

The Role of Serum microRNA-1165-3p in Allergic Asthma

Rana A. Khashaba, Soher A. Abd Elsamee, Fatma R. Omar, Ola S. Elshimi

Department of Clinical and Chemical Pathology, Benha faculty of medicine, Benha University, Egypt.

Correspondence to: Rana A. Khashaba, Department of Clinical and Chemical Pathology, Benha faculty of medicine, Benha University, Egypt.

Email:

ranaatef2207.ra@gmail.com

Received: 10 September 2022

Accepted: 7 November 2022

Abstract

Background: Asthma is a complex, multifactorial, immune-mediated condition with a number of molecular immunopathological mechanisms underlying airway inflammation, hyper reactivity, and bronchial remodeling. MicroRNAs are important regulators in the pathogenesis and progression of chronic respiratory diseases, including bronchial asthma.

Objective: The aim of the study is to investigate the level of expression of serum miR-1165-3p in allergic asthma and its correlation with serum IgE levels. **Subjects and Methods:** A case-control study was conducted on 30 allergic asthma cases and 20 healthy subjects as a control group. The level of expression miR-1165-3p was evaluated by qRT-PCR using the comparative threshold cycle (Ct) method. Serum total IgE levels were measured using enzyme-linked immunosorbent assay (ELISA). **Results:** miR-1165-3p and serum total IgE level were significantly upregulated and elevated, respectively, in asthmatic patients in comparison with control group. The area under the ROC curve of miR-1165-3p showed 0.988 (95% CI 0.967 to 1, P value < 0.001). Asthmatic patients showed a significant positive correlation between the expression level of miR-1165-3p and IgE levels ($r = 0.687$, $P < 0.001$). **Conclusion:** Serum miR-1165-3p along with serum total IgE are valuable biomarkers in predicting and diagnosing asthma.

Keywords: Asthma, miRNA, miR-1165-3p, biomarker.

Introduction

Asthma is one of the most common respiratory diseases. It affects both children and adults. Asthma is a complex, multifactorial, immune-mediated condition

that presents with different clinical phenotypes.¹ Asthma is characterized by episodes of reversible airway constriction and chronic airway inflammation that could be

triggered by infection, environmental allergens, and irritants. The underlying pathogenetic mechanisms of asthma are yet poorly understood.²

Asthma is presented by different respiratory symptoms such as chest tightness, wheezes, shortness of breath, and cough with variable expiratory airflow limitation.³ This variable airflow limitation results from bronchoconstriction, airway edema, mucus secretion, airway hyper-responsiveness, and airway remodeling.⁴ The genetic studies of asthma have focused on genes associated with allergen-specific IgE production, inflammatory mediators generation, airway hyporesponsiveness expression and the determination of Th1 and Th2 ratio immune responses.⁵ Airway inflammation in asthma is due to the imbalance between Th1 cells that secrete IL-2 and IFN- γ and Th2 cells which secrete IL-4, -5, -6 and -13 that are responsible for the allergic immune response.⁵ Thus, two different phenotypes of asthma have been identified; Th2 asthma where patients respond to conventional corticosteroid therapy and non-Th2 asthma where patients show resistance corticosteroids.⁶

MicroRNAs (miRNAs) –the small, noncoding, single-stranded RNAs– play an important role in regulating gene expression by either suppression of protein synthesis or

degradation of RNA.⁷ They are involved in various biological functions, including immune cell maturation, activation, differentiation and maintenance of immune homeostasis.⁸ Studies shows that a wide range of miRNAs are controlling asthma and exploring their functions in the immunopathogenesis of asthma can help in the discovery of novel therapeutic targets.⁹

This study aimed at investigating the role of miR-1165-3p as a biomarker in allergic asthma as well as the correlation between miR-1165-3p expression and serum IgE levels.

Subjects and Methods

Patients and control samples

This case- control study included 50 subjects, 30 were allergic asthma cases with a mean age 52 ± 7 years, 15 males and 15 females, and 20 healthy subjects with a mean age 53 ± 7 years, 11 males and 9 females, were recruited in Zifta Chest Hospital, MOH, Egypt, between October 2019 and October 2020. Allergic asthma was diagnosed according to the published criteria of the Global Initiative for Asthma 2019 (GINA 2019).¹⁰ Any case showed clinical, or laboratory signs of infection was excluded. This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Faculty of Medicine, Benha

University. An informed consent was obtained from each subject before participation. A detailed history was taken from all participants to explore their medication history especially inhaled corticosteroids (ICS). On clinical examination, severity and control level of asthma were determined based on GINA 2019 and asthma control test, respectively. Asthma control refers to the extent to which the effects of asthma can be seen in the patient or have been reduced or removed by treatment. Asthma control can be assessed by symptom control and risk factors for future poor outcomes, particularly flare-ups (exacerbations). GSK[®] Asthma Control Test (<https://www.asthmacontroltest.com>) assess only symptom control.

Laboratory investigations

Venous blood samples were drawn into standard serum tubes, allowed to clot, separated by centrifugation, and stored at -80°C for subsequent testing for IgE levels and molecular testing for miR-1165-3p. and into standard K⁺-EDTA tubes for immediate complete blood count (CBC) analysis.

Determination of total IgE serum levels

Serum levels of total IgE in all studied subjects measured by an ELISA kit (Cat. #: BC-1035, Bio Check Inc., USA), according to the manufacturer's instructions. The assay sensitivity for IgE was 5.0 IU/ml.

miRNA extraction and qRT-PCR for miR-1165-3p

Two steps quantitative real-time PCR (qRT-PCR) was carried after serum miRNA was isolated using a miRNeasy Mini Kit (Cat. #: 217004, Qiagen, Germany) according to the manufacturer's instructions. miRNA purity and quantification were measured at 260/280 nm using NanoDrop Nucleic Acid Quantification (Thermo Fisher Scientific, USA). In the first step -reverse transcription-complementary DNA (cDNA) was produced using HiSenScriptTM RH(-) cDNA Synthesis Kit (Cat. #: 25014, iNtRON Biotechnology, South Korea) according to manufacturer's instructions, on Biometra Thermal cycler (Analytik Jena, Germany) for 45 min at 46 °C. Then, the reaction was terminated by inactivating the enzymes at 85°C for 10 min. In the second step, a quantitative PCR was performed using HERA SYBR qPCR Kit (Cat. #: WF1030400X, Willowofrt, UK), miR-1165-3p with forward primer 5'-AGCAGGCGCAGGGGGTGGTGTGGT-3' and reverse primer 5'-AGCAGGCGCAGGGGGTGGTGTGGT-3' and human GAPDH as internal reference with forward primer: 5'-AAGTCAAGGAAGCCGCAGA-3' and reverse primer: 5'-ATTCCCGCCTTAGCGTCA-3'. The qRT-

PCR reaction was performed on StepOne™ Real-Time PCR System (Thermo Fisher Scientific, USA) for 40 cycles at 95°C for 30 sec and 60°C for 30 sec. The relative quantification of miR-1165-3p was calculated using the Δ CT (cycle threshold) method. Because the relative quantities of the miR-1165-3p was normalized against the relative quantities of the endogenous control (GAPDH gene), fold expression changes were calculated using the equation $2^{-\Delta\Delta CT}$.

Statistical methods

Data management and statistical analysis were done using SPSS version 25. (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using the Shapiro-Wilk test and direct data visualization methods. According to normality testing, numerical data were presented as mean \pm SD or medians and ranges. Independent t-test or Mann-Whitney U test were used to compare between normally and non-normally distributed numerical variables, respectively. Categorical data were presented as numbers and percentages and compared using the Chi-square test. ROC analysis was done for using serum IgE and miR-1165-3p in diagnosing allergic asthma. Area Under Curve (AUC) with 95% confidence interval, best cut-off point, and diagnostic indices were calculated. Correlations were done using Spearman's

correlation. Logistic regression analysis was done for the prediction of allergic asthma. The odds ratio and the 95% confidence interval were calculated. All statistical tests were two-sided. P values less than 0.05 were considered significant.

Results

Characteristics of study population

Basic demographic and clinical characteristics of the studied 30 allergic asthma patients and the 20 age, sex, and body mass index matched healthy control subjects are shown in Table 1. No significant difference was detected between both groups regarding smoking (P 0.470). Most cases had severe asthma (46.7%). Asthma control test revealed that 36.7% of cases were uncontrolled and 33.3% were partially controlled. Only, MCHC and relative monocytes count were significantly higher in allergic asthma patients than controls (P 0.001 and 0.012 respectively).

Table 2

Serum IgE and miR-1165-3p in studied groups

The median IgE serum level was significantly elevated in allergic asthma cases as compared with healthy control subjects, 441.3 IU/ml, and 22.7 IU/ml (P <0.001). Serum miR-1165-3p was significantly 9.2-fold expressed in

allergic asthma patients than controls ($P < 0.001$). Table 2

Moreover, miR-1165-3p expression showed a significant positive correlation with serum IgE level in asthmatic patients ($r = 0.687$, $P < 0.001$). Fig. 1

ROC analysis was performed to assess the performance characteristics of serum IgE and miR-1165-3p in diagnosing allergic asthma. IgE showed an excellent area under ROC curve (AUC) of 0.988 with a 95% confidence interval (CI) (0.967 to 1). At cut-off > 200.7 IU/ml sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 93.3%, 100.0%,

100.0%, and 90.9%, respectively ($P < 0.001$).

The miR-1165-3p; showed an excellent AUC of 0.887 (95% CI 0.792 to 0.981). At the cut-off > 7.1 -fold, sensitivity, specificity, PPV, and NPV were 80%, 100%, 100%, and 76.9%, respectively ($P < 0.001$). Fig. 2

Multivariate logistic regression analysis was done for the prediction of allergic asthma. It revealed that serum Ig E (OR 1.031, 95% CI: 1.007–1.056, $P 0.010$) and miR-1165-3p (OR 1.605, 95% CI: 1.252–2.058, $P < 0.001$) were significant independent predictors for allergic asthma after controlling the effect of age, gender, BMI, and smoking.

Table (1). Demographic and clinical features of studied subjects.

Features	Allergic Asthma (n= 30)	Healthy controls (n= 20)	P
Age (years)	52±7	53±7	0.556
Gender	Males	11 (55.0%)	0.729
	Females	9 (45.0%)	
BMI (Kg/m ²)	28.7±2.8	29.4±2.8	0.379
Smoking	5 (16.7%)	5 (25.0)	0.470
Inhaled corticosteroids	7 (23.3%)	-	-
Asthma severity	Intermittent	3 (10.0%)	-
	Mild	5 (16.7%)	-
	Moderate	8 (26.7%)	-
	Severe	14 (46.7%)	-
Asthma control level	Well	9 (30.0%)	-
	Partial	10 (33.3%)	-
	Uncontrolled	11 (36.7%)	-

Data expressed as mean ±SD or number (percentage). BMI; Body mass index

Table (2). Laboratory findings in studied subjects.

Parameters	Allergic Asthma (n= 30)	Healthy controls (n= 20)	P
RBCs (x10 ⁹ /μl)	4.67 ±0.49	4.59 ±0.43	0.560
Hb (g/ml)	12.3 ±1.5	12.3 ±1.5	0.91
HCT (%)	37.7 ±4	38.4 ±4	0.599
MCV (fl)	80.9 ±5.4	83.6 ±5.8	0.113
MCH (pg)	26.2 ±2.1	26 ±2.2	0.733
MCHC (g/dl)	31.9 ±0.7	31.1 ±0.9	0.001
Platelets (x10 ³ /μl)	246 ±61	245 ±84	0.937
WBC (x10 ³ /μl)	7.2 ±2.3	6.9 ±1.7	0.697
Lymphocyte (%)	41 ±12	38 ±11	0.410
Monocytes (%)	6 ±2	4 ±2	0.012
Granulocytes (%)	53 ±14	57 ±12	0.224
Serum IgE (IU/ml)	441.3 (77.4–700.5)	22.7 (1.7–200.7)	<0.001
miR-1165-3p (fold)	9.2 (0.9–12)	1 (0.9–7.1)	<0.001

Data expressed as mean ±SD or median (range).

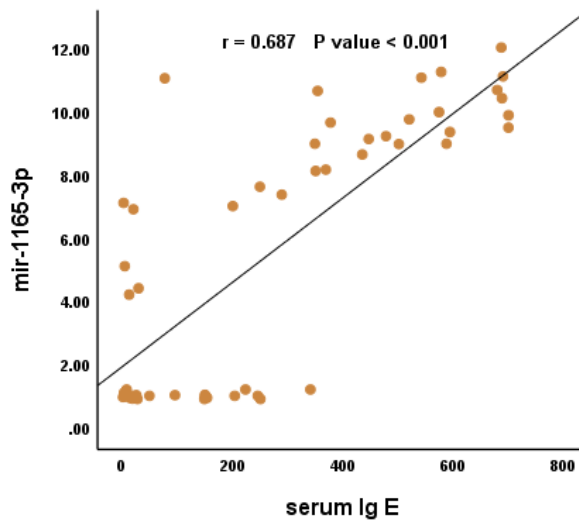


Figure (1). Correlation between miR-1165-3p and serum total IgE in asthmatic patients

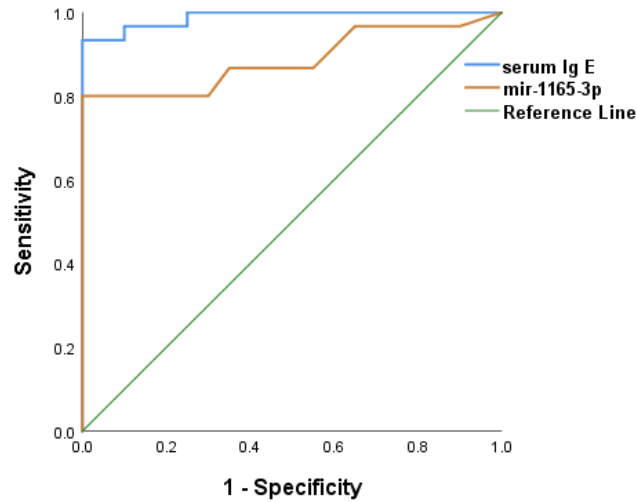


Figure (2). ROC analysis of miR-1165-3p and total IgE for diagnosing allergic asthma

Discussion

Asthma is a common, complex condition with a wide spectrum of symptoms caused by various pathogenesis. The prevalence of asthma increased dramatically in the last decades.¹¹ During the last decade, genome-wide association studies (GWAS) helped to identify the genetic susceptibility of asthma.¹² As genetics are of limited value explaining asthma development and pathogenesis, various studies have been conducted on the epigenetic changes such as microRNA (miRNA) expression, DNA methylation and histone modifications to have a better understanding of the disease's mechanisms.¹³ miRNA serves as posttranscriptional regulators of gene expression.¹⁴ miRNA have been found to play a role in coordinating the

phenotypic programming of immune and airway epithelial cells to increase the production of cytokines and other mediators, leading to inflammatory characteristics of asthma.¹⁵ Serum miR-1165-3p was studied to assess its role as a biomarker for the diagnosis and characterization of allergic asthma.

We found that serum miRNA-1165-3p expression was significantly upregulated in asthmatic patients compared to healthy control subjects ($P < 0.001$). This agreed with the published by Wu and colleagues, who demonstrated higher serum miRNA-1165-3p in of asthmatic patients more than control group.¹⁶ Furthermore, a higher expression of miRNA-1165-3p was detected in asthmatic patients' airways compared with the healthy

individuals, giving a notable evidence on the potential role of miRNA-1165-3p in asthma.¹⁷

We detected a significant positive correlation between miRNA-1165-3p expression and total IgE serum levels ($r = 0.687$, $P < 0.001$) in asthma cases which was on the contrary of findings in Wu et al. study.¹⁶

The diagnostic value of miR-1165-3p was assessed using ROC analysis and revealed an excellent area under the curve (AUC) value of 0.887 ($P < 0.001$) and a cut-off value of 7.1-fold with 80% sensitivity and 100% specificity. Our results were comparable with published data that showed an AUC value of 0.703 ($P 0.0051$) and a cut-off value of 2.41 with 83% and 68.2% sensitivity and specificity, respectively.¹⁶

Along with previous studies, serum total IgE showed an excellent AUC of 0.988 ($P < 0.001$) and a cut-off 200.7 IU/ml with 93.3% sensitivity and 100.0% specificity.^{16,18}

Using multivariate logistic regression analysis, we found serum total IgE and miR-1165-3p significant independent predictors for allergic asthma. This was supported with data published by Wu et al. and Wang et al. who confirmed the role of miR-1165-3p in asthma^{16,19} and Ahmad Al Obaidi et al. who confirmed the predictive value of serum IgE level in asthma, and in differentiating between

asthmatic and non-asthmatic individuals in conjunction with other biomarkers.²⁰

Conclusion

In conclusion, our current study demonstrated that serum miR-1165-3p expression a potential valuable biomarker in predicting and diagnosing asthma especially when combined with serum total IgE.

References

1. Patel SJ, Teach SJ. Asthma. *Pediatr Rev.* 2019;40(11):549-567. doi:10.1542/pir.2018-0282
2. Alizadeh Z, Mortaz E, Adcock I, Moin M. Role of Epigenetics in the Pathogenesis of Asthma. *Iran J Allergy Asthma Immunol.* 2017;16(2):82-91.
3. Tarlo SM, Malo JL. An ATS/ERS report: 100 key questions and needs in occupational asthma. *Eur Respir J.* 2006;27(3):607-614. doi:10.1183/09031936.06.00062105
4. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen MJ, FitzGerald MJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J.* 2008;31(1):143-178. doi:10.1183/09031936.00138707
5. Murphy DM, O'Byrne PM. Recent advances in the pathophysiology of asthma. *Chest.* 2010;137(6):1417-1426. doi:10.1378/chest.09-1895
6. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med.* 2012;18(5):716-725. doi:10.1038/nm.2678
7. Wang JW, Li K, Hellermann G, Lockey RF, Mohapatra S, Mohapatra S. Regulating the Regulators: microRNA and Asthma. *World Allergy Organ J.* 2011;4(6):94-103. doi:10.1186/1939-4551-4-6-94
8. Sonkoly E, Pivarcsi A. Advances in microRNAs:

- implications for immunity and inflammatory diseases. *J Cell Mol Med.* 2009;13(1):24-38. doi:10.1111/j.1582-4934.2008.00534.x
9. Elbehidy RM, Youssef DM, El-Shal AS, Shalaby SM, Sherbiny HS, Sherief LM, et al. MicroRNA-21 as a novel biomarker in diagnosis and response to therapy in asthmatic children. *Mol Immunol.* 2016;71:107-114. doi:10.1016/j.molimm.2015.12.015
10. Reddel HK, FitzGerald JM, Bateman ED, Bacharier LB, Becker A, Brusselle G, et al. GINA 2019: a fundamental change in asthma management: Treatment of asthma with short-acting bronchodilators alone is no longer recommended for adults and adolescents. *Eur Respir J.* 2019;53(6). doi:10.1183/13993003.01046-2019
11. Stern J, Pier J, Litonjua AA. Asthma epidemiology and risk factors. *Semin Immunopathol.* 2020;42(1):5-15. doi:10.1007/s00281-020-00785-1
12. Kabesch M, Tost J. Recent findings in the genetics and epigenetics of asthma and allergy. *Semin Immunopathol.* 2020;42(1):43-60. doi:10.1007/s00281-019-00777-w
13. Brooks C, Pearce N, Douwes J. The hygiene hypothesis in allergy and asthma: an update. *Curr Opin Allergy Clin Immunol.* 2013;13(1):70-77. doi:10.1097/ACI.0b013e32835ad0d2
14. Ambros V. The functions of animal microRNAs. *Nature.* 2004;431(7006):350-355. doi:10.1038/nature02871
15. Xu L, Yi M, Tan Y, Yi Z, Zhang Y. A comprehensive analysis of microRNAs as diagnostic biomarkers for asthma. *Ther Adv Respir Dis.* 2020;14:1753466620981863. doi:10.1177/1753466620981863
16. Wu C, Xu K, Wang Z, Chen Z, Sun Z, Yu W, et al. A novel microRNA miR-1165-3p as a potential diagnostic biomarker for allergic asthma. *Biomarkers Biochem Indic Expo response, susceptibility to Chem.* 2019;24(1):56-63. doi:10.1080/1354750X.2018.1501762
17. Woodruff PG. Subtypes of asthma defined by epithelial cell expression of messenger RNA and microRNA. *Ann Am Thorac Soc.* 2013;10 Suppl(Suppl):S186-9. doi:10.1513/AnnalsATS.201303-070AW
18. Tu YL, Chang SW, Tsai HJ, Chen LC, Lee WI, Hua MC, et al. Total serum IgE in a population-based study of Asian children in Taiwan: reference value and significance in the diagnosis of allergy. *PLoS One.* 2013;8(11):e80996. doi:10.1371/journal.pone.0080996
19. Wang Z, Ji N, Chen Z, Sun Z, Wu C, Yu W, et al. MiR-1165-3p Suppresses Th2 Differentiation via Targeting IL-13 and PPM1A in a Mouse Model of Allergic Airway Inflammation. *Allergy Asthma Immunol Res.* 2020;12(5):859-876. doi:10.4168/aaair.2020.12.5.859
20. Ahmad Al Obaidi AH, Mohamed Al Samarai AG, Yahya Al Samarai AK, Al Janabi JM. The predictive value of IgE as biomarker in asthma. *J asthma Off J Assoc Care Asthma.* 2008;45(8):654-663. doi:10.1080/02770900802126958

To cite this article: Rana A. Khashaba, Soher A. Abd Elsamee, Fatma R. Omar, Ola S. Elshimi. The Role of Serum microRNA-1165-3p in Allergic Asthma. *BMFJ* 2022;39(3):924-932.