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# Circulating miR-21-5p and miR-155-5p as Non-Invasive Biomarkers for Diagnosing and Stratifying Bronchial Asthma Severity

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## ABSTRACT

**Objective:** Bronchial asthma is a heterogeneous chronic inflammatory airway disease driven by T helper 2 (Th2) immunopolarization. The objective assessment of asthma severity remains clinically challenging, particularly in settings with limited access to spirometry, necessitating the identification of reliable, non-invasive molecular biomarkers.

This study aimed to evaluate serum expression levels of miR-21-5p and miR-155-5p in adult patients with bronchial asthma; determine their correlation with total serum immunoglobulin E (IgE), blood eosinophil count (BEC), and disease severity; assess their diagnostic accuracy, and examine the independence of these associations from potential demographic and clinical confounders.

**Materials and Methods:** A case-control study design was employed involving the participation of 120 adults at Al-Diwaniyah Teaching Hospital, Iraq (October 2025-March 2026), consisting of 60 asthmatic patients and 60 healthy controls. Disease severity was confirmed based on disease duration, exacerbation rate, and responsiveness to corticosteroids. Quantification of serum levels of miR-21-5p and miR-155-5p was accomplished using the stem-loop qRT-PCR technique; total IgE was measured through the ELISA test; BEC was estimated based on the CBC test.

**Results:** Serum miR-21-5p and miR-155-5p were significantly elevated in asthmatic patients versus controls ($P<0.001$), with stepwise increases across severity groups. Both correlated positively with total IgE ($r=0.672$; $r=0.719$) and BEC ($r=0.579$; $r=0.756$), and inversely with FEV1% ($r=-0.705$; $r=-0.812$). Asthma severity independently predicted both miRNAs after multivariate adjustment ($\beta=2.12$ and $\beta=2.52$; $p<0.001$). ROC analysis yielded AUCs of 0.97 for miR-155-5p (sensitivity 96.7%, specificity 95.0%), 0.97 for total IgE, and 0.96 for miR-21-5p.

**Conclusion:** Serum miR-21-5p and miR-155-5p were robust, non-invasive biomarkers for diagnosing bronchial asthma and objectively stratifying its severity in an adult Iraqi cohort. Their severity-dependent upregulation is independent of key demographic confounders and is biologically anchored in the Th2-mediated, eosinophilic inflammatory cascade, underscoring their potential utility in resource-limited clinical settings.

**Keywords:** Asthma, IgE, BEC, microRNAs

## INTRODUCTION

Bronchial asthma is a chronic, heterogeneous inflammatory disorder of the airways, characterized by recurrent episodes of wheezing, dyspnea, chest tightness, and cough, associated with variable and reversible expiratory airflow limitation. In accordance with the Global Burden of Disease Study 2021, asthma currently affects over 262 million people worldwide, resulting in 21.6 million disability-adjusted life years (DALYs) each year, with incidence rates expected to remain persistently high through 2050 (1). According to the Global Initiative for Asthma (GINA) 2023 guidelines, asthma severity is classified as mild, moderate, and severe based on spirometric values, symptom frequen-

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cy, and exacerbation rate (2). Given that access to spirometry is frequently limited in low-resource clinical settings, the identification of non-invasive molecular biomarkers capable of diagnosing asthma and objectively measuring its severity constitutes an important research priority (2).

The immunopathogenesis of bronchial asthma is fundamentally driven by the polarization of $CD4^+$ T helper lymphocytes toward the Th2 subset, leading to the production of IL-4, IL-5, and IL-13. These cytokines orchestrate class switching of B lymphocytes to immunoglobulin E, eosinophilic airway inflammation, goblet cell metaplasia, and bronchial hyperresponsiveness (3,4). Total serum IgE, whose production is directly regulated by IL-4 and IL-13, serves as a primary effector molecule in type-2 high (T2-high) allergic asthma (5). Although IgE is inversely correlated with FEV1% predicted and positively correlated with disease severity, it has insufficient specificity to discriminate between asthma phenotypes, emphasizing the need for complementary molecular markers (6,7).

MicroRNAs (miRNAs) are endogenous, single-stranded, non-coding RNAs of 18-22 nucleotides that regulate post-transcriptional gene expression and coordinate complex immune responses (7). Their high stability in the circulation—due to incorporation into exosome vesicles and association with the Argonaut 2 protein complex—makes circulating miRNAs an extremely promising class of non-invasive biomarkers (8). Among the most studied miRNAs in asthma, miR-21-5p functions as a Th2-amplifying miRNA transcriptionally upregulated through the IL-13 and NF-κB pathways. It promotes the Th2 inflammatory cascade by downregulating IL-12p35 mRNA, thereby dismantling the Th1 cytokine axis, and targets phosphatase and tensin homolog (PTEN), activating the PI3K/Akt pathway and driving airway smooth muscle cell proliferation (3,9). Circulating miR-21-5p is significantly upregulated in asthmatic patients, with expression levels correlating with disease severity, total serum IgE, and the inverse of FEV1% predicted (10,11).

The second miRNA of central importance is miR-155-5p, whose overexpression promotes airway inflammation through suppression of SOCS1 and SH2-domain-containing inositol 5-phosphatase 1 (SHIP1), disinhibiting the JAK/STAT and NF-κB pathways and amplifying Th2 and Th17 cytokine production and IgE-mediated mast cell degranulation (12). Serum levels of miR-155-5p positively correlate with total IgE concentration and asthma severity, and negatively with the response to inhaled corticosteroid treatment (11). A comprehensive meta-analysis has identified the combination of serum miR-155-5p and miR-21-5p as the most diagnostically useful microRNA panel for asthma, with a pooled AUC of 0.93 (11).

While the diagnostic value of miR-21-5p and miR-155-5p has been individually addressed by previous studies and meta-analyses, the present study extends this evidence in several important respects. First, this investigation is among the first to systematically evaluate the co-expression of miR-21-5p and miR-155-5p alongside total serum IgE and BEC across the full GINA severity spectrum in an adult Iraqi population (13)—an ethnically and environmentally distinct cohort not previously represented in this body of literature. Second, this study simultaneously integrates serum miRNA levels, total IgE, BEC, spirometric indices (FEV1%, FVC%, FEV1/FVC), and peripheral oxygen saturation within a single, age- and sex-matched case-control design with multivariable adjustment for demographic and clinical confounders. Third, the stringent exclusion of subjects with elevated C-reactive protein (CRP) minimized the confounding effect of non-specific systemic inflammation—a methodological refinement not consistently applied in earlier studies.

This case-control study was conducted to determine the serum levels of miR-21-5p and miR-155-5p in adult patients with mild, moderate, and severe bronchial asthma at Al-Diwaniyah Teaching Hospital, Al-Qadisiyah Governorate, Iraq, along with their diagnostic accuracy and correlations with total serum IgE, BEC, spirometric parameters, and oxygen saturation.

## MATERIALS and METHODS

### Study Design and Patient Selection

This case-control study was conducted at the Department of Chest and Respiratory Diseases and Allergy and Asthma Center, Al-Diwaniyah Teaching Hospital, from October 2025 to March 2026. The study protocol was approved by the Ethics Committee of the College of Medicine, University of Al-Qadisiyah, and all participants provided written informed consent prior to enrollment. A total of 120 subjects were enrolled: 60 patients with physician-diagnosed bronchial asthma and 60 age- and sex-matched healthy volunteers. Bronchial asthma was diagnosed by a consultant pulmonologist on the basis of characteristic symptoms and spirometric findings, in accordance with

**Table I: Disease duration and exacerbation frequency by asthma severity group.**

| Parameter | Mild (n=25) | Moderate (n=20) | Severe (n=15) | H | p-value |
|---|---|---|---|---|---|
| Disease duration (years) | 5.16 ± 2.42 | 11.39 ± 3.38 | 22.05 ± 6.04 | 45.06 | < 0.001** |
| Exacerbations / year | 0.97 ± 0.71 | 3.14 ± 0.63 | 6.14 ± 1.28 | 50.83 | < 0.001** |

**H:** Kruskal-Wallis H statistic; **: Highly significant (p≤0.001); Mean ± SD

**Table II: Corticosteroid response distribution by asthma severity group.**

| Response | Mild n (%) | Moderate n (%) | Severe n (%) |
|---|---|---|---|
| Good | 20 (80.0) | 8 (40.0) | 0 (0.0) |
| Partial | 5 (20.0) | 10 (50.0) | 5 (33.3) |
| Poor | 0 (0.0) | 2 (10.0) | 10 (66.7) |

$\chi^2$=38.48, df=4, p<0.001

**Table III: qPCR primers used in this study.**

| Target miRNA | Primer Type | Sequence (5'→3') |
|---|---|---|
| miR-21-5p | Forward (F) | CGGCTAGCTTATCA-GACTGA |
| | Reverse (R) | GTGCAGGGTCCGAGGT |
| miR-155-5p | Forward (F) | CTCAGACTCGGTTA-ATGCTAATCGTGATAGG |
| | Reverse (R) | GCTGTGGCAGTGGA-AGCGTGATTTATT |

GINA 2023 criteria, including an FEV1/FVC ratio below 0.70 and significant bronchodilator reversibility (increase in FEV1 of ≥12% and ≥200 mL following short-acting bronchodilator administration).

Disease severity was classified according to the GINA 2023 criteria, based on airflow limitation (FEV1% predicted and FEV1/FVC ratio), symptom frequency, and exacerbation frequency requiring escalation of treatment. To further corroborate this classification with treatment-based evidence, data on disease duration, annual exacerbation frequency, and corticosteroid response were obtained from clinical records. The mild, moderate, and severe groups demonstrated a significant progressive increase in disease duration (5.16 ± 2.42, 11.39 ± 3.38, and 22.05 ± 6.04 years, respectively; H=45.06, p<0.001) and exacerbation frequency (0.97 ± 0.71, 3.14 ± 0.63, and 6.14 ± 1.28 episodes/year; H=50.83, p<0.001; Table I). Corticosteroid response differed significantly across groups ($\chi^2$=38.48, df=4, p<0.001): good response was documented in 80.0% of mild patients but in none of the severe patients, whereas poor response was concentrated in 66.7% of severe patients and was absent in the mild group (Table II). Detailed data on specific inhaled corticosteroid (ICS) doses, formal asthma control scores, and use of biologic therapy were not centrally recorded for all participants and are acknowledged as a limitation.

The control group comprised 60 healthy volunteers with no history of respiratory disease, allergy, autoimmune disease, or chronic illness, none of whom were smokers or on regular medication.

### Sample Collection

A total of 5 mL of venous blood was collected from each participant with a single venipuncture. Two milliliters were drawn into K2-EDTA-containing tubes for full blood count and BEC determination using an automated hematology analyser. The remaining 3 mL were drawn into plain gel tubes, allowed to clot at room temperature for 10-20 minutes, and centrifuged at 3,200 rpm for 10 minutes. The resultant serum was used for total RNA extraction, total serum IgE quantification by ELISA, and CRP determination. Subjects with elevated CRP were excluded to ensure that observed differences in miRNA expression were attributable to asthma pathophysiology rather than intercurrent inflammatory processes.

### Quantitative Reverse Transcription Real-Time PCR

Primers for stem-loop RT-qPCR of hsa-miR-21-5p (MIMAT0000076) and hsa-miR-155-5p (MIMAT0000646) were designed in-house using mature miRNA sequences from the miRBase database (www.mirbase.org), as detailed in Table III.

### Total RNA Extraction

Total RNA was extracted from 200 µL of serum using the TransZol Up Plus RNA Kit (Cat. No. ER501, TransGen Biotech, China) per the manufacturer's protocol. The aqueous phase following centrifugation at 10,000 × g was mixed with absolute ethanol, applied to an RNA Spin Column, washed, and eluted in 50-200 µL RNase-free water for storage at −80°C.

**Table IV: Demographic characteristics of asthma patients and healthy controls.**

| Characteristic | Detail | Asthma (n=60) | Control (n=60) | p-value |
|---|---|---|---|---|
| Age (years), n (%) | Mean ± SD | 50.63 ± 15.50 | 43.42 ± 15.35 | 0.238 NS |
| | < 30 yrs | 8 (13.3) | 14 (23.3) | |
| | 30-49 yrs | 18 (30.0) | 24 (40.0) | |
| | ≥ 50 yrs | 34 (56.7) | 22 (36.7) | |
| Sex, n (%) | Male | 26 (43.3) | 27 (45.0) | 0.717 NS |
| | Female | 34 (56.7) | 33 (55.0) | |

**NS:** Non-significant (p>0.05); Independent T-test for age (Mean ± SD); chi-squared test for sex and age group distribution.

### RNA Quantity and Purity Assessment

RNA quantity and purity were assessed using a NanoDrop Spectrophotometer (Thermo Scientific, USA). An A260/A280 ratio between 1.8 and 2.0 was considered indicative of acceptable purity.

### cDNA Synthesis and miRNA Quantification

Complementary DNA (cDNA) was synthesized using the stem-loop method with the EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Cat. No. AE311, TransGen Biotech, China). Cycling conditions comprised denaturation at 65°C for 10 min, cDNA synthesis at 42°C for 15 min, and enzyme inactivation at 85°C for 2 min. Real-time PCR was performed using 2× PerfectStart Green qPCR SuperMix in the MiniOpticon Real-Time PCR System (Bio-Rad Laboratories, USA). Relative expression was calculated using the $2^{-}\Delta\Delta CT$ method (14). U6 snRNA was used as the endogenous reference gene on the basis of its established stability in serum-based miRNA studies.

### Total Serum IgE Measurement

Total serum IgE was quantified using the Human IgE ELISA Kit (Cat. No. E0188Hu, Bioassay Technology Laboratory, China) by the sandwich ELISA method (detection range: 6-2,000 ng/mL; sensitivity: 3.39 ng/mL; $R^2$=0.9986).

### Determination of Blood Eosinophil Count

The percentage of eosinophils and the eosinophil counts were calculated based on a CBC obtained using the Zybio Z50 automated hematology analyzer where all the samples were analyzed within two hours of collecting the samples.

### Statistical Analysis

Data were analyzed using SPSS version 23.0. Continuous variables are expressed as mean ± SD. Group comparisons were performed using the Mann-Whitney U-test (two groups) and the Kruskal-Wallis H-test with Dunn's post hoc analysis (multiple groups). Categorical variables were compared with the chi-squared test. Spearman's rank-order correlation assessed associations between continuous variables. Diagnostic accuracy was evaluated by ROC curve analysis with optimal cut-offs determined by Youden's J statistic (AUC, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)). Multivariable linear regression was performed with miR-21-5p and miR-155-5p fold-change as dependent variables and age, sex, BMI, secondhand-smoke exposure, confirmed allergic comorbidity, and disease severity (ordinal: mild=1, moderate=2, severe=3) as covariates. p<0.05 was considered statistically significant; p≤0.001 was considered highly significant.

## RESULTS

### Demographic and Clinical Characteristics

Demographic characteristics are summarized in Table IV. The mean age of asthmatic patients was 50.63 ± 15.50 years, compared with 43.42 ± 15.35 years in healthy controls, with no statistically significant difference (p=0.238). Sex distribution did not differ significantly between groups, with a slight female predominance observed in both asthmatic patients (56.7%) and healthy controls (55.0%; p=0.717).

### Pulmonary Function Parameters

Pulmonary function parameters and $SpO_2$ showed highly significant stepwise deterioration across study groups (all P<0.001; Table V). FEV1% predicted declined from 90.54 ± 5.52% in controls to 29.49 ± 4.85% in severe asthma, and $SpO_2$ decreased from 98.03 ± 0.56% to 88.57 ± 1.95%.

### Serum miR-21-5p and miR-155-5p Expression

Serum miR-21-5p fold change was significantly higher in asthmatic patients than in healthy controls (3.96 ± 2.85 vs. 1.17 ± 0.53; p<0.001), with a highly significant stepwise increase across severity groups (H=25.189, P<0.001): 2.33 ± 0.80 in mild, 3.80 ± 1.56 in moderate, and 7.31 ± 3.25 in severe asthma (Table VI). Serum miR-155-5p fold change showed a significant stepwise elevation from 1.11 ± 0.56 in healthy controls to 6.90 ± 1.23 in severe asthma (H=35.641, P<0.001; Table VI), with a more pronounced severity gradient than miR-21-5p.

### Total Serum IgE and Blood Eosinophil Count

Total serum IgE was significantly elevated in asthmatic patients (741.37 ± 206.47 ng/mL) versus controls (325.36 ± 86.53 ng/mL; p<0.001), with a significant stepwise increase across severity groups (H=27.190, p<0.001). All pairwise comparisons were significant (mild vs. moderate: P=0.004; mild vs. severe: p<0.001; moderate vs. severe: p<0.001). Blood eosinophil count (BEC) was similarly and significantly elevated across severity groups (H=82.74, p<0.001), ranging from 214.19 ± 47.48 cells/μL in controls to 780.51 ± 263.93 cells/μL in severe asthma, with all pairwise severity comparisons reaching statistical significance (P<0.001). Full data are presented in Table VII.

### Spearman Correlations

Spearman correlation analysis within the asthma group (n=60) revealed strong positive correlations of both miRNAs with total serum IgE (miR-21-5p: r=+0.672; miR-155-5p: r=+0.719; both p<0.001) and significant inverse correlations with FEV1% predicted (miR-21-5p: r=−0.705; miR-155-5p: r=−0.812; both p<0.001). Blood eosinophil count (BEC) demonstrated significant positive correlations with both miR-21-5p (r=0.725, p<0.001) and miR-155-5p (r=0.759, p<0.001). All correlations are summarized in Table VIII and illustrated in Figure 1.

### Multivariable Linear Regression

After simultaneous adjustment for age, sex, BMI, secondhand-smoke exposure, and confirmed allergic co-

**Table V: Pulmonary function parameters and $SpO_2$ across all study groups (Kruskal-Wallis test).**

| Parameter | Control (n=60) | Mild (n=25) | Moderate (n=20) | Severe (n=15) | H | p-value |
|---|---|---|---|---|---|---|
| FEV1 (%) | 90.54 ± 5.52 | 65.91 ± 4.70 | 47.60 ± 5.17 | 29.49 ± 4.85 | 76.379 | < 0.001** |
| FVC (%) | 93.30 ± 7.55 | 76.59 ± 3.18 | 61.88 ± 3.90 | 47.92 ± 4.17 | 74.607 | < 0.001** |
| FEV1/FVC | 0.787 ± 0.034 | 0.698 ± 0.021 | 0.592 ± 0.026 | 0.479 ± 0.037 | 76.211 | < 0.001** |
| $SpO_2$ (%) | 98.03 ± 0.56 | 96.65 ± 0.93 | 94.63 ± 1.04 | 88.57 ± 1.95 | 64.344 | < 0.001** |

**H:** Kruskal-Wallis H statistic; **: Highly significant (p≤0.001); Mean ± SD

**Table VI: miR-21-5p fold change across study groups.**

| Group | Control (n=60) | Mild (n=25) | Moderate (n=20) | Severe (n=15) | H / U | p-value |
|---|---|---|---|---|---|---|
| miR-21-5p Fold Change | 1.17 ± 0.53 | 2.33 ± 0.80 | 3.80 ± 1.56 | 7.31 ± 3.25 | 25.189 H | < 0.001** |
| vs. Controls (U-test) | — | | | | 1840.5 U | < 0.001** |
| miR-155-5p Fold Change | 1.11 ± 0.56 | 2.09 ± 0.43 | 4.99 ± 1.35 | 6.90 ± 1.23 | 35.641 H | < 0.001** |
| vs. Controls (U-test) | — | | | | 1908.5 U | < 0.001** |

**U:** Mann-Whitney U; **H**: Kruskal-Wallis H; **: p≤0.001; Mean ± SD

**Table VII: Total serum IgE levels across study groups.**

| Group | Control (n=60) | Mild (n=25) | Moderate (n=20) | Severe (n=15) | H / U | p-value |
|---|---|---|---|---|---|---|
| Total IgE (ng/mL) | 325.36 ± 86.53 | 585.86 ± 144.25 | 740.41 ± 109.26 | 1001.81 ± 109.20 | 27.190 H | < 0.001** |
| vs. Controls (U-test) | — | | | | 1942.5 U | < 0.001** |
| Blood Eosinophil Count cells/μL | 214.19 ± 47.48 | 323.35 ± 68.18 | 598.52 ± 107.64 | 780.51 ± 263.93 | 82.74 H | < 0.001** |
| vs. Controls (U-test) | — | | | | 2014.5 U | < 0.001** |

**U:** Mann-Whitney U; **H:** Kruskal-Wallis H; **: p≤0.001; Mean ± SD.

**Table VIII: Spearman rank correlations between serum miR-21-5p, miR-155-5p, and clinical biomarkers (IgE, BEC, and spirometric parameters).**

| Gene | Parameter | r (Spearman) | p-value |
|---|---|---|---|
| miR-21-5p | miR-155-5p | 0.746 | < 0.001 |
| | Total IgE | 0.672 | < 0.001 |
| | FEV1 % | −0.705 | < 0.001 |
| | FEV1/FVC | −0.652 | < 0.001 |
| | BEC | 0.579 | < 0.001 |
| miR-155-5p | Total IgE | 0.719 | < 0.001 |
| | FEV1 % | −0.812 | < 0.001 |
| | FEV1/FVC | −0.836 | < 0.001 |
| | BEC | 0.756 | < 0.001 |

r: Spearman's correlation coefficient; **BEC:** blood eosinophil count; **IgE:** total serum immunoglobulin E; **: p≤0.001. All correlations computed within the asthma group (n=60).

morbidity, asthma severity remained an independent and highly significant predictor of both miR-21-5p (β=2.12, 95% CI: 1.17-3.08, p<0.001) and miR-155-5p (β=2.52, 95% CI: 1.98-3.05, p<0.001; Table IX). None of the potential confounders reached statistical significance (all p>0.10).

### ROC Curve Analysis

ROC curve analysis demonstrated excellent diagnostic performance for all four biomarkers (all p<0.001; Table X). miR-155-5p achieved the highest AUC of 0.97 (95% CI: 0.95-0.99; sensitivity 96.7%, specificity 95.0%). Total IgE achieved an equivalent AUC of 0.97 (95% CI: 0.94-0.99). miR-21-5p demonstrated an AUC of 0.96 (95% CI: 0.92-0.99). Blood eosinophil count (BEC) also demonstrated

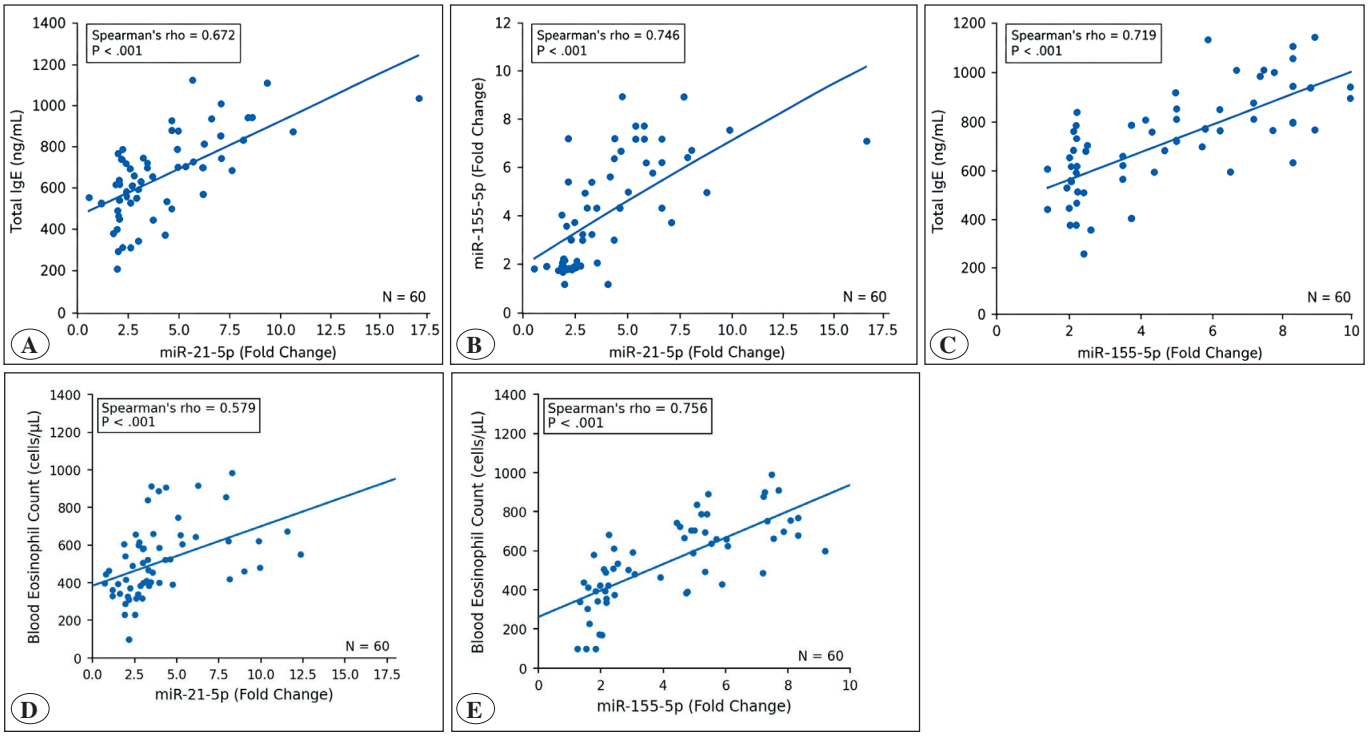


**Figure 1:** Spearman rank correlation scatter plots between serum miR-21-5p, miR-155-5p, total serum IgE, and blood eosinophil count (BEC) in adult asthmatic patients (n=60). Each panel displays individual data points with a linear regression trend line. All correlations were computed within the asthma group only.

**Panel A:** Significant positive correlation between serum miR-21-5p fold change (x-axis) and total serum IgE concentration (ng/mL; y-axis), consistent with the role of miR-21-5p in amplifying IL-13-driven B-cell class switching to IgE (r=0.672, p<0.001).

**Panel B:** Significant positive correlation between serum miR-21-5p fold change (x-axis) and miR-155-5p fold change (y-axis), suggesting co-regulated expression of both miRNAs within the Th2 inflammatory cascade (r=0.746, p<0.001).

**Panel C:** Significant positive correlation between serum miR-155-5p fold change (x-axis) and total serum IgE concentration (ng/mL; y-axis), reflecting the capacity of miR-155-5p to disinhibit JAK/STAT and NF-κB pathways and enhance IgE-mediated mast cell responses (r=0.719, p<0.001).

**Panel D:** Significant positive correlation between serum miR-21-5p fold change (x-axis) and blood eosinophil count (BEC, cells/µL; y-axis), indicating that higher miR-21-5p expression is associated with greater peripheral eosinophilia (r=0.579, p<0.001).

**Panel E:** Strong positive correlation between serum miR-155-5p fold change (x-axis) and blood eosinophil count (BEC, cells/µL; y-axis), the strongest BEC-miRNA association observed in this study, consistent with the established specificity of miR-155-5p for the eosinophilic asthma endotype (r=0.756, p<0.001).

**Table IX: Multivariable linear regression: predictors of miR-21-5p and miR-155-5p fold change (asthma group, n=60).**

| miRNA | Predictor | β | 95% CI | p-value |
|---|---|---|---|---|
| miR-21-5p | Severity (mild→severe) | 2.122 | 1.166 - 3.079 | < 0.001** |
| | Age | −0.019 | −0.054 - 0.015 | 0.267 |
| | Sex (male) | 0.748 | −0.371 - 1.867 | 0.185 |
| | BMI | 0.061 | −0.084 - 0.205 | 0.404 |
| | Secondhand smoke | 0.064 | −1.111 - 1.239 | 0.913 |
| | Confirmed allergy | −0.893 | −1.999 - 0.213 | 0.111 |
| miR-155-5p | Severity (mild→severe) | 2.515 | 1.979 - 3.051 | < 0.001** |
| | Age | 0.003 | −0.016 - 0.022 | 0.775 |
| | Sex (male) | −0.200 | −0.827 - 0.427 | 0.525 |
| | BMI | −0.016 | −0.097 - 0.065 | 0.697 |
| | Secondhand smoke | −0.042 | −0.701 - 0.616 | 0.898 |
| | Confirmed allergy | 0.167 | −0.453 - 0.787 | 0.592 |

**: P≤0.001; **CI:** confidence interval.

**Table X: ROC curve analysis of serum biomarkers for asthma diagnosis.**

| Biomarker | Cut-off | Sensitivity % | Specificity % | PPV % | NPV % | AUC (95% CI) |
|---|---|---|---|---|---|---|
| miR-21-5p | >1.905 | 95.0 | 96.7 | 96.6 | 95.1 | 0.96 (0.92-0.99) |
| miR-155-5p | >1.892 | 96.7 | 95.0 | 96.6 | 95.1 | 0.97 (0.95-0.99) |
| Total IgE (ng/mL) | >451 | 91.7 | 96.7 | 94.8 | 91.9 | 0.97 (0.94-0.99) |
| Blood Eosinophil Count cells/μL | >372 | 90.0 | 88.3 | 88.5% | 89.8 | 0.948 (0.907-0.990) |

**AUC:** Area under the curve; **PPV:** Positive predictive value; **NPV:** Negative predictive value; p<0.001 for all biomarkers.

good diagnostic accuracy with an AUC of 0.948 (95% CI: 0.907-0.990; cut-off>372 cells/μL; sensitivity 90.0%, specificity 88.3%). These individual performances exceed the pooled AUC of 0.93 reported for the combined miR-21/miR-155 panel in the meta-analysis by Xu et al. (11)

## DISCUSSION

This case-control study provides a comprehensive molecular characterization of circulating miR-21-5p and miR-155-5p in adult Iraqi patients with bronchial asthma, extending the existing literature in several clinically meaningful dimensions. Beyond confirming the diagnostic utility of these miRNAs, the study integrates BEC as an eosinophilic phenotype marker, corroborates the GINA severity classification with treatment-response data, and—critically—demonstrates through multivariable regression that the severity-related upregulation of both miRNAs is independent of age, sex, BMI, secondhand-smoke exposure, and allergic comorbidity.

The demographic characteristics of our study population demonstrated no significant age or sex differences between patients and controls, supporting the validity of group comparisons. The mild female predominance observed among asthmatic patients (56.7%) is consistent with the established post-pubertal shift in asthma prevalence toward women, attributed to estrogen-mediated enhancement of Th2 inflammatory responses, including mast cell degranulation and B-cell class switching to IgE (15).

The clinical stratification of disease severity was robustly validated not only by spirometric parameters—with FEV1% declining from 90.54 ± 5.52% in controls to 29.49 ± 4.85% in severe asthma—but also by a significant gradient in disease duration, exacerbation frequency, and corticosteroid response. Good corticosteroid response was documented in 80.0% of mild patients but in none of the severe patients. This treatment-response gradient directly mirrors the GINA framework's emphasis on treatment

requirement as the primary severity determinant (16,17) and provides treatment-based support for the severity classification used in this study.

Serum miR-21-5p levels exhibited a highly significant stepwise increase corresponding to disease severity, rising from a fold change of 1.17 ± 0.53 in controls to 7.31 ± 3.25 in severe asthma. This progressive upregulation is mechanistically coherent with the transcriptional induction of miR-21-5p by IL-13 and NF-κB pathways in T2-high inflammation (18). Our findings corroborate those of ElKashef et al., who reported significantly elevated serum miR-21-5p in an Egyptian adult asthma cohort alongside positive correlations with total IgE and inverse correlations with spirometric function (4). In contrast, Wardzyńska et al. observed no significant miRNA differences in a Polish cohort, attributing this to age-related variation in miRNA responsiveness (19). The age-matched design of the present study effectively mitigates this confounding variable, and our multivariable regression confirms that age was not a significant predictor of miRNA levels (p=0.267 for miR-21-5p; p=0.775 for miR-155-5p).

The expression profile of miR-155-5p was equally informative, demonstrating a pronounced severity-dependent increase from 1.11 ± 0.56 in controls to 6.90 ± 1.23 in severe asthma, with an H-statistic of 35.641 that surpassed that of miR-21-5p. This upregulation reflects the upstream regulatory role of miR-155-5p in suppressing SOCS1 and SHIP1, disinhibiting JAK/STAT and NF-κB pathways and amplifying Th2 cytokine production and IgE-mediated mast cell responses (12). A murine study by Kim et al. demonstrated that pharmacological inhibition of miR-155-5p significantly attenuated granulocytic airway inflammation (20) and Cañas et al. identified elevated miR-155-5p as specific to the eosinophilic asthma phenotype (3). This phenotypic specificity is consistent with the strong miR-155-5p-BEC correlation (r=0.759, p<0.001) observed in the present study.

Total serum IgE demonstrated a parallel severity-dependent elevation, reflecting the cumulative impact of IL-4 and IL-13-driven B-cell class switching.(5) Gil-Martínez et al. affirmed that total IgE serves as a reliable indicator of Th2 burden across the asthma severity spectrum, while also noting that it may lose discriminatory power in non-atopic asthma or during anti-IgE biologic therapy.(7)

Spearman correlation analysis extends prior evidence by demonstrating that the upregulation of both miRNAs is mechanistically coupled with Th2-mediated pathways driving IgE sensitization and structural airway obstruction, with miR-155-5p exhibiting particularly strong inverse associations with FEV1% (r=−0.812) and FEV1/FVC ratio (r=−0.836) (21). These correlations are somewhat stronger than those reported in the meta-analysis by Xu et al. (r=−0.61 for miR-155-5p-FEV1%) (11), a discrepancy attributable in part to the methodological standardization of the present study, including the use of a consistent serum matrix, CRP-based exclusion of non-specific inflammation, and U6 snRNA normalization (22).

Importantly, the association between both miRNAs and asthma severity remained statistically robust after multivariable adjustment for age, sex, BMI, secondhand-smoke exposure, and allergic comorbidity. Severity was the only independent predictor for both miR-21-5p (β=2.12, p<0.001) and miR-155-5p (β=2.52, p<0.001), while none of the potential confounders reached statistical significance. These findings substantially strengthen the causal interpretability of the miRNA-severity associations observed in this study.

The BEC-miRNA correlations provide an additional layer of biological validation absent from prior studies. BEC correlated strongly with both miRNAs in the full sample (r=0.725 and r=0.759 for miR-21-5p and miR-155-5p, respectively) and within the asthma subgroup alone (r=0.579 and r=0.756). Given that BEC is a clinically accessible, guideline-endorsed marker of the eosinophilic endotype (23), its concordance with circulating miRNA levels supports the mechanistic link between miRNA dysregulation and eosinophil-driven T2-high airway inflammation.

The diagnostic performance of the three biomarkers was exceptional. miR-155-5p achieved an AUC of 0.97, surpassing the pooled AUC of 0.93 for the combined miR-21/miR-155 panel reported by Xu et al. (11) and consistent with the designation by Gil-Martínez et al. of miR-155-5p as the most robust single circulating miRNA for asthma diagnosis (7). The superior performance compared with Elbehidy et al. (AUC=0.800 for miR-21) is at least partly attributable to the CRP-based exclusion of participants with non-specific systemic inflammation (24).

This study has several limitations. First, the cross-sectional design precludes the establishment of temporal causality, and longitudinal studies are needed to assess the directionality of miRNA changes over time. Second, formal allergen-specific IgE panels and fractional exhaled

nitric oxide (FeNO) measurements—a validated indicator of eosinophilic airway inflammation (25)—were not obtained, limiting complete T2-high endotype characterization. Third, specific ICS doses, formal asthma control scores (ACQ/ACT), and biologic therapy data were not uniformly recorded. Fourth, the sample size, while adequate for the primary aims, may limit the power of subgroup analyses, and replication in larger, multi-center cohorts is warranted.

## CONCLUSION

This study demonstrates that serum miR-21-5p and miR-155-5p are progressively and significantly upregulated across the full severity spectrum of bronchial asthma in an adult Iraqi cohort, with expression levels positively correlated with total serum IgE and BEC, and negatively correlated with FEV1% predicted. The severity-dependent upregulation of both miRNAs is independent of key demographic and clinical confounders. ROC curve analysis establishes their outstanding diagnostic accuracy, with miR-155-5p achieving an AUC of 0.97. These findings position circulating miR-21-5p and miR-155-5p as minimally invasive, biologically anchored molecular tools for diagnosing and stratifying bronchial asthma severity, particularly in resource-limited clinical settings. Prospective longitudinal studies examining miRNA dynamics in response to ICS and biologic therapies are warranted.

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**Ethical Approval**

This study was conducted in accordance with the ethical standards of the College of Medicine, University of Al-Qadisiyah, and with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to enrollment.

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**Conflict of Interest**

The authors declare no conflict of interest.

**Data Availability**

The raw data are available from the corresponding author upon reasonable request.

**Author Contributions**

Concept: **AMT, OMSA,** Design: **AMT, OMSA,** Data collection or processing: **AMT, OMSA,** Analysis or Interpretation: **AMT, OMSA,** Literature search: **AMT, OMSA,** Writing: **AMT, OMSA,** Approval: **AMT, OMSA.**

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