Expression levels of plasma exosomal miR-124, miR-125b, miR-133b, miR-130a and miR-125b-1-3p in severe asthma patients and normal individuals with emphasis on inflammatory factors

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Research

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Abstract

Background

Identification of molecular markers, such as miRNAs is promising for the diagnosis of asthma and its clinical phenotypes. The aim of this study was to examine the changes in the expression of selected microRNAs in plasma exosomal fractions severe asthma patients. The expression of the miRNAs was determined in relation to the changes in serum immuno-inflammatory markers.

Method

Severe asthma patients (n = 30) and healthy subjects (n = 30) were selected among the individuals referred to the asthma and allergy clinic. Blood was collected from each participant for the estimation of high sensitive C-reactive protein (hs-CRP) and total IgE in serum. The exosomal fraction of plasma was also isolated by ultra-centrifugation and processed for quantitation of miR-124, miR-125b, miR-133b, miR-130a and miR-125b-1-3p expression using quantitative real time-PCR (qRT-PCR).

Results

Serum hs-CRP and total IgE were significantly higher in asthma patients as compared to controls. Expression of miR-124, miR-133b, and miR-130a were down-regulated in asthma patients as compared to controls ($p < 0.0001$). However, the expression of miR-125b was significantly higher in the patients compared to controls ($p < 0.0001$). No major changes in miR-125b-1-3p expression was found in the study groups. Data analysis indicated that among the miRNAs, changes in miR-125b in severe asthma patients was highly correlated with the serum levels of hs-CRP and IgE.

Conclusion

Overexpression of miR-125b that was significantly associated with serum IgE and hs-CRP may suggest that miR-125b is linked to inflammatory reactions in asthma. However, the involvement of miR-124, miR-133b, and miR-130a in asthma complication is ruled out, because they were a poor relationship between these miRNAs and the inflammatory markers.

Introduction

Asthma is a common inflammatory disease distinguished by intermittent airflow obstruction, remodeling, and airway inflammation, affecting 1–18% of the population in different countries. It is characterized by variable and recurring symptoms of wheezing, coughing, chest tightness, and variable expiratory airflow limitation [1]. The prevalence of asthma has been significantly increased in the 20th century [2] and three clinical phenotypes have been defined as; mild persistent, moderate persistent, and severe persistent asthma [3]. The majority of the asthma patients respond to anti-inflammatory treatments, however, 5–10% of patients, so-called severe asthma patients have been identified with uncontrolled asthma [4].

Asthma may occur due to an inflammatory response by activation of various inflammatory cells [5]. In fact, the correlation between inflammatory cells that produce biomolecules and airway smooth muscle cells (ASM) is a causal factor in the pathophysiology of asthma [6]. Hence, the molecules and intermediates that couple with signal transduction pathways play important role in the pathogenesis of asthma. The presence of different clinical asthma phenotypes provides this opportunity to discuss various molecular functions especially cytokines, such as interleukins, and other biomolecules in asthma pathogenesis. Immunoglobulin E (IgE) and highly sensitive C-reactive protein (hs-CRP) are two inflammatory markers that are associated with inflammatory diseases such as asthma [7, 8]. Binding of allergens to mast cells or basophilic cells may lead to cross-linking of the IgE on the cell surface, leading to the release of factors which mediates the reaction so-called type-1 hypersensitivity. In patients suffering from asthma, the potential of serum hs-CRP has been shown in recognizing the inflammatory process and increased serum hs-CRP may be used for assessment of treatment in inflammatory conditions [9].

Increased levels of sphingosine 1-phosphate (S1P) in the airways of asthmatic patients exacerbate the airway smooth muscles (ASM) hyper-responsiveness during the inflammation of the airways in a mast cell-dependent manner [10]. Therefore bioactive molecules such as S1P as a lipid mediator which acts as a messenger in various intercellular communication play important role in airway inflammation and asthma [11, 12]. S1P is generated from sphingosine through the actions of the sphingosine kinase1 and 2 (SphK1 / 2) isoenzymes.

Besides, microRNAs have been identified to be a target for inflammation. MicroRNAs are a group of small (18–22 nucleotides), single-stranded, non-coding RNAs which play a critical role in the regulation of gene expression and immune system functions [13, 14]. MiRNAs also have been reported to affect both the stability and the translation of mRNAs by base-pairing with the complementary sequences within mRNA molecules and effects on methylation or targeting of transcription factors [15].

A group of miRNAs has been shown that target S1P signaling pathway, such as miR-124 which targets Sphingosine Kinase 1 (SphK1) [16], miR-133b and miR-125b-1-3p bind to the Sphingosine-1-phosphate receptor 1 (S1PR1) [17, 18], miR-130a-3p targets Sphingosine-1-phosphate receptor 2 (S1PR2) [19], while miR-125b targets Sphingosine-1-phosphate lyase 1 (SGPL1) messenger RNAs [20]. The expression of these miRNAs inside the exosomes have also been reported [21–25].

Under the a pathological condition such as inflammation, dyspnea, airflow obstruction, and hypoxic pulmonary hypertension the exosomal miRNAs content could be altered which may play a role in the part of the cell stress response [26, 27].

Emerging of reports show the existence of exosomes in bronchoalveolar lavage fluid (BALF) of asthmatics patients with miRNAs that could potentially create inflammatory responses and could play in bronchial hyper-responsiveness [27, 28]. Development of molecular markers, particularly circulating miRNAs are an emerging issue which is promising for the diagnosis of asthma and its clinical phenotypes. Because conventional markers used for early diagnosis of asthma such as pulmonary

function tests are often insensitive and the use of bronchial biopsies for investigation of airway inflammation are invasive and costly to perform in clinics, several biomarkers such as pH, nitric oxides, cytokines, leukotrienes, isoprostanes, and other biomolecules are considered as non-invasive and valuable techniques in diagnosis and management of asthma[29, 30].

Changes in the expression of exosomal miRNA in the blood is promising for differential diagnosis of asthma and other inflammatory diseases. The use of these small molecules as biomarkers could be considered as a novel approach for diagnosis and monitoring the treatment of asthma [31, 32].

The specificity of miRNAs to asthma and its clinical phenotypes is possible by understanding the expression of each miRNA with its related pathway or the inflammatory reactions which are linked to the progression of the disease. In this line, Panganiban et al. have identified 30 miRNAs that were differentially expressed among healthy, allergic, and asthmatic subjects. These miRNAs have been grouped into 5 different expression patterns. In the case of asthmatic patients, 2 subtypes have been identified that differed by high or low peripheral eosinophil levels. Circulating miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b levels were most predictive of allergic and asthmatic status [33].

The aim of this study was to identify miRNAs that may differentially be expressed in severe asthma patients. Thus, based on the databases some microRNAs that are believed to target the genes and pathways in inflammatory reactions have been selected. The abnormal changes in the expression of miR-124, miR-125b, miR-133b, miR130a, and miR-125b-1-3p, in the exosomes derived from plasma of asthma patients, was studied. Besides, changes in the microRNA expression in link to serum CRP and IgE was further evaluated.

Methods

Patients and sample collection

In this case-control study 30 severe asthma patients (aged 23-60 years) referred to the Asthma and Allergy Clinic (Masih Daneshvari Hospital, Tehran, Iran) was enrolled. All the patients were examined and diagnosed with severe asthma by the clinicians according to the clinical guidelines of the Global Initiative for Asthma (GINA, 2019) as described by Reddel et al [34]. Besides, 30 healthy individuals with an age range of 25-58 years (matched to patients) were also enrolled. Exclusion criteria were consumption of immune-mediated inflammatory disease (IMID), lung diseases such as COPD, infections like pneumonia, lung cancer, many other breathing problems, smokers, any type of systemic disease, and neoplasms. The demographic and clinical characteristics of patients and controls are summarized in Table-1.

Blood samples were collected from all the participants and controls from May 2018 to July 2019. This study has been approved by the Medical Ethics Committee of the Tarbiat Modares University (ID: IR.MODARES. REC.1397.047).

Approximately 5 ml of peripheral intravenous blood specimen was collected from each individual. The sample was divided into two aliquots, one in a tube containing an anticoagulant (K3-EDTA), and another aliquot was collected in a clot tube (with gel separator).The sample collected in a k3-EDTA tube(3 mL) was used for exosome separation. In this case, blood was centrifuged for 10 min at 1600 xg at room temperature to separate plasma. Plasma samples were separated and filtered through 0.22-μm syringe filters before transferring into new tubes. Plasma samples were further centrifuged at 16000 xg for 10 minutes at room temperature to remove residual dead cells, cellular debris, and apoptotic bodies as described by [35]. As well as blood collected in a clot tube (2mL) were centrifuged in 3000rpm for 15 min at 4°C and then the separated serum was used to determine the level of hs-CRP and total IgE as described before.

Estimation of serum C-reactive protein (CRP) and IgE

The total serum IgE was measured in all the samples by Electrochemiluminescence immunoassay (ECLIA) technique using fully automated immunoassay analyzer (Cobas e 411, Roche Diagnostics GmbH, Germany) following the manufacturer's instructions. IgE concentration was considered positive at >100 IU/mL for adults as recommended (Biomedical Laboratory Inc.)[36].

serum High-Sensitive C- reactive protein (hS-CRP) was also estimated based on turbidimetric assay on COBAS INTEGRA® 400 plus (Roche Diagnostic, Mannheim, Germany) autoanalyzer at wavelength of 552 nm. The reference interval was <5 mg/L for adults (Biomedical Laboratory Inc.)[37, 38].

Isolation of exosomes from cell-free plasma

Exosomes were isolated from cell-free plasma samples by ultracentrifugation as described by [39]: Briefly, 500 μl of plasma sample was diluted with an equal volume of phosphate-buffered saline (PBS) and centrifuged for 30 min at 12000 ×g at 4˚C. The supernatant was separated and transferred into an ultracentrifuge tube and centrifuged for 70 min at 100,000 ×g at 4◦C (VS-35SMTi, Vision Scientific Co. Ltd., South Korea). The pellet obtained from each sample was re-suspended in 1 ml of PBS, and centrifuges again at 100,000 ×g at 4˚C for 70 min to wash the pellet. The supernatant was discarded and the exosomal fraction was collected and re-suspended in 150 μl PBS and stored at −80˚C for further use.

Protein determination

The total protein content of the exosomal fraction was measured using the BCA Protein Assay kit (Thermo Scientific Pierce™, USA). This method is based on bicinchoninic acid for the colorimetric detection of total protein according to the manufacturer's instruction. Finally, the protein concentration was calculated using a linear standard curve drawn with bovine serum albumin (BSA) standard provided with the assay kit.

Characterization of the exosomes

Different approaches were used to check the quality and quantity of the isolated exosomes. The samples were subjected to the dynamic light scattering method (DLS) with Zetasizer Instrument (Malvern Zetasizer, Nano Series, UK). Then the samples were subjected to Transmission electron microscopy (TEM) analysis. In this experiment, the exosome-containing solution was mixed with glutaraldehyde solution (25%) and dried onto 100 mesh carbon-coated TEM grids. After fixation, the grids were negatively stained with phosphotungstic acid (PTA) and imaged were recorded on a Zeiss-EM10C transmission electron microscopy (TEM, Zeiss–EM10C–100 kV, Germany) operating at an accelerator voltage of 100KV.

The exosomes were also subjected to flow cytometry analysis as described earlier [40]. For this, 10 µg of exosomes was coupled with 20 µl of aldehyde/sulfate latex beads (4 µm in diameter), (Invitrogen, USA) for overnight at 4°C on an orbital rotator for the blocking of remained sites. The product was then incubated with 100 mM glycine (Sigma-Aldrich, USA) for 30 min and after washing with FACS buffer, stained with CD63-PE antibody or isotype control and CD81-FITC antibody or isotype control (eBioscience, San Diego, CA, The USA). Then 10000 events were measured by a Flow cytometer (FACS Calibour, BD, USA). Data were analyzed using FlowJo software (FlowJo 7.6).

RNA extraction and cDNA synthesis

Total RNA was extracted from each exosomal sample using TRIzol solution (Invitrogen, USA). The exosomal RNA concentration (ng/µL) was quantified using the NanoDrop-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Absorbance at 260nm was used for the determination of total RNA concentration in the samples. The 260/280 nm (1.8 - 2) and 260/230 nm(1.8 – 2.2) ratio were used to assess RNA purity. The RNA samples were then resuspended in 20 µl DEPC water and stored at -20 ˚C for further use. Then 5 µg of each RNA sample was subjected to reverse transcription using the BON-miR miRNA 1st-Strand cDNA Synthesis Kit based on Poly (A)-Tailed universal Reverse Transcription method using poly(A) polymerase (PAP) as described earlier [41].

Real-Time Quantitative PCR (qRT-PCR) for miRNA expression

For this experiment, 5 microRNA molecules were selected based on their target genes identified by the TargetScan, miRTarBase and miRDB databases, and based on the target genes.

The exosomal miRNA expression was determined using the BON-miR High-Specificity miRNA qPCR Kit and miRNA-specific primers (synthesized by Stem Cell Technology Research Center, Tehran, Iran, BON209001) using LightCycler® 96 PCR system (Roche Applied Science, Germany). A two-step real-time PCR protocol was adopted using an initial denaturation step of 2 min at 95°C, followed by 40 amplification cycles including a denaturation step (5s at 95°C), and an annealing step (the 30s at 60°C) based on the kit brochure. The melting curve was prepared for all the reactions to confirm the precision of each sample. The cycle threshold (Ct) values for the samples were normalized to the U6 expression as an internal control and results were then converted into fold change using the 2^-ΔΔCt formula.

Statistical analysis

The qPCR data were analyzed using The 2^-ΔΔCt formula by the Livak method[42]. All the assays were performed in duplicate and the results are presented as mean ± SD of samples from patients and normal individuals. The data were analyzed using the Graphpad Prism Software (version 8.0). The differences between groups were compared by Student's t-test and Mann Whitney U test for significance ($p < 0.05$). In other parts of the paper, data are shown as means (95% confidence intervals; 95% CI). The relationship between inflammatory factor concentrations and miRNAs expression in severe asthma patients was assessed using Spearman's rank correlation test.

Results

Characterization of plasma exosomes

The exosomes prepared from plasma samples of asthma patients and normal individuals were found to be spherical shape particles as shown by TEM (Fig 1A). The average size of the particles was 55 nm as determined by the DLS method (Fig 1B). Flow cytometry analysis showed that the isolated exosomes were positive for CD81 and CD63 surface markers (Fig 1C).

Expression of the micro RNAs in exosomal fraction

As shown in figure-2, the qRT-PCR data show that the expression of miR-124 in plasma exosomes was down-regulated in the patients suffering from severe asthma compared to that measured in normal individuals (normal individuals, 2.12±1.14, and patients, 0.39±0.196). Likewise the expression of miR-130a (normal, 1.69±1.364; asthma patients:0.29 ±0.22), and miR-133b (normal: 2.38±0.94, asthma patients: 0.47±0.18) were significantly lower in patients as compared to controls.

In contrast, the expression of miR-125b in plasma exosomes of the asthma patients was substantially increased when compared to healthy control samples (normal:0.52±0.33; asthma patients: 2.188±1.53). The expression of miR-125b-1-3p remained within normal range (normal: 0.48±0.34; patients: 0.37±0.26).

The expression data presented above were normalized to internal control (U6) prior to analysis. The values are presented as Mean±SD.

The expression level of each microRNA is presented as the relative expression (fold change). The differences between the controls and patients were presented as Mean ± SD. The results show that the expression of miR-124, miR-133b, and miR-130a significantly down-regulated in the patients' group. Whereas, the expression of miR-125b was significantly up-regulated. The expression of miR-125b-3p remained unchanged in asthmatic patients compared to the healthy group (Table 2 and Fig. 3).

The data obtained from the Receiver Operating Characteristic (ROC) curve show that expression of miR-124 with AUC=0.96 (95% CI: 0.92–1.00), the miR-125b with AUC=0.91 (95% CI: 0.84–0.98), the miR-133b

with AUC=0.99 (95% CI: 0.97–1.00) and miR-130a with AUC=0.87 (95% CI: 0.79–0.96) were correlated with the asthma condition ($p<0.05$) (Table 3 and Fig. 4).

Comparison of serum hs-CRP and total IgE level in asthmatic patients and control

Comparison of the serum levels of CRP and IgE between the control subjects and severe asthma patients are as shown in figure 5. Serum hs-CRP (controls=1.075 ± 0.21; patients= 6.659 ± 0.94) and IgE (controls=39.32 ± 5.33; patients=23.8 ± 25.79) levels in asthma patients were significantly higher than that of healthy subjects ($p<0.0001$).

Correlation between hs-CRP and IgE with the exosomal miRNAs

As shown in Table-5, correlation analysis showed that the expression level of exosomal miRNA125b is significantly correlated with the serum hs-CRP and total IgE levels (CRP; $r=0.86$, $p<0.0001$ and IgE, $r=0.68$, $p<0.0001$). The relationship between the expression of other miRNAs and serum levels of IgE and hs-CRP in asthma patients was statistically not significant (Figure6).

Discussion

In the current study, serum levels of hs-CRP and total IgE as the immuno-inflammatory markers with the expression of exosomal miRNAs (miR-124, miR-125b, miR-130a, miR-133b, and miR-125b-1-3p) were evaluated in severe asthma patients. The current results clearly show that among the selected miRNAs, only the expression of plasma exosomal miR-125b was significantly increased which was correlated with the inflammatory indices measured in serum. Although the exact targets related to severe asthma complications for these miRNAs are not well known, changes in expression of a single miRNA in severe asthma could be considered an important finding. Over-expression of plasma exosomal miR-125b in asthma patients and its correlation with IgE and hsCRP levels could be considered important as a marker or for understanding the pathophysiology of severe asthma. This finding is in agreement with other reports showing that the expression of miR-125b is over-expressed in inflammatory diseases including asthma [33]. Similarly, the expression of this type of miRNA has been elevated in ulcerative colitis[43], Encephalitis[44], and preeclampsia [20].

To our knowledge, this is the first report showing over-expression of miR-125b in severe asthma patients is linked with serum levels of hsCRP and total IgE. These data may suggest that miR-125b is a good candidate as a molecular marker for the diagnosis of severe asthma besides clinical signs of disease.

Down regulation of other miRNAs namely; miR-124, miR-125b, miR-130a, miR-133b without a significant relationship with hsCRP and total IgE in serum samples further confirm the importance of miR-125b in inflammatory diseases. However, the question remains as to what is the impact of miR-125b in severe asthma and its clinical phenotypes. Association of the expression of miR125b with serum levels of hs-CRP and total IgE in asthma patients suggest that this particular miRNA could directly be involved in inflammatory reactions and allergy process. Over-expression of miR125b as the circulating microRNAs in

patients with allergic rhinitis and asthma further support our data in asthma patients has been reported[33].

Besides, down-regulation of plasma exosomal miR-124, miR-130a and miR-133b in asthma patients indicates that these molecules are probably negative regulators of inflammatory and allergic reactions and related target genes. These miRNAs were poorly related to hs-CRP and IgE levels in asthma patients. It appears that the miRNAs which apparently target common pathways in asthma and respiratory infections are down-regulated in exosomal fraction of asthma patients, although may show increased expression in monocytes of asthma patients [45].

It has been reported that the sphingosine kinase 1 (SPHK1), which balances the inter-conversion of ceramide, sphingosine, and S1P can be directly targeted with miR-124 [15]. Perhaps there are no sufficient evidences to assign the expression of miRNAs to certain disease condition. However, it appears that down regulation of miR-124 in plasma exosomal samples from patients with severe asthma is linked to regulation of the S1P pathway which is considered as a potent lipid mediator that can induce airway inflammation and asthma.

The relationship between the inflammatory factors and expression of miR-130a which regulates S1PR2 protein has been described. Suppression of miR-130a is linked to inflammatory mediators such as TNF-α which may be responsible for increased inflammatory gene expression [46]. Therefore, it seems that a decrease in plasma exosomal miR-130a expression in asthma patients regardless of the high levels of serum IgE and CRP can be effective in increasing the inflammatory process in inflammatory diseases such as asthma. Decreased expression of circulating form of miR-133b in patients with allergic rhinitis and asthma[33], further support our data showing that miR-133b expression is decreased in plasma exosomes.

Involvement of the signal transducer and activator of transcription 3 (STAT3), which is activated by the S1PR1 pathway in causing inflammation and cancer are well established [47]. According to Nan Cheng et al, miR-133b plays a significant role in controlling S1PR1 protein expression. These information suggest that changes in miR-125b-1-3p which target S1PR1 is common in the pathogenesis of inflammatory diseases such as preeclampsia [17]. Likewise evidences show that down-regulation of miR-130a and miR-133b expression is not limited to the pathogenesis of severe asthma, but could be common for other inflammatory conditions.

In the present study, the impact of the miRNAs, namely, miR-124, miR-125b, miR-130a, and miR-133b was on the immuno-inflammatory factors such as serum IgE and CRP was also examined. Comparison of the expression of miRNAs and their relationship to the inflammatory factors revealed that among the miRs studied, there was a highly significant correlation between the expression of miR-125b and CRP/IgE levels ($r = 0.68$; $p < 0.0001$) in asthma patients. The association between these mediators with the exosomal miR-125b could possibly be considered as an evidence for the role of miRNAs in the pathogenesis of severe asthma. This finding may suggest the role of miR-125b in regulation of genes in asthma related inflammatory pathways.

In fact, the limited data presented in this study on the expression of five different miRNAs in plasma exosomal samples failed to show a specific profile for severe asthma condition compared to normal individuals. However, a considerable over-expression of miR-125b in the patients compared to normal is promising for diagnosis as well as its possible role in the asthma-related inflammatory reactions.

Based on the data obtained from the ROC curve, the changes in expression of miRNA-125b can be considered as a promising biomarker in diagnosis of severe asthma patients. However, the diagnostic value of a specific miRNA such as miRNA-125b relies on other factors such as the clinical stage and the clinical signs of the disease.

Evidences presented in terms of asthma treatment protocols may suggest that highly expressed miR-125b could be considered a putative molecular target for the management of corticoid unresponsive form of asthma. In conclusion, the data presented here may suggest that expression of miR-125b in the exosomal fraction of plasma and its correlation with serum inflammatory markers (CRP) and allergic (total IgE) is promising for molecular diagnosis of severe asthma. Moreover, changes in the expression of microRNAs in plasma exosomes could be useful for discrimination of clinical phenotypes of asthma. Further studies with a larger sample size could help to identify gene targets and pathways related to specific microRNA involved in the pathophysiology of severe asthma.

Abbreviations

miRNAs
microRNAs
S1P
Sphingosine-1-phosphate
GINA
Global Initiative for Asthma
ROC
Receiver operating characteristic
S1PR
Sphingosine-1-phosphate receptor
ASM
Airway smooth muscles
SphK1
Sphingosine kinase 1
SGPL1
Sphingosine-1-phosphate lyase 1
PBS
Phosphate-buffered saline
BSA
Bovine serum albumin

DLS
Dynamic light scattering
TEM
Transmission electron microscopy
PAP
Poly(A) polymerase
hs-CRP
High-sensitivity C-reactive Protein
IgE
Immunoglobulin E
BMI
Body mass index
BCA
Bicinchoninic acid

Declarations

Ethics approval and consent to participate

The research was performed according to the Declaration of Helsinki of the World Medical Association. The study was approved by the Medical Ethics Committee of the Tarbiat Modares University (ID: IR.MODARES. REC.1397.047).

Consent for publication

All authors agree with the publication.

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no competing interest

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Author Contributions

Mr. M. Atashbasteh has performed blood sample collection and processing. He has also carried out most of the assays, designed the molecular assays and analyzed the data. He has also prepared the first draft of the manuscript.

Prof. A. Allameh has supervised the research project, experiments and data analysis. He has reviewed and edited the manuscript and finalized the paper.

Dr. E. Mortaz: The original idea and concept of this research has been given by E.M. He has also supervised the assays. He has also reviewed and edited the final draft of the manuscript.

Dr. S.A. Mahdaviani and Dr. H. Jamaati have examined the patients and selected the asthma patients according the criteria described in the paper.

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Tables

Table 1

Demographic and clinical characteristics of patients and normal individuals.

Samples	Age	Sex (M/F)	FEV1% ± SD	FVC% ± SD	BMI (Mean±SD)
Control(n=30)	39.23±10.1	13/17	ND	ND	22.46±2.2
Severe asthma (n=30)	42.13±9.4	12/18	51.37± 16.1	63.50± 14.8	23.30±2.0

Normal individuals selected for this study had no history of predisposing chronic or inflammatory diseases. Forced expiratory volume in 1 second (FEV1%) and Forced vital capacity (FVC %) were not detected (ND) in the control group due to clinical limitations. BMI=body mass index.

Table 2

Relative expression (fold change) of different microRNAs in exosomes isolated from asthma patients and controls.

miRNA	Relative expression	P-value	overall expression
miR 124	0.19 ± 0.09	<0.0001*	DOWN
miR 125b	4.17 ± 2.92	<0.0001*	UP
miR 133b	0.20 ± 0.08	<0.0001*	DOWN
miR 130a	0.17 ± 0.14	<0.0001*	DOWN
miR 125b-3p	0.76 ± 0.54	0.156	No change

Purification and characterization of plasma exosomes have been described in the 'Methods' section. Fold change is presented as Mean ±SD. *p<0.0001 is considered as significant. The overall expression shows differences in expression between the asthma patients and controls.

Table 3

ROC curve predictive values obtained from AUC (Area under the curve) and CI (95% confidence interval) data in severe asthma samples.

miRNA	AUC	CI 95% (p<0.05)
miR 124	0.96	0.92-1.00
miR 125b	0.91	0.84-0.98
miR 133b	0.99	0.97-1.00
miR 130a	0.87	0.79-0.96

Table 4

Relationship of the expression of exosomal miRNAs and serum hs-CRP and IgE in severe asthma patients.

	hs-CRP (log)		IgE level (log)	
microRNAs	*r-value*	*P-value*	*r-value*	*P-value*
miR-124	0.22	0.23	0.07	0.73
miR-125b	0.86	<0.0001*	0.68	<0.0001*
miR-133b	0.29	0.12	0.35	0.06
miR-130a	0.34	0.06	0.29	0.12

$p < 0.05$ indicates the Spearman's rank correlation with statistical significance.

Figures

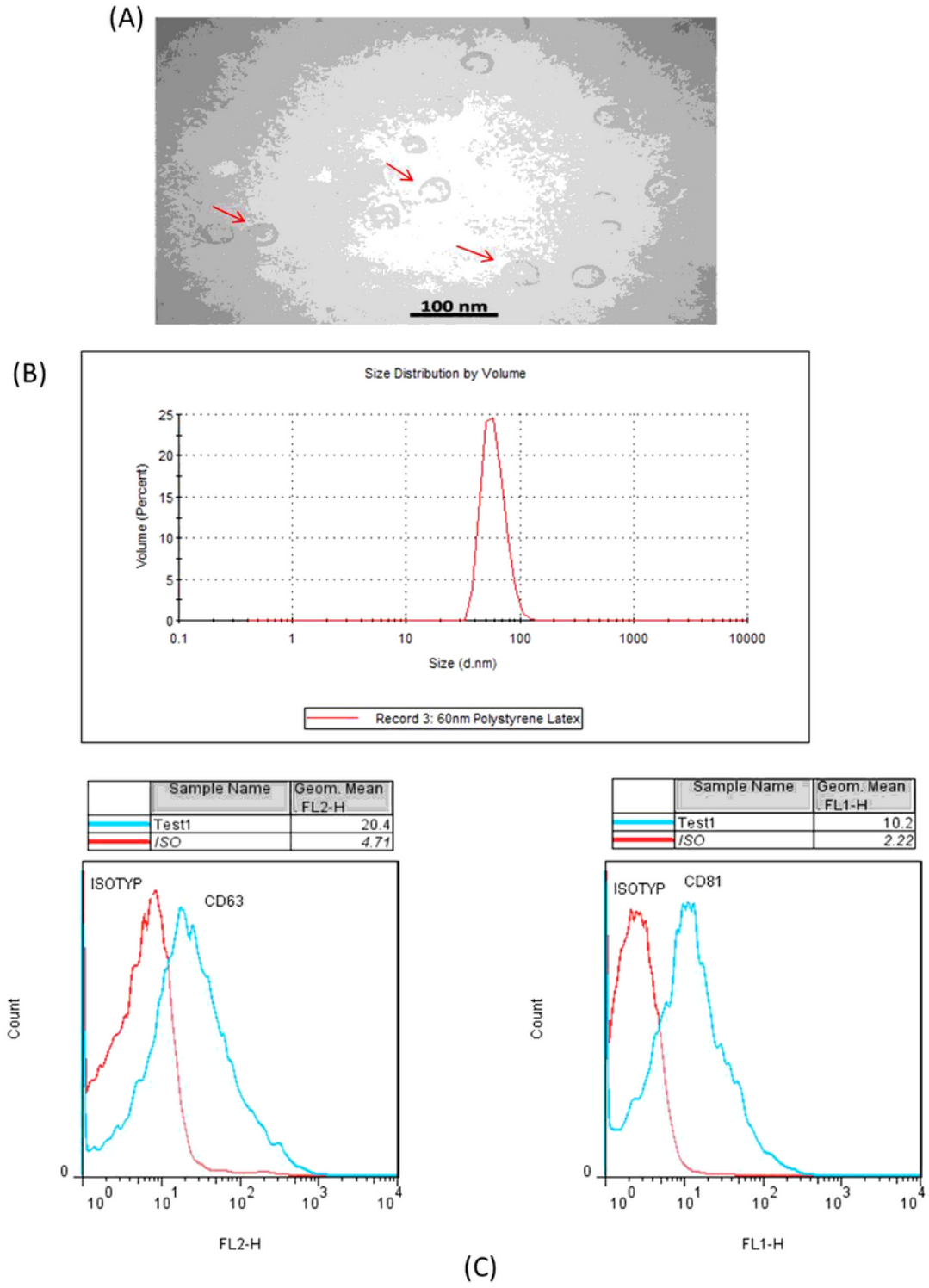

Figure 1

Characterization of the plasma-derived exosomes. Purification and characterization of plasma exosomes have been described in the 'Methods' section. A) The arrows in the transmission electron microscopy (TEM) image show the spherical shape of the exosomes. Scale bar represents 100 nm. B) Shows the size distribution of the exosomes analyzed by Dynamic light scattering. The average size (Z-Average) was

55nm. C) Flow cytometry data show the expression of surface markers on the exosomes; CD81 and CD63.

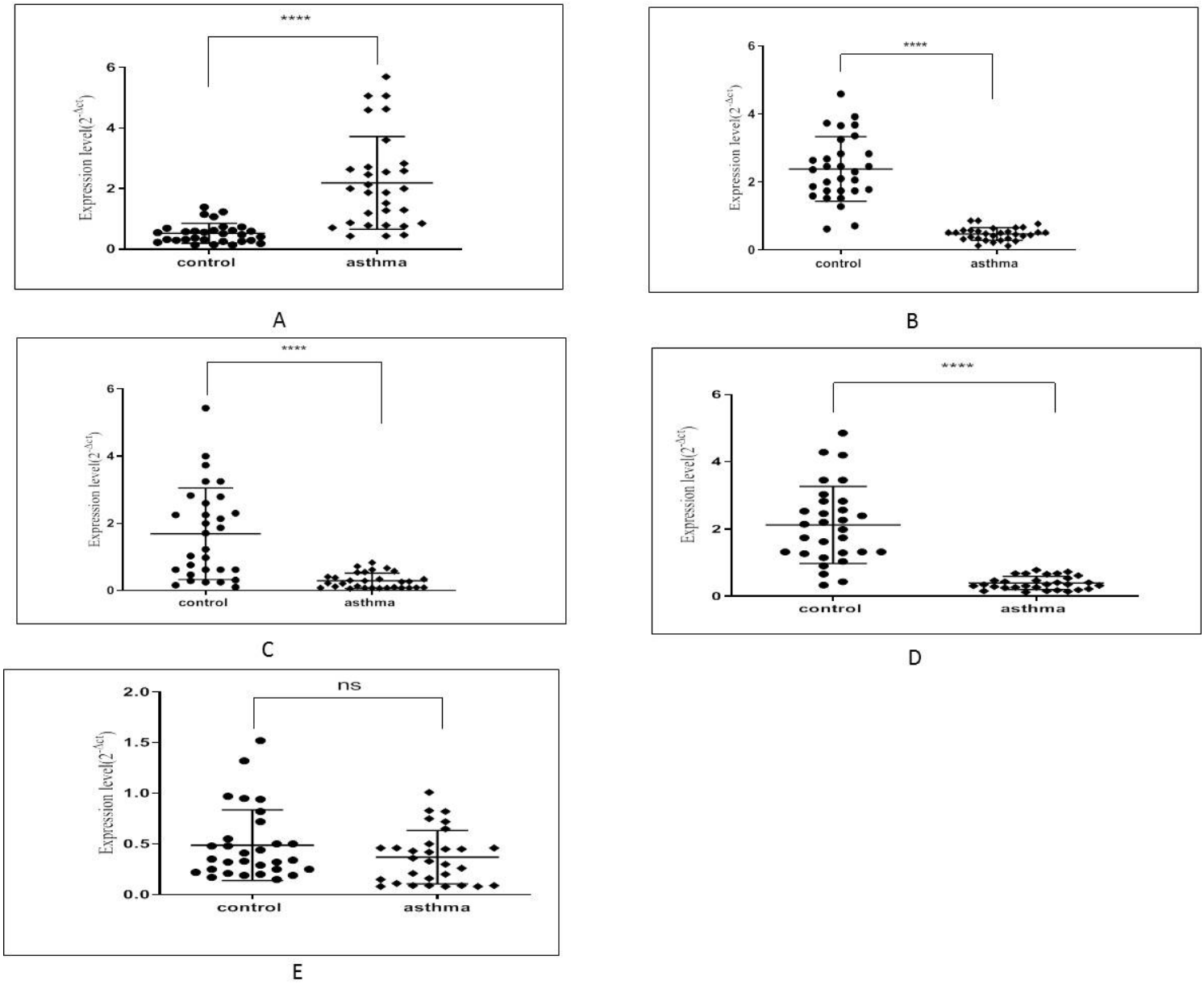

Figure 2

miRNAs expression level in plasma exosomes derived from severe asthmatic patients and healthy controls. (A) The qRT-PCR for miR125b, (B) miR133b, (C) miR130a, (D) miR124 and (E) miR125b-3P expression in severe asthma patients. The results are indicated the mean values of normal controls (n=30) and asthma patients (n=30). * indicates statistically significant difference between the groups.

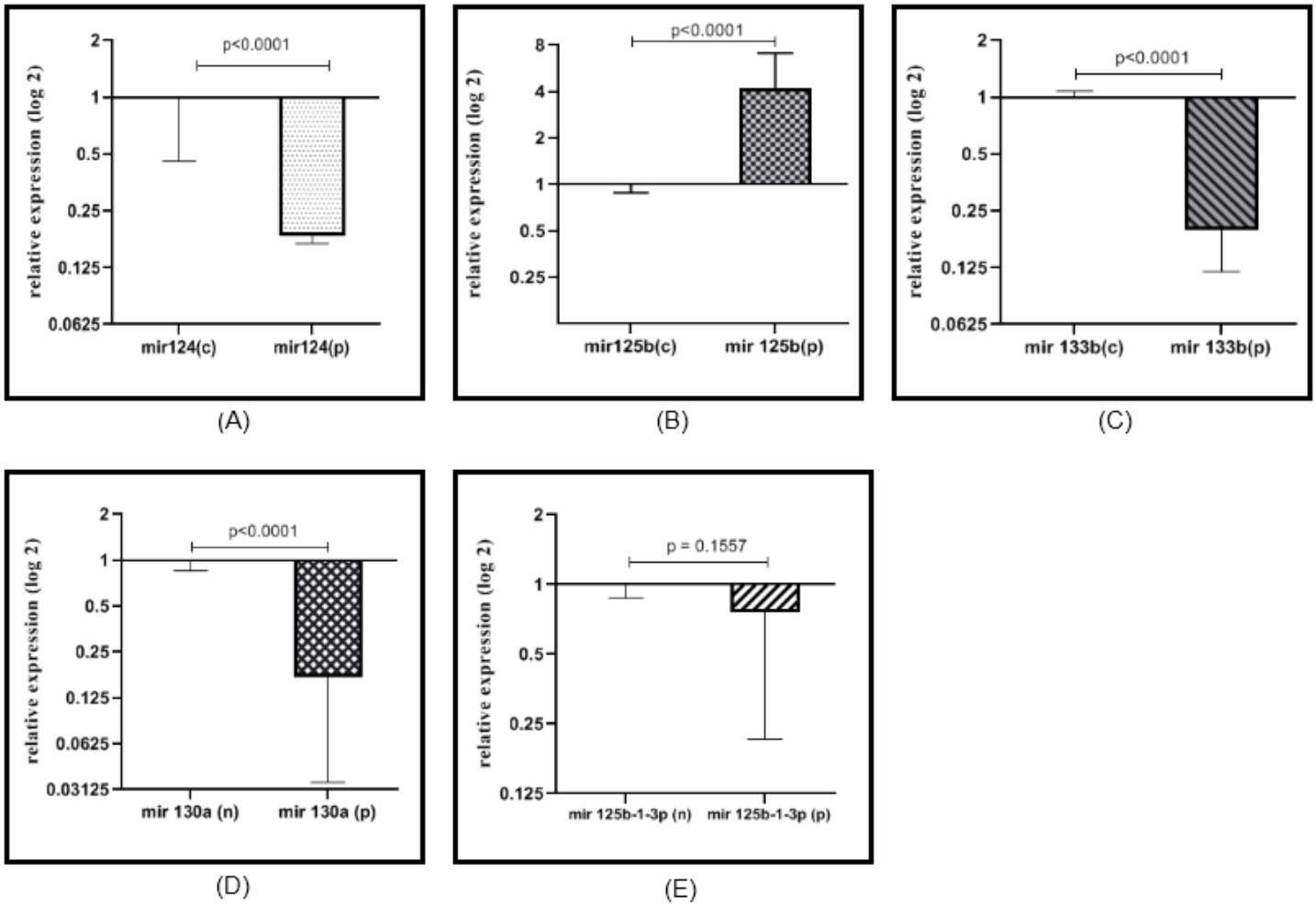

Figure 3

The relative expression (fold of change) of exosomal (A) miR-124, (C) miR-133b, and(D) miR-130a in 30 severe asthmatic patients (p) compared to 30 control subjects(n), indicated significantly down-regulation in patients group ($p < 0.0001$). (B) MiR-125b up-regulated in severe asthma and (E) miR-125b-3p had no significantly different compared to control. Data represented mean ± SD from 30 patients in each group.

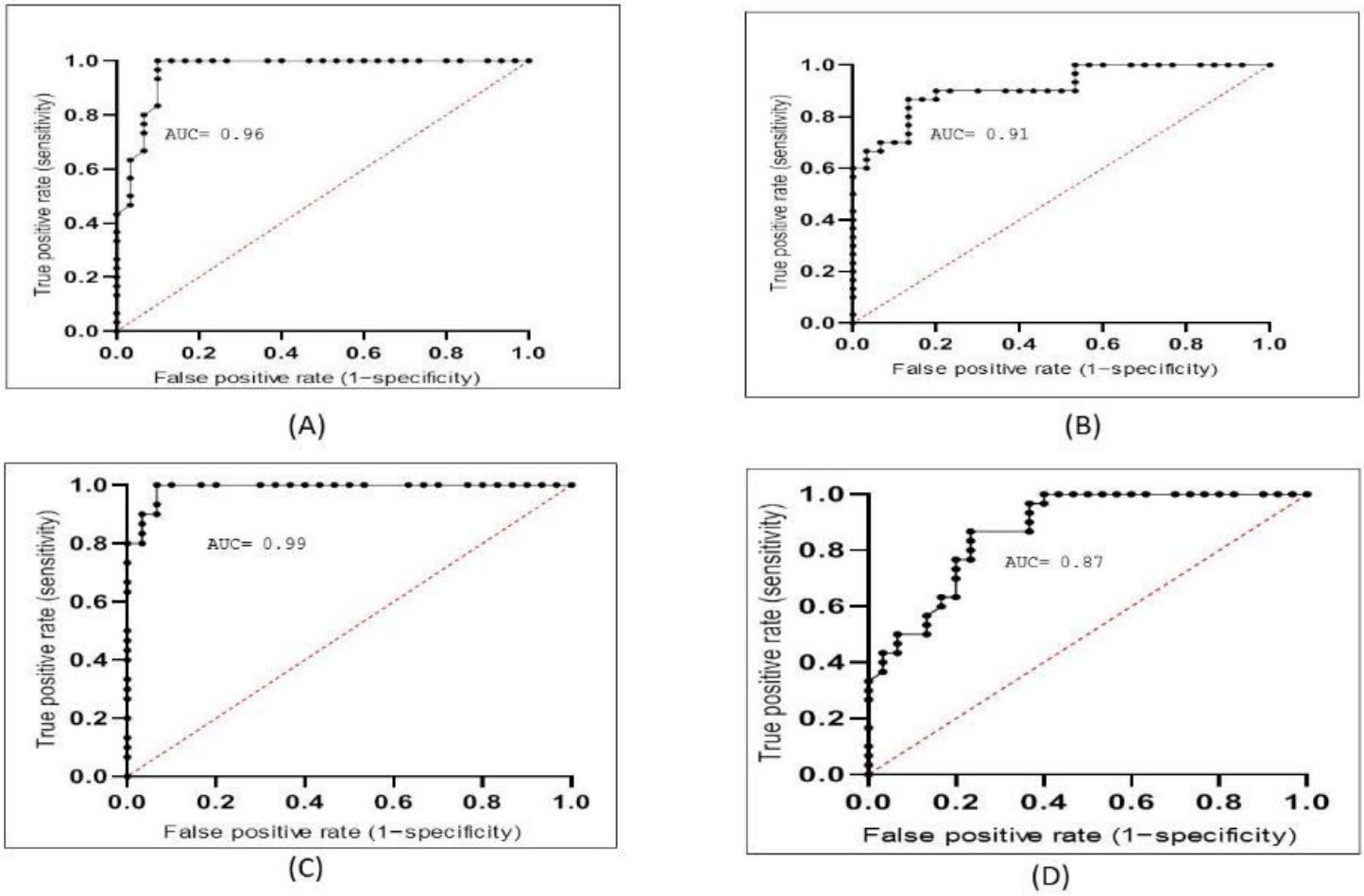

Figure 4

The diagnostic power of plasma exosomal miR-124 (A), miR-125b (B), miR-133b (C) and miR-130a (D) designated by Receiver Operating Characteristic (ROC) curve analysis. The results are shown as the area under ROC curve (AUC) for the sensitivity and specificity of each miRNA.

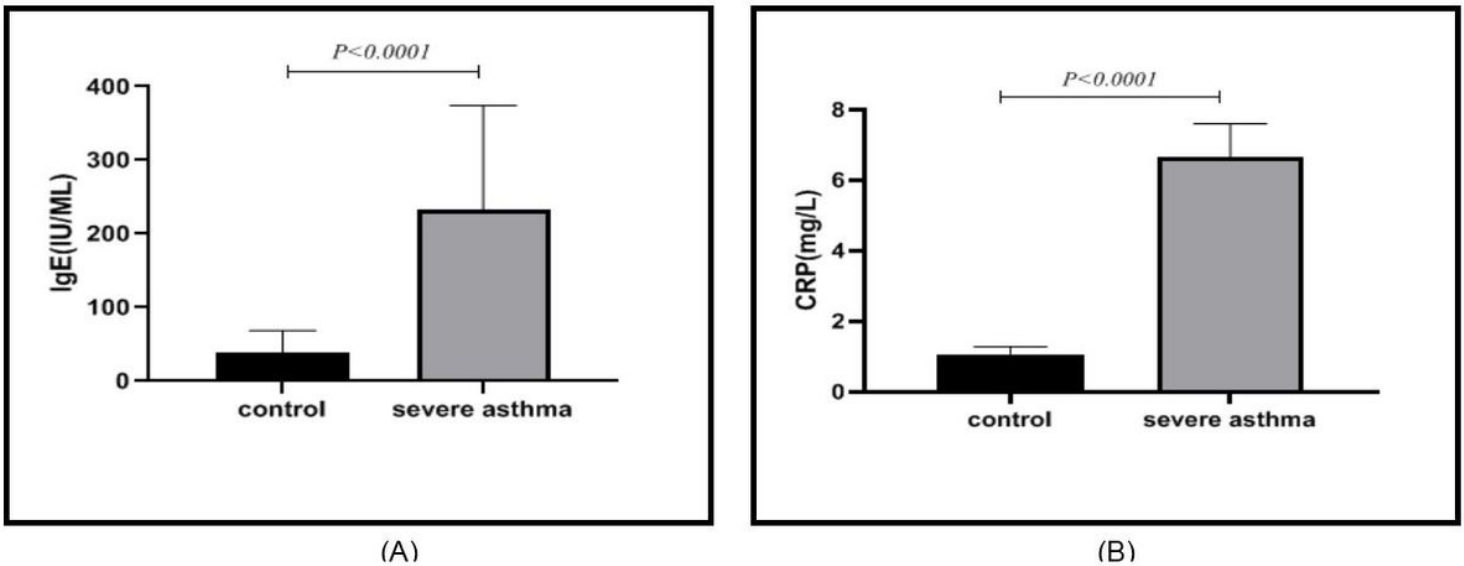

Figure 5

Comparison of the serum hs-CRP (section A) and total IgE (section B) levels in severe asthmatic patients and healthy controls. The data are presented as mean values ± SD of all the samples in each group. The p-value<0.05 is considered significant.

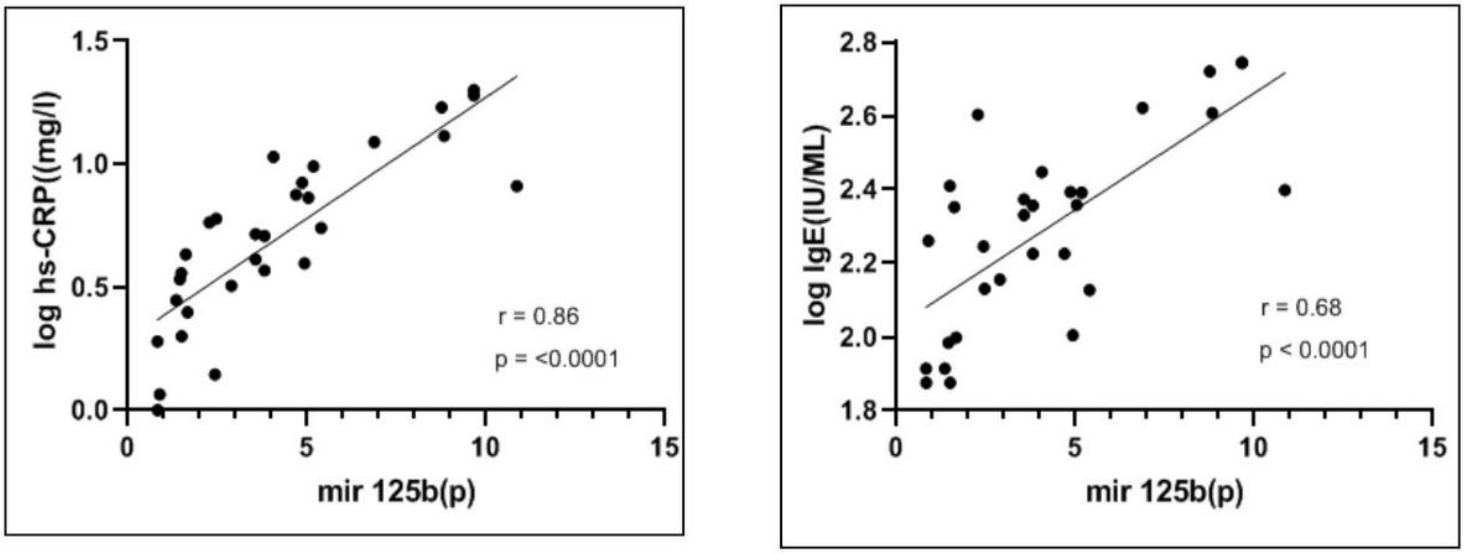

Figure 6

Relationship between the plasma exosomal miR 125b and inflammatory factors. Section (A) indicates the correlation of exosomal miR125b and IgE and Section (B) shows the correlation of miR125b and high sensitivity CRP. . r=correlation coefficient, P-value is calculated by Spearman's rank correlation test.