

Research Communication

Effect of high fat diet on NF- κ B microRNA146a negative feedback loop in ovalbumin-sensitized rats

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Abstract

The present study aimed to investigate the role of microRNA-146a and its adapter proteins [interleukin-1 receptor-associated kinase 1 (IRAK1) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6)] in the pathogenesis of ovalbumin (OVA)-sensitized rats in association with the diet-induced obesity condition. Twenty male Wistar rats were divided into four groups: control with normal diet (ND), OVA-sensitized with normal diet (S + ND), high-fat diet (HFD), and OVA-sensitized with high-fat diet (S + HFD). All the animals were fed for 8 weeks with standard pellets or high-fat diet, and were then sensitized and challenged with OVA or saline for another 4 weeks. The tracheal responsiveness to methacholine, serum protein levels, and lipid profile levels was measured by the ELISA method. Moreover, the gene expression level of microRNA-146a (miR-146a) was

measured in the lung tissue of the rats using the real-time PCR method. Maximum response to methacholine increased in the S + HFD group in compared with ND, S + ND, and HFD groups ($P < 0.05$ to $P < 0.001$). Moreover, in the S + HFD group the mRNA expression levels of miRNA-146a increased in the lung tissue ($P < 0.001$). In addition, the protein analysis results showed that IRAK1, TRAF6, NF- κ B, and IL-1 β protein levels were high in the S + HFD group compared to the ND and HFD groups; however, in compared with the S + ND group, only the IL-1 β protein level was higher in the S + HFD group ($P < 0.05$). These results suggest that a defect in the NF- κ B–miR-146a negative feedback loop may be involved in the pathogenesis of obesity associated with OVA-sensitized condition. © 2018 BioFactors, 9999(9999):1–10, 2018

Keywords: asthma; miRNA-146a; NF- κ B; IL-1 β ; obesity

Abbreviations: AHR, airway hyper-responsiveness; AP-1, activator protein 1; BF%, percentage of body fat; DIO, diet-induced obesity; EC₅₀, the effective concentration of methacholine generating 50% of peak response; ELISA, enzyme-linked immunosorbent assay; gF/TW, gr force/mg tissue weight; HDL-C, high-density lipoprotein cholesterol; HFD, high fat diet group; IgE, immunoglobulin E; IL-(1, 6, 8), interleukin-(1, 6, 8); IRAK1, interleukin-1 receptor-associated kinase 1; LDL-C, low-density lipoprotein cholesterol; miRNA, microRNA; ND, a normal diet group; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; OVA, ovalbumin; PCR, polymerase chain reaction; RANTES, Regulated on Activation, Normal T cell Expressed and Secreted; S + HFD, high-fat diet with OVA-sensitized group; S + ND, normal diet and OVA-sensitized group; TC, total cholesterol; TG, triglycerides; TNF- α , tumor necrosis factor- α ; TRAF6, tumor necrosis factor (TNF) receptor-associated factor 6.

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1. Introduction

Obesity is considered as an important risk factor for chronic diseases such as coronary heart disease, high blood pressure, type II diabetes, cancers, and asthma [1]. Epidemiological data show that weight gain increases the risk of asthma and exacerbates its symptoms [2]. Although the underlying mechanism for the association of obesity with asthma is not well defined, there are some factors that indicate this association such as common genetic basis [3], asthma comorbidities [4], effects of obesity on pulmonary volumes [5], chronic systemic inflammation [6], and adipokines [7].

Indeed, obesity is a low-grade systemic inflammation, which is usually characterized by increasing serum concentration of cytokines, chemokines, adipokines, and acute phase proteins [6,8,9]. It has been reported that there is a relationship between tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and obesity with asthma [10,11]. It is likely that specific cytokines, such as IL-1 β , are involved in altering the airway responsiveness in asthma [12]. IL-1 β is a pro-inflammatory cytokine that plays a key role in local and systemic immune responses. Studies have shown that the airway smooth muscle tissue has the potential role to synthesize and secrete IL-1 β , which can affect the contraction of the airways [13]. One of the most important intracellular mediators for inflammatory response to IL-1 β is the nuclear factor kappa-light-chain enhancer of activated B cells (NF- κ B) [14]. In the signaling pathway for the activation of NF- κ B via IL-1 β , two molecules are involved, which include interleukin receptor-associated kinase 1 (IRAK1) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) [14].

On the other hand, microRNAs (miRNAs) are a collection of small RNA molecules (with 18–25 nucleotides), which are made by the RNA polymerase II and III in the nucleus cells and then migrated to the cytoplasm [15]. New findings confirm the important role of specific miRNAs in biological processes such as proliferation, cell differentiation and evolution, modulation of immune responses in macrophages, and differentiation of heart and muscle cells [16–19]. The role of miRNAs in the pathogenesis of various pulmonary diseases, including pulmonary fibrosis, obstructive pulmonary disease, lung cancer as well as asthma has been proven [20]. Among the miRNAs involved in the progression of asthma, miRNA-146a can be mentioned. Evidence suggests that miRNA-146a can inhibit the expression of IRAK1 and TRAF6 as well as the impairment of NF- κ B activity, and suppress the expression of NF- κ B target genes including IL-6, IL-8, IL-1 β , and TNF- α [21]. By activating NF- κ B, the expression of miRNA-146a increases and results in negative feedback effects on IRAK1 and TRAF6 as well as reduced activity of NF- κ B [22]. IL-1 β induces the miRNA-146a expression in alveolar epithelial cells and negatively regulates the release of IL-8 and RANTES (regulated on activation, normal T cell expressed, and secreted) [22].

Considering that in previous studies [23,24], we showed that expression levels of IRAK1, TRAF6, NF- κ B, and IL-1 β mRNAs under obesity associated with the asthma condition

increased in the tracheal tissue, in this study, we investigated the effects of post translation events on the amount of proteins levels of IRAK1, TRAF6, NF- κ B, and IL-1 β in the lung tissue. We also investigated miRNA-146a as a negative regulator of the IRAK1-TRAF6-NF- κ B pathway in the lung tissue. Accordingly, the goals of the current study in obesity associated with asthma condition include (1) Determination of changes in protein levels of IRAK1, TRAF6, NF- κ B, and IL-1 β in the lung tissue, (2) The effect of obesity on the expression level of miRNA-146a in lung tissue under the OVA-sensitization condition, and (3) The effect of miRNA-146a expression levels on the amount of IL-1 β pathway proteins (IRAK1, TRAF6, and NF- κ B) as negative regulator of this pathway in obesity associated with asthma condition.

2. Materials and methods

2.1. Animals and diets

The present study was performed on 20 mature male Wistar rats weighing approximately 160 g (8 weeks old), obtained from the Animal House of Tabriz University of Medical Sciences, Tabriz, Iran. The animals were cared in cages under controlled conditions of 12:12 h light–dark cycle at $22 \pm 2^\circ\text{C}$. Food and water were accessible *ad libitum* during the accommodation and experimental period.

Subsequently, all the animals were randomly subclassified into four main groups (5 rats in each group), including: a normal diet group (ND), normal diet and OVA-sensitized group (S + ND), high-fat diet group (HFD), and high-fat diet with OVA-sensitized group (S + HFD). In order to have a diet-induced obesity (DIO) model, we used the model in our previous study [24]. Briefly, the animals in the DIO groups (HFD and S + HFD) received a high fat diet (42% energy from fat, 19% energy from protein, and 39% energy from carbohydrate), and the animal in the normal diet groups (ND and S + ND) received standard rat chow (11% energy from fat, 28% energy from protein, and 61% energy from carbohydrate).

The rats were fed for 8 weeks in accordance with the abovementioned diet and were used for induction of sensitization with ovalbumin in the S + ND and S + HFD groups from the following protocol. The experiment continued for 4 weeks for the OVA-sensitized group with the previous regime.

2.2. Animal sensitization

The rats in the sensitized groups (S + ND and S + HFD) were intraperitoneally sensitized with 1 mg OVA dissolved in 0.9% saline and 200 mg aluminum hydroxide gel (Sigma) on days 1 and 8. The animals from the day 14 were then exposed to aerosol particles of 4% OVA for 17–19 days produced by a nebulizer (CX3, Omron Health Care Europe B.V., the Netherlands) 15 min/daily in a closed chamber (dimensions: $30 \times 20 \times 20$ cm). The results of previous studies with this model of sensitization have shown that some aspects of OVA-sensitization such as airway hyper-responsiveness (AHR), airway remodeling, and pathologic changes [25–27]. The rats of the ND and HFD groups were treated similarly with saline instead of the OVA solution.

The ethical committee of the Ardabil University of Medical Sciences confirmed the study protocol.

2.3. Body weight

At the end of the study, after induction of anesthesia, the animals were weighed and measured for their naso-anal length, that is, the distance between nose and anus. To calculate the obesity indices in the rodents, factors such as final body weight, percentage of body fat (BF%), and Lee index were determined. The following formula indicates the calculation of all the obesity indices [7]:

- (1) Percentage of body weight changes (%) = [(Final weight - initial weight)/ initial weight] × 100
- (2) Lee index (mg/mm) = Final weight^{0.33} / naso-anal length
- (3) Percentage of body fat (%) = 0.69 (Lee index-274.8)

2.4. Tissue preparation

The rats were first anesthetized by intraperitoneal injection of ketamine 50 mg/kg and xylazine 30 mg/kg. Then, the chest was opened and approximately 2 cm of trachea was isolated along with the connective tissue. After removing the peripheral tissue around the trachea, it was cut into several 5 mm segments (5–6 ring cartilages). Each tracheal segment was immediately settled between two horizontal stainless-steel hooks suspended in a 20-mL organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing the Krebs–Henseleit solution of the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5, and dextrose 11. The Krebs solution was maintained at 37 ± 1 °C and gassed with 95% O₂ and 5% CO₂. The isometric tension was 1 g throughout the experiment, and the equilibrium period was at least 1 h. The bath solution was replaced with fresh Krebs solution every 15 min. Contraction responses were measured using an isometric transducer (ADInstruments, Spain) connected to a power lab system (ML-750, 4-channel recorder, March-Hugstetten, Germany).

2.5. Assessment of tracheal responsiveness to methacholine

In each experiment, a cumulative log concentration–response curve of methacholine hydrochloride (Sigma Chemical Ltd, UK) was obtained in each tracheal segment by adding consecutive concentrations (10^{−8} to 10^{−3} mM) every 2 min. To plot the curve, the percentage of contraction of the tracheal smooth muscle caused by each methacholine concentration in proportion to the maximum contraction acquired by its final concentration was plotted against log concentration of methacholine. Then, EC₅₀ as the effective concentration of methacholine generating 50% of peak response was measured from the methacholine response curve in each experiment using 50% of maximum response in the *Y* axis and by measuring the dose of methacholine causing this response in the *X* axis. The contractility response to 10 μM methacholine was also determined as the magnitude of contraction. At the end of the experiments, the tissues were weighed. Afterwards, the contraction force induced by methacholine (at the plateau level) was calculated

to express as the gr force/mg tissue weight (gF/TW) to compare the contraction activity of the spasmogen between the groups.

2.6. Biochemical measurements

Blood samples (3–5 ml per rat) were collected from the heart of the fasted rats after they were anesthetized with ketamine and xylazine. Plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations were measured using an automatic blood analyzer (Bayer corp.). Triglycerides, total cholesterol, and HDL-C kits were obtained from Pars Azmoon CO, Iran. Low-density lipoprotein cholesterol (LDL-C) was evaluated using the Friedewald formula: LDL-C = TC − [HDL-C + TG/5] [23].

2.7. Tissue sampling and protein measurement

After anesthesia induction, the animals were sacrificed and their lung tissue was isolated and frozen with nitrogen. The tissues were kept at −70 °C until the study parameters were measured. Levels of IRAK1, TRAF6, NF-κB, and IL-1β were measured by the enzyme-linked immunosorbent assay (ELISA, Hangzhou Eastbiopharm co., Ltd). The tests were done according to the manufacturer's instruction. Briefly, after weighing, the tissue samples were homogenized in the PBS (Ph 7.2–7.4) and centrifuged at 3000 rpm at 4 °C for 20 min. Then, the supernatants were isolated and proteins were extracted.

2.8. Lysate preparation from lung tissue

The isolated lungs were immediately weighed and the recommended amount of tissue was removed. The tissue was grinded, and the homogenized tissue was provided. Then, after adding 600 μL RNase-free water and 20 μL reconstituted Proteinase K, lysate was incubated and spun for 1 min. For the RNA binding to columns, about 650 μL of lysate with the ethanol mixture was loaded on the columns and centrifuged for 1 min. Afterwards, DNase I treatment was performed, followed by wash step. RNA was eluted from the column and the purified RNA sample was kept at −70 °C for real time-PCR analysis.

2.9. Real-time PCR

RNA content and purity were measured using Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington, DE). For determination of microRNA 146a expression levels, the revert aid first-strand cDNA synthesis kit (Ferment as GmbH, Leon-Rot, Germany) with the aid of random hexamer primers and MMLV reverse transcriptase (as a complete system for efficient synthesis of first-strand cDNA from mRNA or total RNA templates) was used.

By using the SYBR green master mix (Exiqon), each cDNA was used as a template for separate assays for mRNA quantitative real-time PCR. The locked nucleic acid forward and reverse primer sets (Exiqon) for miRNA-146a include: miRNA-146a (target sequence: UGAGAACUGAAUCCAUGGGU and product name: hsa-miR-146a, LNA PCR primer set, UniRT [Access number: MIMAT0000449]), and rno-miR-191 (target sequence: CAACG-GAAUCCCAAAAGCAGCUG and product name: hsa-miR-191, LNA PCR primer set, UniRT [Access number: MIMAT0000440]). Real-

time PCR reactions were performed on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia).

The PCR products were normalized with housekeeping rno-miR-191 for the miR-146a samples. The $2^{-(\Delta\Delta C_t)}$ method was used to determine relative quantitative levels of the study miRNA. Results were expressed as fold change versus the relevant controls [23].

2.10. Statistical analysis

Analysis of variance (ANOVA) was used to compare between the different groups with Tukey–Kramer *post hoc* test. The correlation between the miRNA-146a level and study variables was analyzed with Pearson's correlation coefficient. The results are presented in mean $\pm$ SD. *P* values less than 0.05 were considered significant.

3. Results

3.1. Body weight

Figure 1A shows the weight courses in the experimental groups. The animals fed the high-fat diet gained more weight than those in the control groups. This was associated with the raised final body weight, Lee index, and percentage of body fat in both the DIO groups (Table 1).

Moreover, the final body weight, Lee index, and percentage of body fat were significantly higher in the HFD and S + HFD groups than in the ND and S + ND ones ($P < 0.001$).

3.2. Serum lipid profile

As seen in Fig. 1B, the lipid profiles (TC, TG, HDL-C, and LDL-C) in the obese groups (HFD and S + HFD) were significantly higher than those in the control groups ($P < 0.05$ to $P < 0.001$). Results did not show any significant differences in the lipid profile test among the normal diet (ND versus S + ND) and obese (HFD versus S + HFD) groups.

3.3. Tracheal response to methacholine

Based on Fig. 2A, there were leftward shifts in methacholine concentration–response curves in the groups S + HFD and S + ND compared to the groups ND and HFD. Interestingly, after DIO in the OVA sensitized group, this leftward shift became markedly evident.

The EC_{50} in the tracheal segments was significantly lower in the S + HFD animals (0.70 ± 0.07) than in the ND (1.20 ± 0.27 , $p < 0.01$) and HFD groups (1.16 ± 0.18 , $P < 0.01$) (Fig. 2B). Furthermore, the EC_{50} in the tracheal segments was significantly lower in the S + ND (0.79 ± 0.12) than in the ND and HFD groups ($P < 0.05$ for both, Fig. 2B). On the other hand, although EC_{50} in the S + HFD group was less than that in the S + ND, there was no significant difference between the groups ($P = 0.856$). In addition, no significant difference was observed in EC_{50} between the ND and HFD animals ($P = 0.984$).

Contractility results showed that the gr force/mg tissue weight (gF/TW) was significantly higher in the S + HFD animals (37.11 ± 7.51) than in the ND (16.34 ± 1.85 , $P < 0.001$), HFD (18.61 ± 1.06 , $P < 0.001$), and S + ND (28.27 ± 5.62 , $P < 0.05$)

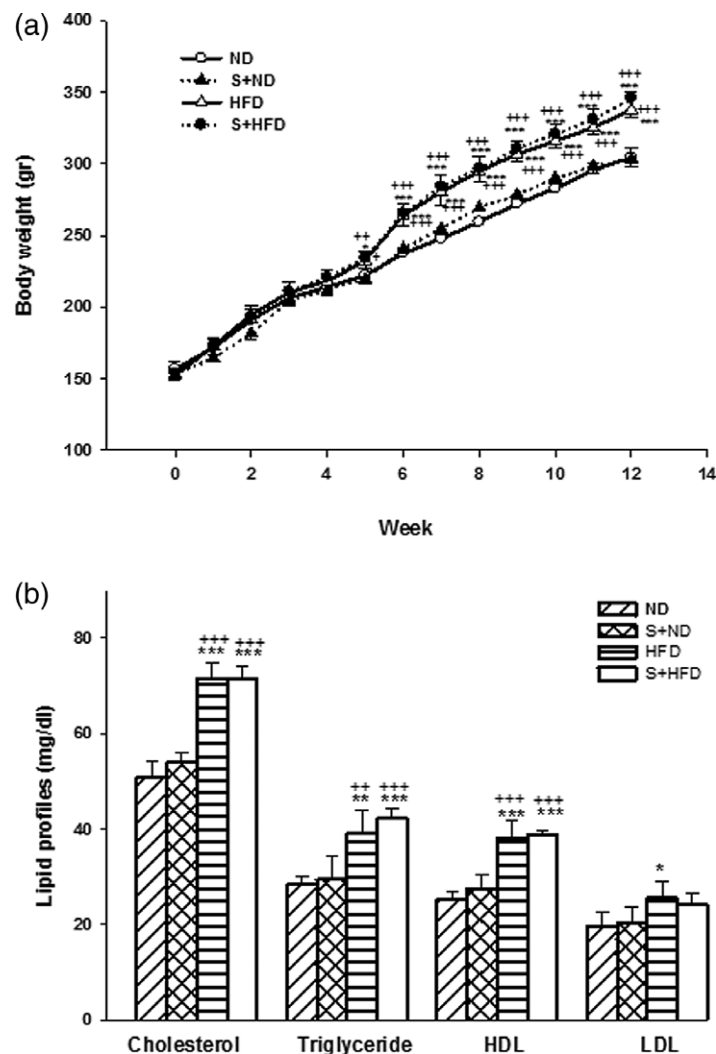


FIG 1

Weight courses (A) and serum levels (B) of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), and low density lipoprotein (LDL-C) in the experimental groups; values are represented as mean $\pm$ SD. ND: normal diet; S + ND: OVA-sensitized with normal diet; HFD: high-fat diet; S + HFD: OVA-sensitized with high-fat diet. Differences between the results of ND and those of the other groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Differences between the sensitized normal diet group and obese groups: ** $P < 0.01$, *** $P < 0.001$. For each group, $n = 5$.

groups (Fig. 2C). Moreover, gF/TW was significantly higher in the S + ND animals than in the ND ($P < 0.01$) and HFD ($P < 0.05$) ones. Additionally, there were no significant differences in gF/TW between the ND and HFD groups (Fig. 2C).

3.4. Protein levels of IRAK-1, TRAF-6, NF- κ B, and IL-1 β in the lung tissue

The results showed that levels of the proteins IRAK1 (Fig. 3A), TRAF6 (Fig. 3B), NF- κ B (Fig. 3C), and IL-1 β (Fig. 3D) in the lung tissue were higher in the S + HFD group than in the ND and HFD groups ($P < 0.01$ to $P < 0.001$, respectively). In addition, it

TABLE 1

Weight changes and obesity indexes in the experimental groups

Variables	ND	S + ND	HFD	S + HFD
Initial body weight (g)	155.60 ± 5.50	155.00 ± 3.08	152.20 ± 3.11	153.40 ± 4.21
Final body weight (g)	302.20 ± 9.09	304.00 ± 6.51	338.20 ± 5.40 ^{a,b}	345.80 ± 4.54 ^{a,b}
Percentage of body weight change (%)	93.05 ± 5.42	96.15 ± 3.83	122.24 ± 3.09 ^{a,b}	125.54 ± 6.32 ^{a,b}
Lee index (mg/mm)	299.83 ± 1.47	302.37 ± 4.24	315.55 ± 3.28 ^{a,b}	313.91 ± 2.16 ^{a,b}
Percentage of body fat (%)	13.88 ± 1.07	15.74 ± 3.09	25.36 ± 2.39 ^{a,b}	24.16 ± 1.58 ^{a,b}

Values are represented as mean ± SD: ND; control with normal diet, S + ND; OVA-sensitized with normal diet, HFD; control with high-fat diet, S + HFD; OVA-sensitized with high-fat diet.

Differences between the results of ND with those of the other groups.

^a*P* < 0.001. Differences between sensitized normal diet with obese groups.

^b*P* < 0.001. For each group, *n* = 5.

was determined that levels of the proteins IRAK1 (Fig. 3A), NF-κB (Fig. 3C), and IL-1β (Fig. 3D) were higher in the S + ND group than in the ND and HFD groups (*P* < 0.05 to *P* < 0.001, respectively). Furthermore, level of the protein IL-1β in the S + HFD group was higher than that in the S + ND group (*P* < 0.05, Fig. 3D); however, there were no statistical differences between the other proteins in the two groups.

3.5. MicroRNA-146a expression levels in lung tissue

The miRNA-146a expression level increased more than twofold in the S + HFD group compared to that ND, S + ND, and HFD groups (*P* < 0.001 for all, Fig. 4). In addition, the miRNA-146a expression levels were higher in the S + ND group than in the ND and HFD groups (*P* < 0.05 for both, Fig. 4).

3.6. Relationship between lipid profile and protein levels with miRNA-146a expression levels

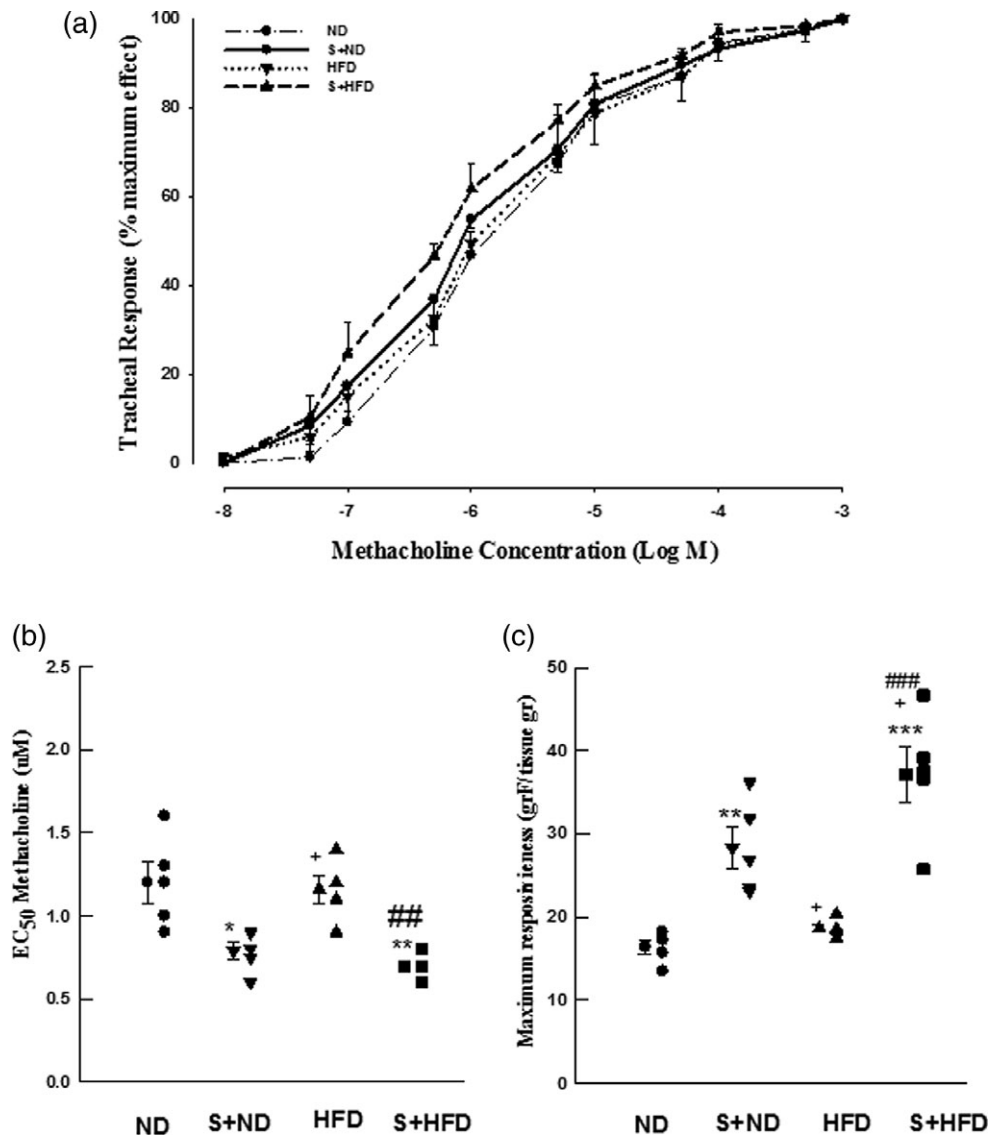
Pearson correlation analyses of the study variables and miRNA-146a levels are summarized in Table 2. All the parameters were found to be positively correlated with miRNA-146a (excluding the EC₅₀ that had a negative correlation). Moreover, there was no significant relationship between the LDL and total cholesterol with the miRNA-146a levels (Table 2).

4. Discussion

The main finding of the current study was the increase in miRNA-146a levels in the lung tissue of ovalbumin-sensitized obese rats when compared with other groups. This increase was not associated with a decrease in levels of the proteins IRAK1, TRAF6, and NF-κB in the ovalbumin-sensitized obese rats compared to the ovalbumin-sensitive lean rats. On the other hand, the results showed that the IL-1β protein levels were higher in the ovalbumin-sensitized obese rats compared to the ovalbumin-sensitive lean rats.

Asthma is particularly characterized by reversible airway obstruction, airway hyperresponsiveness (AHR), and chronic airway inflammation [28]. The AHR is a common feature of obese mice, and the effect of obesity is greater in mice than in humans. Regardless of the method of inducing obesity in mice, the increased response of the airway to methacholine was evident [29,30]. The results of this study revealed that there was a significant increase in the airway responsiveness to methacholine and airway contractility in the OVA-sensitized groups compared to the control groups, which are consistent with the results of previous studies [31]. Interestingly, the maximum response of trachea to methacholine was significantly higher in the OVA-sensitized obese group than that in the sensitized group with the normal diet. Indeed, the sensitization with OVA associated with diet induced obesity showed a change in the responsiveness of trachea, which, in our previous study, was also observed in female mice [32]. Several factors have contributed to the AHR in obesity associated with asthma, including mechanical factors and systemic inflammation [33,34].

Obesity is a chronic systemic pro-inflammatory condition characterized by the production of various cytokines, chemokines, acute phase protein, adipokines, inflammatory cells, and elevated oxidative stress markers [34]. Studies have shown that systemic and local inflammation in the lung tissue in obesity with asthma can be a factor for worsening AHR [32,34]. Regardless of the potential effects of pro-inflammatory products, certain cytokines such as IL-1β may play a role in interacting with altered response in asthma. IL-1β is an important cytokine that has important functions in local and systemic immune responses in asthma and obesity [35]. In the signaling pathway, IL-1β through IRAK1 and TRAF6 activates various transcription factors, such as NF-κB and AP-1 (activator protein 1), and increases the expression of various inflammatory genes [14]. In our previous study [24], we showed that the expression levels of IRAK1, TRAF6, NF-κB, and IL-1β were higher in the


FIG 2

Cumulative log concentration–response curves of methacholine-induced contraction of the isolated trachea in male Wistar rats. Moreover, individual values and mean + SD of (B) tracheal response to methacholine (EC₅₀, the effective concentration of methacholine, causing 50% of maximum response) in male Wistar rats and (C) tracheal contractility to methacholine (gr force/tissue gr) (contractility response to 1 mM methacholine) in the experimental groups. ND: normal diet; S + ND: OVA-sensitized with normal diet; HFD: high-fat diet; S + HFD: OVA-sensitized with high-fat diet. Differences between the results of the ND and those of the other groups: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Differences between the sensitized normal diet group and obese groups: * $P < 0.05$. Difference between the results of the HFD and S + HFD: ## $P < 0.01$, ### $P < 0.001$. For each group, $n = 5$.

obese OVA-sensitized group than in the lean OVA-sensitized group. In this study, the results showed that levels of the proteins IRAK1, TRAF6, and NF- κ B in the obese associated with asthma group were higher than those in the control groups. However, the comparison of the levels of these proteins between the obese and lean associated with OVA-sensitization condition, despite high levels in the obese-OVA sensitized group, did not show statistically significant difference. Although, the significant increase in the IL-1 β protein levels in the obese OVA-sensitized group was comparable to that in the lean OVA-sensitized group. In a study by Lu et al., an increase

in the IL-1 β expression in the lung tissue was shown in some genetic models of obesity [36]. These findings suggest that DIO in OVA-sensitized mice can induce IL-1 β mRNA and may have autocrine effects on airway cells. Increasing the expression and protein level of IL-1 β and its autocrine effects on the lung tissue may increase the synthesis and release of other mediators and ultimately lead to worsening of pulmonary inflammation.

Recent studies have shown that miRNAs affect the regulation of cell gene expression at post-translation levels. Although the structural expression of miRNAs in tissues and during evolution indicates their importance in preserving cell phenotypes,

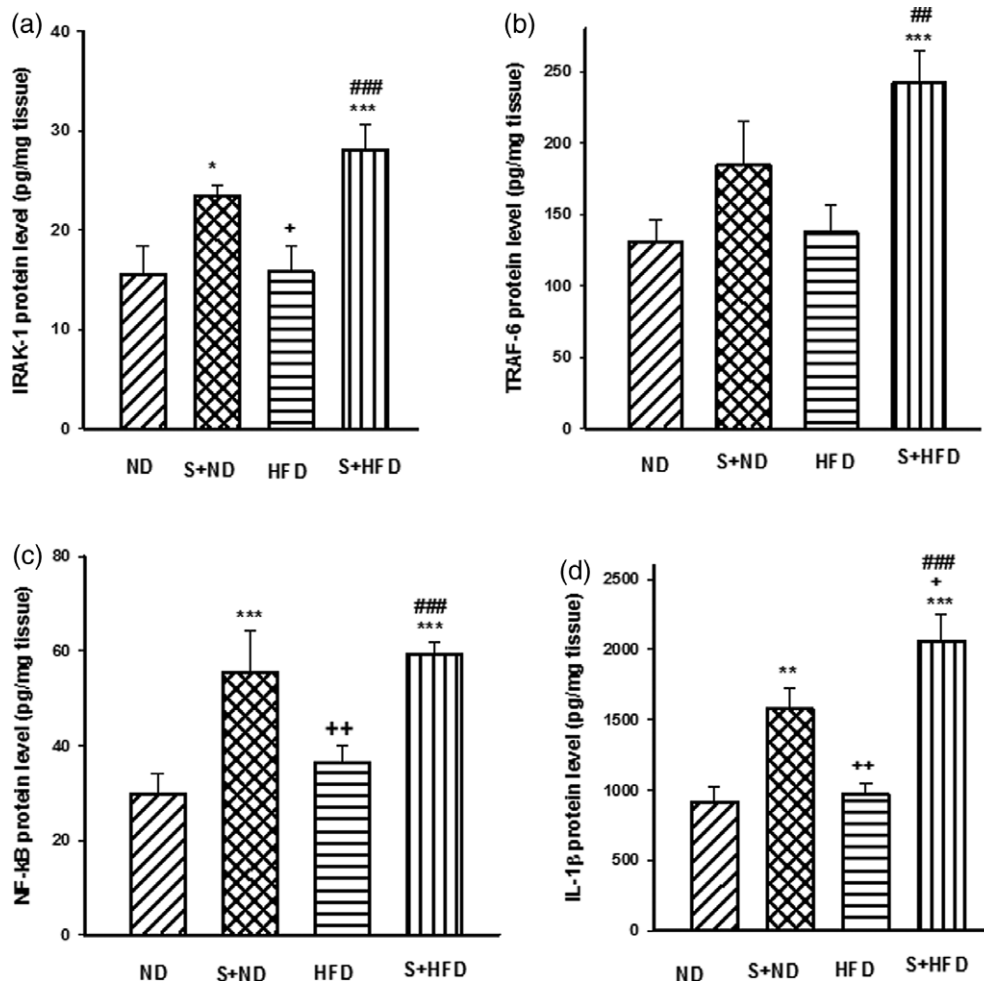
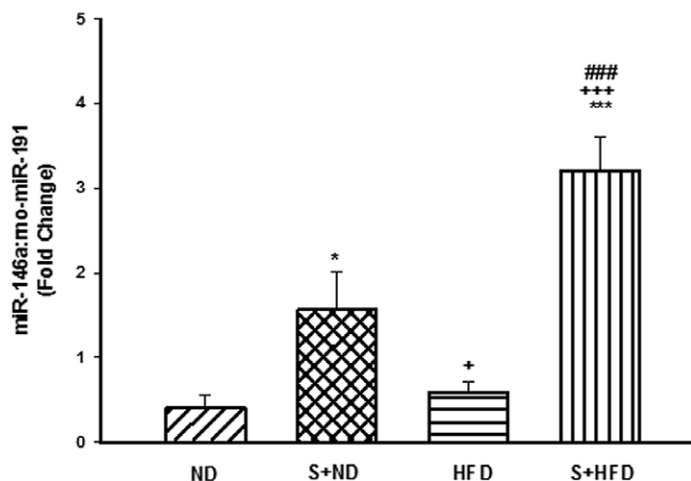


FIG 3

The ELISA of the concentrations of IRAK-1 (A), TRAF-6 (B), NF-κB (C), and IL-1β (D) in the lung tissue; bars represent the mean ± SD (n = 5). Statistical significance for the difference between the data of the ND and other groups: *P < 0.05, **P < 0.01, ***P < 0.001. Statistical differences between S + ND and obese groups: *P < 0.05, **P < 0.01. Difference between the results of the HFD and S + HFD: ##P < 0.01, ###P < 0.001.

nevertheless, the physiological roles of most miRNAs are unclear [22]. Since asthma is an airway inflammatory disease, the inflammatory process is closely associated with the regulation of the immune system. There is little information currently available on miRNA performance during inflammatory responses; nevertheless, a number of recent researches has shown that miRNAs regulate inflammatory responses along with intrinsic and acquired immunity [37]. Many pieces of evidence suggest that asthma is affected by the regulatory functions of miRNAs [38]. In a study, Tananovo et al. suggested that miRNA-146a and miRNA-146b regulate the activity of intrinsic immune response through negative feedback on IRAK1 and TRAF6 [39]. In this study, after the sensitization with ovalbumin, the expression level of miRNA-146a in the lung tissue significantly increased in compared with the control group. Increased expression of miRNA-146a was similar to that of previous studies in monocyte cells stimulated with lipopolysaccharide and TNF-α [39,40]. On the other hand, the high-fat diet markedly increased the expression level of miRNA-146a in the

OVA-sensitized group in comparison with the lean OVA-sensitized group. It is thought that miRNA-146a expression is regulated by the inflammatory transcription factor NF-κB [39]. Increasing the expression and protein of NF-κB in our previous and current study, at least in part, suggests that increased miRNA-146a could be the result of this effect. In fact, under severe inflammatory conditions, NF-κB regulates its activity through negative miRNA-146a feedback on its adaptor molecules, IRAK1 and TRAF6 [41]. These molecules are the basic components of the Toll-like and IL-1 receptor signaling system. Although the anti-inflammatory effects of miRNA-146a have been reported by a number of studies, the opposite results are also reported in this regard. The results of our study showed that, despite a significant increase in miRNA-146a in the obesity associated with asthma group, there was not significant down-regulation in levels of the proteins IRAK1 and TRAF6 in the lung tissue, as compared to the control groups. In a study by Perry et al. [22] on lung alveolar epithelial cells, it was shown that stimulation with IL-1β, levels of the proteins IRAK1 and


FIG 4

The real-time quantitative RT-PCR analysis of miR-146a in the lung tissue; bars represent the mean $\pm$ SD ($n = 5$). Statistical significance for the difference between the data of the ND and other groups: * $P < 0.05$, *** $P < 0.001$. Statistical differences between the S + ND and obese groups: * $P < 0.05$, *** $P < 0.001$. Difference between the results of the HFD and S + HFD: ### $P < 0.001$.

TRAF6 did not decrease with this condition. The authors suggested that miRNA-146a acts as an inflammatory regulator at the translation suppression level independent of the IL-1 signaling pathway involved with IRAK1. Moreover, in a study by Larner-Svensson et al. [41] on smooth muscle cells of the tracheal in rats, they showed that by IL-1 β stimulation, despite a decrease in the expression levels of IRAK1 and TRAF6 mRNA, this decrease was not observed in the protein levels. The reason for this may be that IRAK1 and TRAF6 may be under other control levels or they may have different responses in different amounts of cytokine stimuli to induce the expression of miRNA-146a. It is also conceivable that exposure to the prolonged and sustained lung tissue with high concentrations of cytokines under obesity associated with asthma condition may be a reason in miRNA-146a-NF-kB axis deregulation. In fact, studies have shown that increased expression of NF-kB as well as over-expression of miRNA-146a can have a significant effect on tissue function [42].

It has been shown that in moderate miRNA-146a induction, most of its anti-inflammatory effects is evident through inhibition of its transducer molecules (IRAK1 and TRAF6) [42]. In contrast, in the prolonged and strong induction of miRNA-146a in the presence of a variety of cytokines, it may have deleterious effects on cell survival by inhibiting other targets in signaling pathway [42]. In addition, Garbacki et al. [43] showed that in the murine model of asthma, the amount of miRNA-146a expression in the lung tissue greatly increased, resulting in an increase in immunoglobulin E (IgE) levels. The authors concluded that miRNA-146a has a proinflammatory role in these conditions. Further, Feng et al. [44] showed that treatment with dexamethasone in murine model of asthma caused decreased miRNA-146a expression level in the lung tissue. Based on these

results, the authors concluded that the anti-inflammatory effect of dexamethasone may be due to miRNA-146a expression levels. In general, under obesity associated with asthma condition, the systemic and local increase in cytokines and adipokines has led to a significant increase in miRNA-146a levels, and these conditions can contribute to its pro-inflammatory effects to its anti-inflammatory effects, which require more studies. Also, the results of this study showed that there was a correlation between the expression level of miRNA-146a and study parameters such as EC₅₀, maximum response, IL-1 β , IRAK1, TRAF6, NF-kB, triglyceride, and HDL. In DIO groups, serum levels of TG, TC, LDL, and HDL were remarkably increased compared with those in the normal diet groups, indicating the occurrence of hyperlipidemia, which was similar to previous studies [45,46]. Given that it has been shown that free fatty acids can activate inflammatory pathways in tissues [7], the authors do not rule out the effect of lipid profiles in alteration of cellular pathways. Increasing the miRNA-146a as well as NF-kB in the current study and there was the association between miRNA-146a with lipid profile, at least in part, reflects the fact that free fatty acids may have been involved in these pathways, which requires further studies. Finally, it should be noted that the absence of some of the expected results is maybe due to the limitations of the asthma induction model with ovalbumin, which requires further studies.

In conclusion, miRNA-146a acts as a fine tuner and this agent then acts as a negative feedback on the production of IRAK-1 and TRAF-6 and possibly contributes to the modulation of severe immune responses. As studies have shown, increased expression of miRNA-146a occurs in severe inflammation conditions [47,48]. The results of the current study indicated that

TABLE 2

Pearson correlation analysis of the study parameters with miRNA-146a in the lung tissue

Variables	miRNA 146a	
	<i>r</i>	<i>P</i> value
EC50	−0.702	0.001
Maximum response (%)	0.952	0.000
IL-1 β (pg/mg tissue)	0.840	0.000
IRAK-1 (pg/mg tissue)	0.906	0.000
TRAF-6 (pg/mg tissue)	0.846	0.000
NF-kB (pg/mg tissue)	0.813	0.000
TC (mg/dl)	0.412	0.071
TG (mg/dl)	0.515	0.020
HDL (mg/dl)	0.447	0.048
LDL (mg/dl)	0.144	0.544

level of the proteins TRAF6 and IRAK1 did not decrease with increasing the expression of miRNA-146a in the obesity associated with asthma condition. It is possible that in obesity associated with asthma condition, impairment in the miRNA-146a-mediate negative loop provides conditions that activate NF- κ B and direct its target cells to inflammation or apoptosis. Based on the study results, it is unclear whether miRNA-146a is a causal factor in obesity with asthma. However, its function seems to depend on various factors such as tissue type, inflammation intensity, systemic and local levels of cytokines, and possibly adipocytokines in obesity associated with asthma condition.

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Conflict of interest

The authors have declared that there is no conflict of interest.

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