Heliyon 10 (2024) e27694

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The role of matrix metalloproteinase-2 and miR-196a2 in bronchial asthma pathogenesis and diagnosis

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ARTICLE INFO

Keywords:
miR-196a2
Asthma
Pathophysiology
Matrix metalloproteinase-2
Diagnosis
IL-6

ABSTRACT

Background: Bronchial asthma is a persistent inflammatory respiratory condition that restricts the passage of air and causes hyperresponsiveness. Chronic asthma can be classified into three categories: mild, moderate, and severe. Remodeling took place as the extracellular matrix accumulated in the walls of the airways. Inflammation occurs as a result of the damage caused by matrix metalloproteinase-2 (MMP-2) to basement membrane type IV collagen. The severity of asthma may be associated with miR-196a2. The objective of our study was to investigate the underlying mechanisms and clinical relevance of miR-196a2 and MMP-2 serum levels in relation to the severity of asthma.

Methods: This study recruited 85 controls and 95 asthmatics classified as mild, moderate, or severe. Expression of miR-196a2 was measured by quantitative reverse transcriptase PCR. Using the enzyme-linked immunosorbent assay (ELISA), MMP-2, IL-6, and total immunoglobulin E (IgE) levels in the serum of asthmatics of various grades were compared to a control group. MMP-2's

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https://doi.org/10.1016/j.heliyon.2024.e27694
Received 4 November 2023; Received in revised form 4 March 2024; Accepted 5 March 2024
Available online 12 March 2024

diagnostic and prognostic potential was determined using ROC curve analysis. This study also measured blood Eosinophils and PFTs. We examined MMP-2's connections with IgE, blood Eosinophils, and PFTs.

Results: The current investigation found that miR-196a2 expression was significantly higher in the control group than in asthmatic patients as a whole. The study found that severe asthmatics had higher MMP-2, IL-6, and IgE serum levels than healthy controls. We identified the MMP-2 serum concentration cutoff with great sensitivity and specificity. Significant relationships between MMP-2 serum level and miR-196a2 expression in the patient group with severe asthmatics were found. The MMP-2, IL-6, and IgE serum levels were considerably higher in mild, moderate, and severe asthmatics than controls. The miR-196a2 expression and MMP-2 serum concentration correlated positively with IgE and blood eosinophils % and negatively with all lung function tests in the asthmatic patient group.

Conclusion: the study revealed that the elevated miR-196a2 expression and serum concentration of MMP-2, IL-6, and IgE associated with elevated blood eosinophils % is associated with pathophysiology and degree of asthma severity. The miR-196a2 expression and MMP-2 serum concentration have a promising diagnostic and prognostic ability in bronchial asthma.

1. Introduction

Asthma is a persistent inflammatory condition affecting the bronchial airways, which is characterized by heightened sensitivity to stimuli and the ability to reverse airflow restriction [1]. It is defined by recurrent episodes of respiratory symptoms such as chest tightness, wheezing, cough, and shortness of breath that vary over time and in intensity [2]. The determination of bronchial asthma diagnosis often relies on the observation of asthma symptoms, examination of medical history, and assessment of pulmonary function by tests such as forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) [3]. Asthma is recognized as a disease with multiple phenotypes that differ in severity, atopy, pathological findings, and response to therapy [4].

Recently, the prevalence of asthma has elevated at an alarming rate. Asthma affects up to 300 million people worldwide [5]. Annually, more than 250,000 deaths from bronchial asthma were recorded [6].

The lung airways of individuals with asthma have structural alterations known as airway remodeling, which encompasses all elements of the airway wall. These changes are believed to be initiated by inflammatory processes [7]. The extracellular matrix (ECM) is the primary constituent of the airway wall, and its buildup inside the airway walls serves as the primary catalyst for airway remodeling, a process intricately associated with the development of permanent airflow restriction [8]. The process of airway remodeling is distinguished by the infiltration of mast cells into airway smooth muscle (ASM) cells, leading to an increase in bronchial hyper-reactivity and the worsening of asthma symptoms [9]. The ASM cell is well recognized as a pivotal cell in the process of airway remodeling in asthma. It is responsible for the secretion of matrix metalloproteinases (MMPs), which play a crucial role in the regulation of ECM composition [10].

MicroRNAs (miRNAs, miRs) act as epigenetic mediators by binding to the 3′ or 5′ untranslated sections, gene promoter region, or coding sequence of their target mRNA and causing translational inhibition or transcript destruction, which has a negative effect on protein levels [11]. Various illnesses have been associated with alterations in the expression of miRNAs, which play a regulatory role in numerous vital disorders including cancer [12], liver disorders [13], bone diseases [14] and asthma [15]. They are involved in regulating signaling pathways of allergy and inflammatory responses of asthma as well as modulating therapy response [16,17].

MiRNAs are crucial for regulating a wide range of biological functions. Undoubtedly, these regulatory molecules contribute to the development of asthma. However, there is also the potential to utilise them as targets for therapeutic interventions [18]. Due to the involvement of miRNAs in the post-transcriptional regulation of numerous mRNAs, their functions heavily depend on the surrounding environment. These functions are affected by several aspects, including as miRNA-gene interaction, miRNA-target mRNA abundance and binding strength, cell type, miRNA expression level, and subcellular localization of miRNA expression [19].

In the field of asthma research, miR-196a2 has received a lot of interest lately [20]. Nevertheless, most of these studies had assessed miR-196a2 on DNA level via gene polymorphisms. These studies had revealed the association of miR-196a2 rs11614913 mutant C allele polymorphism with susceptibility to asthma. Moreover, a vital associated was observed between miR-196a2 minor genotypes (C) and incidence of eosinophilic asthma, an increased sputum eosinophil count, and higher frequency of nocturnal asthma. The function of the miR-196a2 rs11614913C allele was found to enhance the expression level of miR-196a2, which can target the anti-inflammatory protein annexin A1 [21,22].

The MMPs are a diverse collection of endopeptidases that rely on calcium and zinc for their activity [23]. The family of MMPs includes several subtypes, including gelatinases, collagenases, elastases, stromelysins, membrane-type MMPs, and other members [24]. The overexpression of MMPs is often seen in pathological states such as inflammatory disorders and cancer [25]. The proteolytic activity of MMPs is subject to stringent regulation, particularly via the action of tissue inhibitors of metalloproteinases (TIMPs) [26].

Matrix metalloproteinase-2 (MMP-2) is an enzyme synthesized by cells in various parts of the body and incorporated into the extracellular matrix. The extracellular matrix is a complex network of proteins and other molecules that fills the gaps between cells [23]. MMP-2 is alternatively referred to as 72 kDa type IV collagenase and gelatinase A. The matrix metalloproteinase (MMP) family, which includes MMP-2, is responsible for breaking down proteins in the extracellular matrix, specifically collagen. These proteins have important functions in various physiological processes, such as embryonic development, reproduction, and tissue remodeling.

However, they also contribute to disease processes, such as arthritis and metastasis. MMP-2 plays a crucial role in multiple physiological processes, such as the degradation of the uterine lining during menstruation, angiogenesis, tissue regeneration, and inflammatory responses. MMP-2 also contributes to bone remodeling, a physiological process involving the breakdown of old bone and the subsequent formation of new bone to replace it.

In recent times, there has been a notable emphasis on the function of MMPs in the development of asthma, mostly attributed to their impact on the migration and behavior of inflammatory cells, as well as their involvement in the destruction and remodeling of the ECM [8]. The levels of MMPs are increased in the respiratory airways of individuals diagnosed with asthma, especially during episodes of heightened asthma symptoms [27].

The proteolytic activity of MMPs has the capability to destroy the ECM and transmit signals to the cells that are embedded within it, therefore enhancing their responsiveness to various stimuli [28]. Furthermore, it has been suggested that MMPs could be involved in the impairment of lung elastic recoil seen in individuals suffering from chronic persistent asthma [27].

The MMP-2 is a member of the MMPs family that is encoded by the MMP-2 gene. Its primary function is the degradation of type IV collagen and gelatin found in the basement membranes [29]. The MMP-2 is the primary matrix metalloproteinase generated from airway ASM. Both ASM cells and bronchial epithelial cells have been shown to manufacture MMP-2, which plays a role in regulating their proliferation [28]. In addition, it is noteworthy that eosinophils, which are classified as inflammatory cells, have a significant role in the production of MMP-2 [30]. Furthermore, it has been shown that MMP-2 may be produced by T-cells, macrophages, and granulocytes in the context of inflammation [31].

The current research aimed to evaluate the miR-196a2 expression and serum concentration of MMP-2 in individuals with asthma in comparison to a control group of healthy individuals. Additionally, we investigated the potential diagnostic significance of miR-196a2 expression and MMP-2 serum concentration in individuals with asthma. Furthermore, we assessed the miR-196a2 expression and serum concentrations of MMP-2 in patients with mild, moderate, and severe persistent asthma to investigate the potential role of miR-196a2 expression and MMP-2 serum concentration as a prognostic indicator for bronchial asthma. In addition, we conducted an analysis to examine the relationship between the serum concentration of MMP-2 and the average levels of blood eosinophils and lung functions in a cohort of individuals diagnosed with asthma.

2. Methods

2.1. Subjects

A total of 180 Egyptian participants were included in the study, including 95 persons diagnosed with asthma and 85 healthy nonsmokers who were matched in terms of age and gender. The study recruited patients diagnosed with asthma from both inpatient and outpatient clinics associated with the Chest Department at Benha University Hospitals, Faculty of Medicine, as well as Benha Chest Hospital. All participants in the study provided written informed permission, and the research protocol received approval from the ethics committee of Badr University in Cairo. The study included individuals diagnosed with stable asthma and airway obstruction (FEV1/FVC $<70\%$) who exhibited a certain level of reversibility in FEV1 ($\geq 12\%$ or 200 ml) after using a bronchodilator, as per the criteria outlined in the Global Initiatives for Asthma (GINA) 2011 guidelines [32]. All individuals diagnosed with asthma were prescribed inhaled corticosteroids. The research categorized asthmatic patients into three groups depending on the severity of their asthma, as outlined by the GINA criteria [32].

Specifically, there were 16 patients classified as having mild asthma, 30 patients classed as having moderate asthma, and 49 patients defined as having severe persistent asthma. Participants who had lung illnesses other than asthma, active malignancy, history of parasite infection, immunological problem or inflammatory condition, chronic obstructive pulmonary disease, or smoked ≥ 10 packs per year were excluded from the study.

All participants underwent a comprehensive history-taking session, which included a thorough questionnaire regarding the clinical symptoms of asthma. Additionally, a complete physical examination, along with a plain chest X-ray and pulmonary function tests (specifically spirometry pre and post-bronchodilator administration) were carried out at Benha University Hospitals. Laboratory investigations, including the measurement of blood eosinophil count, serum MMP-2, interleukin-6 (IL-6), and serum total IgE concentrations, were performed at the Biochemistry Department at Badr University in Cairo.

2.2. Blood sampling and storage

During the clinic visit, venous blood samples were obtained from all participants and then split into two types of tubes: a gel-separating tube for serum samples and an EDTA-containing tube for whole blood samples. Serum samples were prepared by inducing blood coagulation in a gel-separating tube at ambient temperature for a duration of 30 min. Subsequently, centrifugation was performed at a speed of 4000 rpm for a period of 15 min. The resulting sera were then separated, split into two equal portions (referred to as aliquots), and preserved at a temperature of $-80\ ^{\circ}C$ until the time of analysis.

2.3. Evaluation of miR-196a2 expression level

The level of miR-196a2 expression was measured via quantitative reverse transcription polymerase chain reaction (qRT-PCR).

2.3.1. Total RNA isolation

The RNA was purified using the RNeasy Mini Kit (QIAGEN, Germany) according manufacturer's isolation protocol. The RNA concentration (ng/μL) and puritie were determined using NanoDrop® 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The resulting RNA samples were frozen at −80 °C till the reverse transcription step.

2.3.2. Reverse transcription step

Complementary DNA (cDNA) was synthesized from isolated RNA using a miScript reverse transcription (RT) kit (Qiagen, Germany) according to the producer's directives using a thermocycler (Biometra GmbH, Göttingen, Germany), according to the following thermal program: the reverse transcription (RT) reaction was carried out at 25 °C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 95 °C. Afterward, the reaction tubes containing cDNA were collected on ice until being used for cDNA amplification.

2.3.3. Quantification of miR-196a2 using quantitative real-time polymerase chain reaction

Using the miScript SYBR green PCR kit (Qiagen, Germany), we were able to discover miR-196a2 (Assay ID MS00008960) using StepOne® quantitative real-time polymerase chain reaction analysis. PCR findings were tested for accuracy using melting curves. A comparison to the endogenous control RNU6B (U6) primer was used to derive the miRNA data. The real-time polymerase chain reaction primers had the following sequences: miR-196a (target) forward primer, 5′-CGTCAGAAGGAATGATGCACAG-3′; reverse primer, 5′-ACCTGCGTAGGTAGTTTCATGT-3′; and U6 (reference) forward primer, 5′-CTCGCTTCGGCAGCACA-3′ and reverse primer, 5′-AACGCTTCACGAATTTGCGT-3′. The melting curve was obtained from 65 °C to 95 °C. The relative expression of miR-196a2 to U6 was calculated using equation $2^{-\Delta Ct}$, where ΔCt = (Ct miR-196a2 - CtU6). The fold change or relative expression of miR-196a2 was determined by the $2^{-\Delta\Delta Ct}$ method.

2.4. Serum matrix metalloproteinase-2, IL-6, and total immunoglobulin E measurements

The enzyme-linked immunosorbent assay (ELISA) technique was used to determine the serum concentration of MMP-2. A commercially available human MMP-2 ELISA Kit (Catalog No. EK0459, BBT Co., Ltd., Pleasanton, California, USA) was used in accordance with the instructions provided by the manufacturer. The serum concentration of IL-6 was determined using the ELISA technique, using a commercially available IL-6 kit (Catalog No. CRS-B001, ClinMax, Inc., USA), by the manufacturer's instructions The serum concentration of total IgE was determined using ELISA technique, using a commercially available total IgE kit (Catalog No.BC-1035, BioCheck, Inc., California, USA), in accordance with the manufacturer's instructions.

2.5. Determination of blood eosinophils % and pulmonary function tests (PFTs)

The count of blood eosinophils was conducted on a 2 ml aliquot of whole blood collected in ethylenediamine tetraacetic acid (EDTA), using the Sysmex XP-300 counting chamber manufactured by Sysmex, USA. The respiratory functions during stable asthma were assessed using the Sensor-medics spirolab III spirometer (Italy). The findings were computed as a percentage of both predicted and actual values, with the exception of the ratio between forced expiratory volume in 1 s and forced vital capacity percentage (FEV1/FVC%). To assess the reversibility of airway blockage, PFTs were conducted on each patient both before and after the administration of a bronchodilator inhalation [33].

2.6. Statistical analysis

This study analyzed data with GraphPad Prism 8.0 (San Diego, CA, USA). Both asthmatic and control group data were analyzed statistically. For parametric analysis data, a one-way ANOVA was followed by Tukey's multiple comparison tests. Kruskal-Wallis and Dunn's multiple comparisons tests were employed for non-parametric data. A Chi-squared test was employed to analyze nominal data. Relationships between variables were examined using Spearman's or Pearson's correlation coefficients. The data were provided as means ± SEM unless otherwise noted. Differences with a P value below 0.05 were considered significant. The cutoff value from Receiver Operating Characteristics (ROC) analysis of asthmatic patients and controls was used to calculate sensitivity, specificity,

Table 1
the baseline characteristics of the studied groups.

Variables		Control subjects n = 85	Total asthmatic patients n = 95			*P* value
			Mild persistent n = 16	Moderate persistent n = 30	Severe persistent n = 49	
Sex						
Male (%)	No.	25 (29.5%)	6 (37.5%)	12 (40%)	9 (18.5%)	0.42
Female (%)	No.	60 (70.5%)	10(62.5%)	18 (60%)	40(81.5%)	0.84
Age (years)	M±SEM	48.61 ± 1.1	43.0 ± 3.8	46.8 ± 1.7	48.6 ± 1.6	0.23
BMI (Kg/m^2)	M±SEM	28.8 ± 0.6	30.5 ± 0.7	28.3 ± 1.1	30.6 ± 1.0	0.30

BMI: body mass index, M ± SEM: mean ± standard error of Mean, a one-way ANOVA test was used to assess age and BMI while a Chi-squared test was used to analyze sex distribution and significant P value < 0.05.

positive predictive value, and negative predictive value. The ROC curve area for the tested protein was compared to AUC 0.5.

3. Results

3.1. The demographic data

Table 1 presents the baseline characteristics of the study participants, including both individuals with asthma and control persons. There were no statistically significant variations seen in terms of age, sex distribution, body mass index (BMI), and ethnicity between patients with mild, moderate, and severe persistent asthma and healthy control participants.

3.2. miR-196a2 expression level

The present study revealed a notable disparity in the expression levels of miR-196a2 between the control group and the group of individuals diagnosed with asthma. Specifically, the control group exhibited considerably greater expression levels of miR-196a2 compared to the asthmatic patients (Fig. 1). We showed that there were significant associations between MMP-2 blood level and miR-196a2 expression in the patient group with severe asthmatics.

3.3. Serum matrix metalloproteinase-2

In the present study, we found a significant increase in the serum concentration of MMP-2 of total asthmatic patients as compared to healthy controls (30.35 ± 0.40 ng/ml vs 20.53 ± 0.41 ng/ml, $p < 0.0001$). Moreover, significantly higher serum concentration was observed in severe persistent asthmatic patients as compared to mild, moderate persistent asthmatic patients and healthy controls. Furthermore, both mild and moderate persistent asthmatic patients showed a significant increase in serum concentrations of MMP-2 as compared to healthy controls as illustrated in Fig. 2.

The ROC curve for the serum concentration of MMP-2 of total asthmatic patients and healthy controls was shown in Fig. 3. Interestingly, the ROC curve analysis showed that the AUC for the serum concentration of MMP-2 was significantly higher than 0.5 (AUC = 0.96, $P < 0.0001$). The cutoff value of MMP-2 serum concentration was determined as (25.95 ng/ml) with high sensitivity and high specificity as shown in Table 2. The positive predictive value and negative predictive value of the MMP-2 serum concentration are illustrated in Table 2.

3.4. Serum Interleukin-6

Interestingly, our results showed a significant increase in the IL-6 serum concentration of total asthmatic patients as compared to healthy controls. In addition, significantly higher serum concentration was observed in severe persistent asthmatic patients as compared to other studied groups. moderate persistent asthmatic patients showed a significant increase in serum concentrations of IL-6 as compared to both mild persistent asthmatic patients and healthy controls Also, mild persistent asthmatic patients showed a significant increase in serum concentrations of IL-6 as compared to healthy controls as illustrated in Fig. 4.

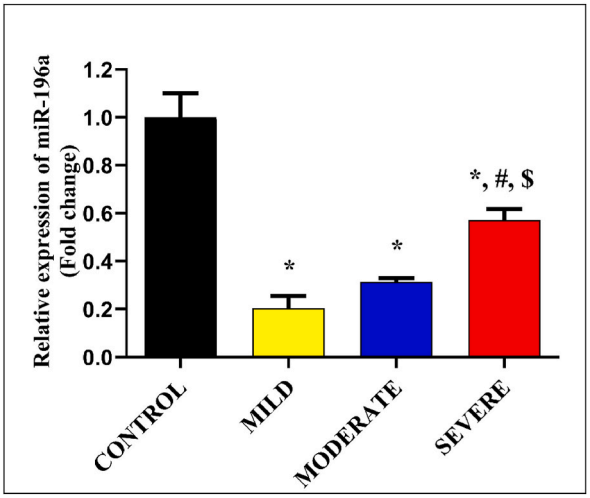

Fig. 1. miR-196a2 expression level in all the studied groups. * significant P value < 0.05 vs. control group, # significant P value < 0.05 vs. mild persistent asthmatic patients group, $ significant P value < 0.05 vs. moderate persistent asthmatic patients group and values are represented as mean ± SEM.

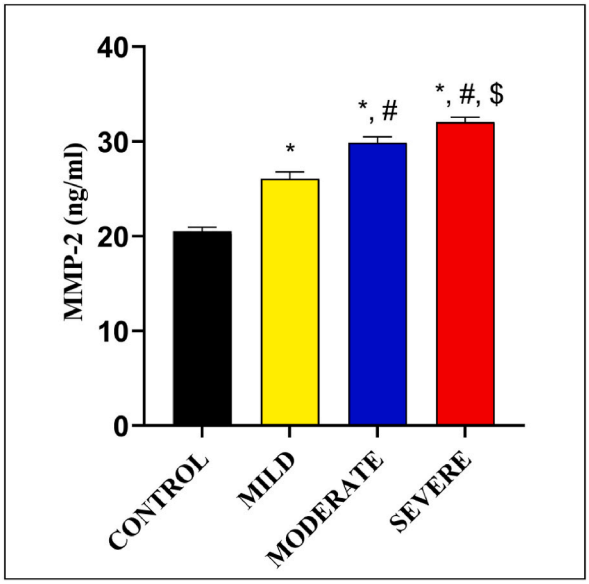

Fig. 2. Serum MMP-2 (ng/ml) concentrations in all the studied groups. MMP-2: matrix metalloproteinase-2, * significant P value < 0.05 vs. control group, # significant P value < 0.05 vs. mild persistent asthmatic patients group, $ significant P value < 0.05 vs. moderate persistent asthmatic patients group and values are represented as mean $\pm$ SEM.

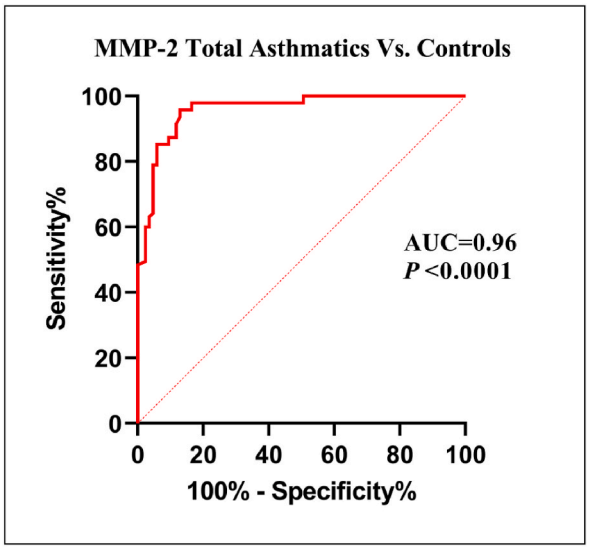

Fig. 3. ROC curve analysis of MMP-2 (ng/ml) serum concentration in discriminating asthmatic patients from non-asthmatic subjects. The cutoff value of MMP-2 serum concentration was 25.95 (ng/ml) with 87% sensitivity and 90% specificity. MMP-2: matrix metalloproteinase-2, ROC: Receiver operating characteristics, AUC: area under the curve.

3.5. Serum total immunoglobulin E

As expected, a significantly higher serum concentration of total immunoglobulin E (IgE) was observed in total asthmatic patients as compared to healthy controls (175.2 $\pm$ 8.1 IU/ml vs 46.9 $\pm$ 2.8 IU/ml, $p < 0.0001$). Furthermore, Mild, Moderate and severe persistent asthmatic patients had significantly higher serum concentrations of total IgE as compared to healthy controls. Moreover, severe persistent asthmatic patients showed the highest serum concentrations of total IgE when compared to all the studied groups as shown in Fig. 5.

Table 2
ROC analysis of MMP-2 serum concentration.

Cutoff	25.95 ng/ml
Sensitivity % (95% C.I.)	0.87 (0.78–0.94)
Specificity % (95% C.I.)	0.90 (0.81–0.96)
PPV%	89.74
NPV%	87.8
AUC	0.96
Standard error	0.013
95% Confidence interval	0.94 to 0.99
***P* value**	<0.0001

MMP-2: matrix metalloproteinase-2, ROC: Receiver operating characteristics, C.I.: confidence interval, AUC: Area under the ROC curve, PPV%: Positive predictive value, NPV%: Negative predictive value, significant P value < 0.05.

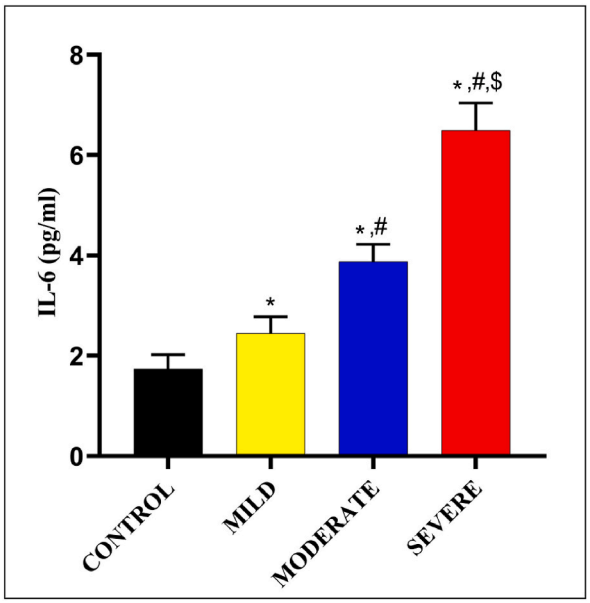

Fig. 4. Serum IL-6 (pg/ml) concentrations in all the studied groups. IL-6: interleukin −6, * significant P value < 0.05 vs. control group, # significant P value < 0.05 vs. mild persistent asthmatic patients group, $ significant P value < 0.05 vs. moderate persistent asthmatic patients group and values are represented as mean ± SEM.

3.6. Pulmonary function tests

In this study, the researchers observed a significant decrease in various pulmonary function tests among those who had asthma compared to a control group of healthy individuals. These tests included prebronchodilator forced expiratory volume in 1 s (PRE-FEV1), forced expiratory volume in 1-s percent of predicted (FEV1%), prebronchodilator forced vital capacity (PRE-FVC), forced vital capacity percent of predicted (FVC %), and the ratio of forced expiratory volume in 1 s to forced vital capacity percent (FEV1/FVC %). These findings are presented in Table 3.

We found a significant decrease in the mean values of PRE-FEV_1, FEV_1%, PRE-FVC, FVC%, and FEV_1/FVC % in severe persistent asthmatic patients as compared to mild, moderate persistent asthmatic patients and healthy controls. Moreover, both moderate and mild persistent asthmatic patients showed a significant decrease in the mean value of PRE-FEV_1 as compared to healthy controls. Furthermore, moderate persistent asthmatic patients showed a significant decrease in the mean value of FEV_1%, PRE-FVC, FVC%, and FEV_1/FVC % as compared to controls. A significant decrease in the mean values of FVC% was observed in moderate asthmatic patients as compared to mild asthmatic patients as illustrated in Table 3.

3.7. Blood eosinophils %

A significant increase was observed in blood eosinophils % in total asthmatic patients than healthy controls. Moreover, a significantly higher blood eosinophils % was observed in severe persistent asthmatic patients as compared to mild persistent asthmatic

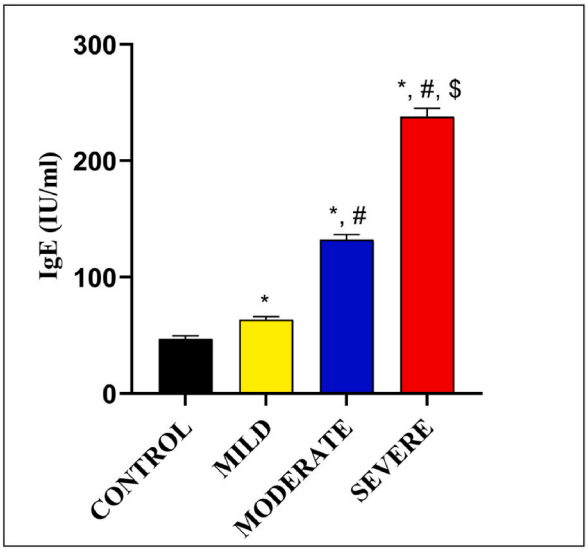

Fig. 5. Serum IgE (IU/ml) concentrations in all the studied groups. IgE: immunoglobulin E, * significant P value < 0.05 vs. control group, # significant P value < 0.05 vs. mild persistent asthmatic patients group, $ significant P value < 0.05 vs. moderate persistent asthmatic patients group and values are represented as mean ± SEM.

Table 3
Pulmonary function tests and blood eosinophils % of the studied groups.

Variables	Control subjects (n = 85	Mild asthmatic patients (n = 16)	Moderate asthmatic patients (n = 30)	Severe asthmatic patients (n = 49)	*P* value
PRE-FEV_1 (liters)	3.75 ± 0.1	2.69 ± 0.2*	2.05 ± 0.1*	1.05 ± 0.1$^{*,\#,\$}$	<0.0001
FEV_1%	88.5 ± 0.6	83 ± 0.9	66.4 ± 1.04*	38.7 ± 1.7$^{*,\#,\$}$	<0.0001
PRE-FVC (liters)	4.04 ± 0.1	3.86 ± 0.2	3.16 ± 0.2*	2.18 ± 0.1$^{*,\#,\$}$	<0.0001
FVC %	86.5 ± 0.6	90.8 ± 0.7	75.8 ± 1.01$^{*,\#}$	49.4 ± 1.6$^{*,\#,\$}$	<0.0001
FEV_1/FVC %	102.5 ± 0.5	69.8 ± 0.3	64.9 ± 0.5*	47.8 ± 1.4$^{*,\#,\$}$	<0.0001
Blood eosinophils %	2.54 ± 0.1	3.79 ± 0.5	4.68 ± 0.1*	5.075 ± 0.1$^{*,\#}$	<0.0001

PRE-FEV_1: prebronchodilator forced expiratory volume in 1 s, FEV_1%: forced expiratory volume in 1-s percent of predicted, PRE-FVC: pre-bronchodilator forced vital capacity, FVC %: forced vital capacity percent of predicted, FVC/FEV_1 %: ratio of forced expiratory volume in 1 s to forced vital capacity percent, * significant P value < 0.05 vs. healthy control group, # significant P value < 0.05 vs. mild persistent asthmatic patients group, $ significant P value < 0.05 vs. moderate persistent asthmatic patients group and values are represented as mean ± SEM.

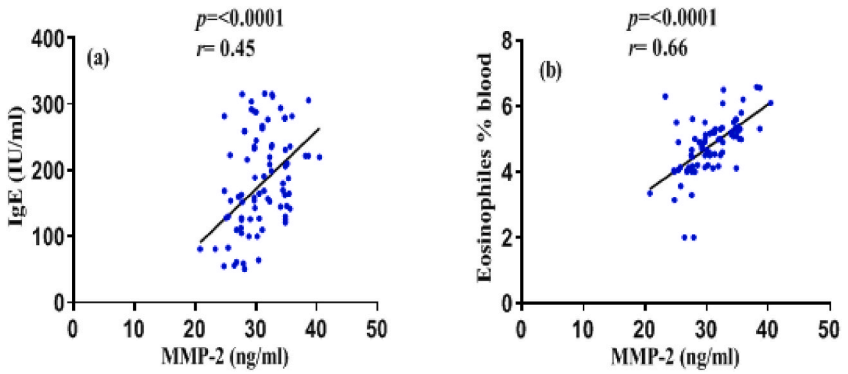

Fig. 6. Positive correlation observed between MMP-2 (ng/ml) and IgE (IU/ml) serum concentrations (a) and between MMP-2 (ng/ml) and blood eosinophils % (b) in total asthmatic patients (n = 95). MMP-2: matrix metalloproteinase-2, IgE: immunoglobulin-E, r: correlation coefficient, significant P value < 0.05.

patients and healthy controls. Moderate persistent asthmatic patients offered a significant increase in blood eosinophils % as compared to healthy controls as shown in Table 3.

Interestingly, significantly positive correlations were observed between the serum concentration of MMP-2 and the mean values of the serum IgE and blood eosinophils % in the patient population of the current study as shown in (Fig. 6a and b).

Furthermore, significantly negative correlations were noticed between the serum concentration of MMP-2 and the mean values of PRE-FEV_1 (Fig. 7a), PRE-FVC (Fig. 7b), FEV_1% (Fig. 8a), FVC% (Fig. 8b) and FEV_1/FVC % (Fig. 8c) in the total asthmatic patients.

4. Discussion

The baseline features of all groups were not significantly different, according to the study. These findings support Matsusaka et al. [34], who found no significant variations in age, sex distribution, BMI, or ethnicity between asthmatic patients and controls. This was done to prevent these factors from affecting study results as risk factors.

Deregulation of miR-196a2 has been linked to asthma and several forms of cancer, and previous research has shown that it can target many genes involved in cell cycle regulation and death. Asthma researchers have recently shown a great deal of interest in miR-196a2. Targeting several molecular and signaling pathways involved in inflammatory and immunological biological processes may contribute to the pathophysiology of the bronchial asthma [20].

According to the results presented here, miR-196a2 is expressed at a lower level in the serum of people with asthma than in the control group. In congruence with our results, an Egyptian study reported a decreased expression level of miR-196a2 in asthmatic patients when compared to healthy subjects [20].

Regarding the asthmatic patients group, a significant increase in the expression level of miR-196a2 in severe asthmatic patients when compared to both mild and moderate asthmatic patients. While no statistically significant difference was observed between mild and moderate asthmatic patients groups. Conversaly to our results, Ibrahim et al., reported an elevation in miR-196a2 expression level in moderate asthmatic patients when compared to severe asthmatic patients [20] which may explained by smaller sample size of the studied patients.

Different studies suggested the role of the miR-196a2 in the mechanism of eosinophilic asthma. Their results showed that higher level of miR-196a2 which can target the antiinflammatory and immunosuppressive protein annexin A1 leading to increasing airway hyperresponsiveness, respiratory secretions and a wheezy chest and airway inflammation in asthma [20–22,35,36].

The current study found that asthmatics had significantly higher MMP-2 serum levels than healthy controls. This shows that MMP-2 serum levels may be a biomarker for asthmatics and non-asthmatics. Xuan et al. [8], found increased blood MMP-2 in acute and chronic asthmatic children compared to the control group.

In this study, the serum MMP-2 AUC curve was created to assess its clinical use in diagnosing bronchial asthma. The serum MMP-2 AUC was statistically significant, indicating good sensitivity and specificity in distinguishing asthmatics from healthy controls. This study confirms the therapeutic utility of MMP-2 serum levels as a novel biomarker for bronchial asthma.

We believe our study group is the first to assess MMP-2 serum concentration's clinical relevance in bronchial asthma diagnosis and prognosis. Compared to a control group without asthma, patients with mild, moderate, and severe chronic asthma had higher MMP-2 serum concentrations. It was also found that severe persistent asthmatics had greater blood MMP-2 levels than moderate and mild asthmatics. Positive correlation between asthma severity and MMP-2 serum level rising. This study shows that asthma severity increases MMP-2 serum levels.

Xuan et al. [8] were the only study to compare asthmatic blood MMP-2 levels to healthy controls, and their findings match ours. Our findings are similarly consistent with Felsen et al. [37], who found increased MMP-2 activity in a rat model of acute asthma and chronic airway remodeling.

Furthermore, a significantly higher level of MMP-2 was observed when it was measured in the sputum and bronchoalveolar lavage fluid of asthmatic patients as compared with healthy individuals [38,39] which may support our results.

Thus, our work demonstrates that MMP-2 serum levels may indicate asthma severity. More MMP-2 in the blood means more asthma worsening and severity. Thus, increased serum MMP-2 levels indicate poor prognosis and progressive bronchial asthma.

Multiple factors cause chronic inflammation in bronchial asthma. Our findings demonstrated that asthmatic patients had significantly higher serum IL-6 than healthy controls. In addition, severe chronic asthma had greater IL-6 serum levels than the study group. Dimitrova et al. found that moderate and severe asthmatics have higher IL-6 levels than controls. IL-6 levels are higher in asthmatics than in healthy controls due to persistent inflammation and innate and adaptive immune response, notably in moderate and severe persistent asthma, supporting our findings [40].

This study found that asthmatics had higher blood total IgE levels than controls. Another major study found similar results [41]. Our findings supported an important Anupama et al. study [42] that found significantly higher serum total IgE levels in mild, moderate, and severe persistent asthmatic patients than in healthy controls. Thus, this study shows a definite link between asthma severity and IgE serum concentration. IgE serum levels rise with asthma severity. This implies that IgE serum levels may help diagnose and prognose bronchial asthma.

This investigation found a statistically significant positive connection between serum MMP-2 concentration and total IgE serum level, showing that MMP-2 and IgE play multiple roles in airway remodeling and asthma pathogenesis [43,44].

Airway inflammation is a prominent feature of asthma characterized by the infiltration of eosinophils and other inflammatory cells into the airways [45]. Many types of cells are involved in the pathophysiology of asthma [46]. Eosinophilic inflammation is a predominant feature of asthma [47]. Under normal physiological settings, eosinophils are present in the bloodstream in very small quantities [48]. While elevated levels of blood eosinophils are seen in conjunction with episodes of moderate and severe asthma

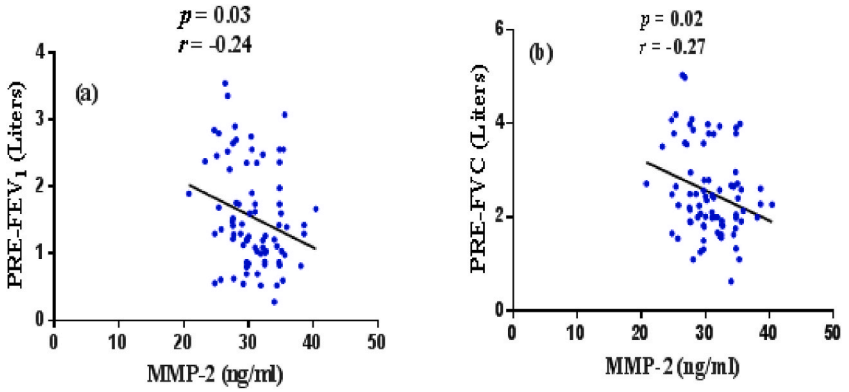

Fig. 7. Negative correlation observed between (a) MMP-2 (ng/ml) serum concentration and PRE-FEV_1 (liters) and between (b) MMP-2 (ng/ml) serum concentration and PRE-FVC (liters) in total asthmatic patients (n = 95). MMP-2: matrix metalloproteinase-2, PRE-FEV_1: prebronchodilator forced expiratory volume in 1 s, PRE-FVC: prebronchodilator forced vital capacity, *r*: correlation coefficient, significant *P* value < 0.05.

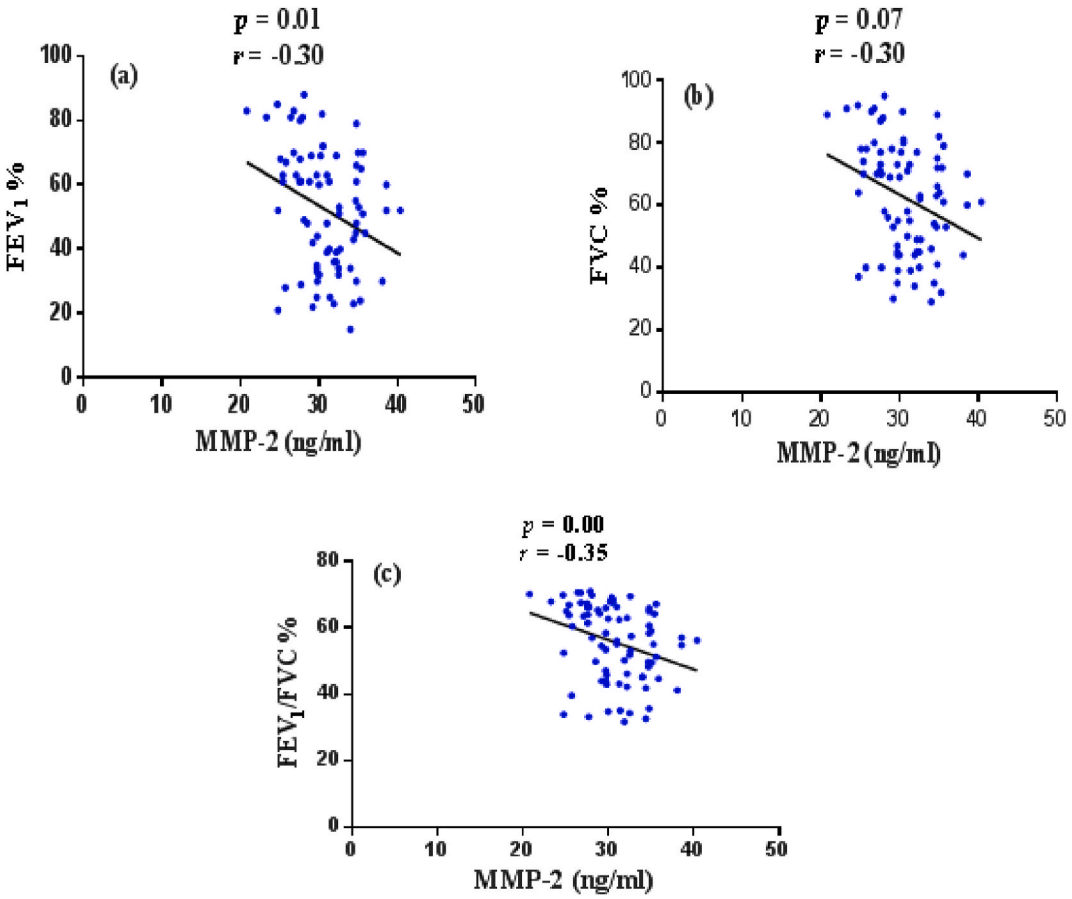

Fig. 8. Negative correlation observed between (a) MMP-2 (ng/ml) serum concentration and FEV_1 % and between (b) MMP-2 (ng/ml) serum concentration and FVC % in total asthmatic patients and between (c) MMP-2 (ng/ml) serum concentration and FEV_1/FVC % (n = 95). MMP-2: matrix metalloproteinase-2, FEV_1%: forced expiratory volume in 1-s percent of predicted, FVC %: forced vital capacity percent of predicted, FEV_1/FVC %: ratio of forced expiratory volume in 1 s to forced vital capacity percent, r: correlation coefficient, significant P value < 0.05.

exacerbation [49].

Our study found that asthmatics had more blood eosinophils than controls. The prior investigation by Signe et al. [49] found similar results. Blood eosinophils were significantly higher in severe persistent asthmatics than in moderate asthmatics. Blood eosinophils were significantly higher in severe and moderate chronic asthmatics than healthy controls. These findings supported the new study by

Westerhof et al. [50] that found blood eosinophils could accurately classify severe, moderate, and mild asthma phenotypes.

MMP-2 levels in the blood were positively correlated with eosinophil counts. Inflammation and asthma severity are positively correlated with MMP-2 serum levels. Chu et al. explained that eosinophils are a major source of MMP-2. Thus, as blood eosinophils rise, MMP-2 rises with asthma severity [51].

Our study found that overall asthmatics had a statistically significant decline in pulmonary function tests (PFTs), specifically PRE-FEV1, FEV1 %, PRE-FVC, FVC%, and FEV1/FVC %. Two significant research studies confirmed the anticipated outcomes [52,53]. Severe persistent asthmatics had significantly lower mean PFT values than moderate, mild, and healthy controls. Mean FVC% values were significantly lower in moderate asthmatics than in mild asthmatics. The mean PFT values of moderately persistent asthmatics were also significantly lower than those of healthy controls. Compared to healthy controls, moderate and mild chronic asthmatics had significantly lower PRE-FEV1 readings. These results were consistent with two other studies [7,9] which reported the same differences among patients with mild to moderate asthma, patients with severe asthma, and control subjects.

In total asthmatics, a strong negative correlation was found between MMP-2 serum concentration and PFTs such as PRE-FEV1, FEV1%, PRE-FVC, FVC %, and FEV1/FVC %, indicating that lung function declines with MMP-2 serum concentration. These findings imply that MMP-2 serum levels indicate pulmonary function decrease. The experimental results of An et al. supported this correlation [54].

5. Conclusion

In conclusion, this research revealed a significant elevation in MMP-2, IL-6, and IgE serum concentrations in asthmatic patients with different severities as compared to healthy controls. While, a significant reduction in miR-196a2 expression level was noticed in asthmatic patients when compared to healthy controls. In severe asthmatics, miR-196a2 expression is significantly higher than in mild and moderate asthmatics. There was no statistically significant difference between mild and moderate asthmatics. Moreover, the higher MMP-2, IL-6, and IgE concentrations may be associated with a higher degree of asthma inflammation, airway remodeling, and consequently asthma pathophysiology and severity. In addition, the MMP-2 serum concentration may be used as a potential biomarker for bronchial asthma with promising diagnostic and prognostic abilities and high sensitivity and specificity.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval and consent to participate

This study was conducted according to the Declaration of Helsinki and approved by the Badr University in Cairo (Approval number: IACUC/PHA/147/A/2023; May 12, 2023). informed consent was obtained from the participants. The authors have no ethical conflict.

Data availability statement

The study data are available on request from the corresponding author.

CRediT authorship contribution statement

Osama A. Mohammed: Writing – original draft, Investigation, Formal analysis, Data curation. **Ahmed S. Doghish:** Writing – review & editing, Validation, Methodology. **Mohannad Mohammad S. Alamri:** Writing – review & editing. **Muffarah Hamid Alharthi:** Resources. **Jaber Alfaifi:** Validation, Project administration. **Masoud I.E. Adam:** Writing – review & editing, Methodology, Conceptualization. **Abdullah Hassan Alhalafi:** Investigation, Formal analysis, Data curation. **AbdulElah Al Jarallah AlQahtani:** Writing – review & editing, Validation, Methodology. **Assad Ali Rezigalla:** Validation, Conceptualization. **Magaji Garba Taura:** Validation, Methodology. **Adamu Imam Isa:** Investigation, Formal analysis. **Ahad Fuad Binafif:** Writing – review & editing, Validation. **Mohammed A. Attia:** Investigation. **Elsayed A. Elmorsy:** Writing – review & editing, Visualization, Formal analysis. **Ayman A. Yousef:** Writing – review & editing, Validation, Methodology, Conceptualization. **Mustafa Ahmed Abdel-Reheim:** Investigation, Formal analysis, Data curation. **Mohamed A. Elkady:** Writing – review & editing, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are thankful to the Deanship of Graduate Studies and Scientific Research at University of Bisha for supporting this work through the Fast-Track Research Support Program. The authors would like to thank the Deanship of Scientific Research at Shaqra University for supporting this work.

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