vs. non-allergic asthma), genetic predispositions, and environmental exposures [27, 28].

From an immunopathological standpoint, elevated IL-12 levels in asthma parallel observations in other immune-mediated diseases, such as psoriasis and Crohn's disease, supporting its role in immune dysregulation. Consequently, IL-12 has been proposed as a potential early biomarker for disease activity and therapeutic responsiveness. In agreement with this, earlier studies have reported higher IL-12 concentrations during acute asthma exacerbations compared with remission phases, suggesting a compensatory mechanism aimed at counterbalancing Th2-driven inflammation by enhancing IFN-γ production and inducing apoptosis of CD4+ T cells in the airways [29]. Moreover, although the correlation was not statistically significant, the

positive association between IL-12 levels and pulmonary function (FEV1%) observed in the current study suggests a possible protective role for IL-12 in modulating airway inflammation and improving lung function [30].

ROC curve analysis in this study confirmed the diagnostic utility of IL-12, with a cutoff value above 953.0 pg/mL achieving 100% sensitivity and specificity. This finding is consistent with [31], who demonstrated that serum IL-12 could differentiate between intrinsic and extrinsic asthma phenotypes in children, albeit with moderate accuracy. Additionally, [32] suggested that combining IL-12 measurements with other cytokines, such as IL-5, could further improve phenotype classification.

Regarding miRNA-140-5p, the study revealed significantly lower expression levels in asthma patients compared with healthy controls.

These results support the growing body of evidence indicating that miRNA-140-5p exerts anti-inflammatory effects and prevents airway remodeling by targeting genes such as TGFBR1 and SMAD3 [33]. Consistent with these findings, [34] reported that inflammatory stress suppresses miRNA-140-5p expression in lung cells, leading to upregulation of TGF-β1 and activation of fibroblasts, processes central to chronic asthma pathogenesis. Furthermore, [35] proposed that miRNA-140-5p could serve as a biomarker for chronic lung diseases owing to its regulatory role in inflammation. Thus, the present findings align with previous literature and reinforce the concept that miRNA-140-5p functions as an inhibitory regulator of airway inflammation in asthma [36].

Conclusions:

The study showed that elevated IL-12 and reduced miRNA-140-5p levels may serve as complementary non-invasive biomarkers reflecting airway inflammation and remodeling in asthma.

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