Lung Altered Expression of IL-1β mRNA and Its Signaling Pathway Molecules in Obese-asthmatic Male Wistar Rats

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ABSTRACT

Epidemiological and clinical studies indicate a close relationship between obesity and asthma. Here, we determined the impact of diet-induced obesity on the expression levels of IL-1β, IRAK-1 and TRAF-6 mRNA as well as IL-1β protein level and pathological changes in male Wistar rat’s lung after sensitization with ovalbumin (OVA).

Twenty male Wistar rats divided into four groups, control with normal diet (C+ND), OVA-sensitized with normal diet (S+ND), control with high-fat diet (C+HFD), and OVA-sensitized with high-fat diet (S+HFD). All rats fed for 12 weeks with standard pellets or high-fat diet while sensitization and challenging with OVA or saline were done for groups in the last month. In the end of intervention, lung was isolated and tested for the expression levels of IL-1β, IRAK-1 and TRAF-6 mRNA with real time-PCR method, and pathological changes were determined.

Diet-induced obesity groups showed increased weight, obesity indexes and lipid profiles. The expression levels of IL-1β mRNA in OVA-sensitization groups (S+ND and S+HFD) showed a significant increase compared with other groups. Also in S+HFD group, expression level of IRAK-1 and TRAF-6 mRNA were markedly higher than other groups (p<0.001). The pathological changes were marked in sensitized groups compared to non-sensitized groups; with marked increase in obese sensitized rat.

The results showed that high fat diet caused overexpression of IL-1β, IRAK-1 and TRAF-6 mRNA as well as IL-1β protein in an experimental model of asthma. Our results suggest that obese-asthmatic conditions may lead to the local production and activation of pro-inflammatory agents.

Keywords: Asthma; IL-1β; IRAK-1; Lung; Obesity; TRAF-6

INTRODUCTION

Obesity is an excess abdominal body fat state.
which is considered as a risk factor for asthma. In the human studies it has become apparent that most people with asthma are overweight or obese subjects. Accordingly, clinical reports have shown that obesity caused increased prevalence of asthma and worse the clinical outcomes. In addition, weight loss in obese people with asthma caused a decrease in intensity of asthma symptoms. Different mechanisms describe how obesity impact on asthma, nevertheless, the exact mechanism of asthma-obesity relationship remains uncertain. Obesity is associated with a chronic low-grade systemic inflammation, characterized by presence of circulating leukocytes, production of abnormal cytokines/chemokines and activation of inflammatory signaling pathways. The effects of obesity on lung tissue involve in the inflammatory changes in pulmonary microenvironment. Therefore, it is important to understand the lung inflammation differences between obese and lean subjects.

Inflammatory mediators and adipokines produced by adipose tissue exert their biological function in an autocrine, paracrine and systemic manner. It is thought that these mediators and adipokines contribute to the immune response in lung. A variety of inflammatory moieties are increased in the obese people. IL (interleukin)-1β is a key cytokine involved in systemic and local immune responses in obesity and asthma. IL-1β is primarily produced by monocytes and macrophages, but other cells including epithelial, endothelial, B and T lymphocytes, have also been shown to release IL-1β. Externally injection of IL-1β to isolated guinea pig airways has disrupted the isoproterenol-induced relaxation response. Also, treatment of animals with IL-1 receptor antagonist (IL-1ra) could decrease hyper-responsiveness to histamine and substance P, and reduce lung inflammation and prevent leukocyte infiltration. Several evidences are available indicating the synthesis and non-regulated release of IL-1β in chronic inflammatory conditions such as inflammatory bowel diseases, psoriasis and rheumatoid arthritis. On the other hand, the impact of IL-1β on contraction and relaxation responses in asthmatic patient through the direct effect on airways has been shown. Taken together, IL-1β has key role in the pathophysiology of asthma. In signaling pathway, IL-1β forms a ligand-induced complex with IL-1 type I receptor (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP). Then, IL-1 receptor associated kinase (IRAK)-1 attaches to receptor complex. Finally, following the activation of IRAK-1, it interacts with tumor necrosis factor receptor associated factor (TRAF)-6 which is required for the activation of IL-1-induced NF-κB. This study was designed to determine the expression levels of IL-1β, IRAK-1 and TRAF-6 mRNA as well as IL-1β protein in the lung tissue and evaluate the differences between the mRNA expressions in obese and lean male Wistar rats in experimental asthmatic model.

MATERIALS AND METHODS

Animals and Diets

Twenty male Wistar rats (8 weeks old, weighing approximately 160 g) were obtained from animal house of Tabriz University of Medical Science. All animals were placed in cages under controlled conditions at 22°C with a 12-12h light-dark cycle and 50-60% humidity. Food and water throughout the accommodation and experimental period was provided ad libitum.

After a week of accommodation, all rats randomly divided to four groups (5 rats in each group), including: control with normal diet (C+ND), OVA-sensitized with normal diet (S+ND), control with high-fat diet (C+HFD), and OVA-sensitized with high-fat diet (S+HFD). Diet-induced obesity (DIO) model was prepared according to the method of previous study. Briefly, in DIO groups (C+HFD and S+HFD), rats were fed with high fat diets (42 % energy from fat, 19% energy from protein and 39% energy from carbohydrate) and other rats (C+ND and S+ND) were fed standard rat chow (11% energy from fat, 28% energy from protein and 61% energy from carbohydrate).

After 8 weeks, sensitization of animals to ovalbumin (OVA) in S+ND and S+HFD groups was performed with the following protocol. The experiment continued for four weeks by OVA sensitization along with the previous regime.

Animal Sensitization

Sensitization of animals to OVA in S+ND and S+HFD groups was performed using the method described previously. Briefly, 1 mg OVA and 200 mg Al (OH)₃ (Sigma) per 1 ml of saline solution were injected intra-peritoneal (i.p) on day 1 and 8. From day 14, the animals were placed in a closed chamber, with dimensions 30x20x20 cm, for exposing to an aerosol of 4% OVA (solution in 2 ml of saline) for 18 ± 1 days.
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during 15 min daily using a nebulizer (CX3, Omron Health care Europe B.V., and the Netherlands). All control animals (C+ND and C+HFD) were treated similarly but saline was used instead of OVA solution. The study was approved by the ethical committee of Tabriz University of Medical Sciences (Acceptance number: 92/4-6/9).

Body Weight
The animals were weighted weekly on a certain day (at 16.00) during the experiment. At the end of study, rats were anesthetized by 50 mg/kg ketamine and xylazine (i.p). Then they were weighted and naso-anal length; the distance between nose and anus of rats; measured. Final body weight, Lee index, and percentage of body fat (BF %) were also determined for calculation of obesity indexes used in rodents. All obesity indexes described previously (reference for each index shown in Table 1). Briefly, Table 1 shows how all obesity indexes were calculated.

Biochemical Measurements
The fasted rats then anesthetized with ketamine and xylazine, blood samples (3-5 ml per rat) were collected from the heart into tubes without EDTA. The plasma total cholesterol (TC), triglycerides (TG) and HDL-C concentrations were measured using an auto blood analyzer (Bayer corp. USA). Triglyceride, total cholesterol and HDL-C kits were obtained from pars azmoon Co., Iran. LDL-C was evaluated using the freedewald formula: LDL-C= TC-[HDL-C+TG/5].25

Lung Histopathological Evaluation
Rats were anesthetized by ketamine and xylazine (i.p) and sacrificed. Their lungs and trachea were isolated and placed into 10% buffered formalin (37%, Merck, Germany). After seven days, the tissues were dried by passage through 70% to 100% ethanol. Then the tissues were cleared by xylene and embedded in paraffin. Finally the specimens were cut into 4-μm sections using an autotechnicon apparatus and stained with hematoxylin and eosin (H&E stain). The tissues were then evaluated under a light microscope. The pathologic changes in the lung of all groups including the lipid aggregation, the emphysema, lymphoid hypertrophy, airway smooth muscle hyperplasia and edematous changes were recorded. The pathological changes were scored according to the following grades: no pathologic changes=0; patchy changes=1; local changes=2; scattered changes=3 and severe changes (in the most parts of the lungs)=4.

Lysate Preparation from Lung Tissue
After anesthetized, the chest and the neck of the rats were opened and the lung was isolated for weighing and removing an appropriate amount of tissue. Tissue was grinded and homogenized for subsequent RNA extraction. After adding 600 μL of RNase-free water and 20 μL reconstituted proteinase K, lysate was incubated and spun for 1 min. To extract the RNA, about 650 μL of mixtures of lysate and ethanol was loaded to total RNA extraction column and centrifuged for 1 minute. Then, DNase I treatment performed followed by washing step. RNA was eluted from column and the purified RNA sample kept at −70°C for real time-PCR analysis.

Real-Time PCR
RNA content and purity were measured using Nanodrop 1000 spectrophotometer (Thermo scientific, Wilmington, DE, USA). For determination of IL-1β, IRAK-1 and TRAF-6 mRNA expression levels RevertAid First-Strand cDNA Synthesis Kit (Fermentas 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) were used.

Each cDNA was used as a template for separate assay for mRNA quantitative real-time PCR by using SYBR Green master mix.

Table 1. Obesity indexes formula

<table>
<thead>
<tr>
<th>Obesity index</th>
<th>Formula</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of body weight changes (%)</td>
<td>(Final weight-initial weight)/initial weight) x100</td>
<td>(22)</td>
</tr>
<tr>
<td>Lee index (mg/mm)</td>
<td>Final weight/naso-anal length</td>
<td>(50)</td>
</tr>
<tr>
<td>Percentage of body fat (%)</td>
<td>0.73(lee index-280.8)</td>
<td>(50)</td>
</tr>
</tbody>
</table>
Table 2. Primer set list for analyzing the gene expressions

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Accession number</th>
<th>Primer sequencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>NM_031512</td>
<td>Forward Primer 5'- AGA GTG TGG ATC CCA AAC AA -3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse Primer 5'- AGT CAA CTA TGT CCC GAC CA -3'</td>
</tr>
<tr>
<td>TRAF-6</td>
<td>NM_001107754.2</td>
<td>Forward Primer 5'- CAG TCC CTC GCA CAT -3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse Primer 3'- GAG GCA TCG CAT -3'</td>
</tr>
<tr>
<td>IRAK-1</td>
<td>NM_001127555</td>
<td>Forward Primer 5'- GAG AGT GTT CCT GGC CTC TC -3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse Primer 5'- GCT GGG TTT ATG ATG ATC TG -3'</td>
</tr>
<tr>
<td>Beta Gusp</td>
<td>NM_017015</td>
<td>Forward Primer 5'- GGGGGAATATGACCTGCA -3'</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse Primer 5'- GGAACCCCTGTTAGAACGT -3'</td>
</tr>
</tbody>
</table>

a. Sequences were derived from NCBI (www.ncbi.nlm.nih.gov)

Table 2 shows locked nucleic acid (LNA) forward and reverse primer sets (Exiqon) for analyzing the gene expressions. Real-time PCR reactions were performed on a Rotor-Gene 6000 instrument (Corbett Life Science, Australia).

The amount of PCR products of mRNA extracted samples was normalized to the PCR products of housekeeping beta-glucuronidase gene. The \(2^{-\Delta \Delta Ct}\) method was used to determine relative quantitative levels of IL-1β, IRAK-1 and TRAF-6 mRNA. The results were expressed as fold change versus the relevant controls.

### Tissue Sampling and Protein Measurement

At the end of study period, rats were anesthetized with an i.p injection of ketamine (50 mg/kg) and xylasine (5 mg/kg) and sacrificed. Then lung tissues were removed and after quick freezing in liquid nitrogen, all tissues transferred to -70 °C temperature until IL-1β measurement.

Tissue samples were weighted, homogenized in PBS (pH 7.2-7.4) and centrifuged for 20 min at the speed of 3000 rpm and 4°C. Then supernatants were removed and IL-1β protein was measured using sandwich rat ELISA kits according to the manufacturer’s instructions (Boster Biological Technology Co., Ltd, catalog: CA, 94566).

### Statistical Analysis

Results are given as the mean ±SD. The data between different groups were compared using one way analysis of variance (ANOVA) with Tukey-Kramer post hoc-test. \( p \) less than 0.05 was considered significant. The correlation between IL-1β, IRAK-1 and TRAF-6 mRNA levels with weight were calculated by pearson’s correlation coefficient.

### RESULTS

#### Body Weight

Table 3 showed the mean (±SD) of initial body weight, final body weight, Lee index, and percentage of body fat for all groups. In the present study, results showed that diet-induced obesity caused significant increase in final body weight, Lee index, and percentage of body fat in C+HFD and S+HFD animals compared to C+ND and S+ND animals (\( p<0.05 \) to

### Table 3. Weight changes and obesity indexes in experimental groups

<table>
<thead>
<tr>
<th>variables</th>
<th>C+ND</th>
<th>S+ND</th>
<th>C+HFD</th>
<th>S+HFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (gr)</td>
<td>159±5.43</td>
<td>152.80±8.25</td>
<td>154.80±4.81</td>
<td>156±7.96</td>
</tr>
<tr>
<td>Final body weight (gr)</td>
<td>317.20±25.05</td>
<td>318.00±24.89</td>
<td>355.40±10.13</td>
<td>371±28.12*</td>
</tr>
<tr>
<td>Percentage of body weight change (%)</td>
<td>99.28±9.75</td>
<td>108.48±11.70</td>
<td>129.78±10.13*</td>
<td>137.83±13.92***</td>
</tr>
<tr>
<td>Lee index (mg/mm)</td>
<td>305.29±6.83</td>
<td>307.58±6.16</td>
<td>320.04±2.53**</td>
<td>321.15±2.87**</td>
</tr>
<tr>
<td>Percentage of body fat (%)</td>
<td>17.87±4.98</td>
<td>19.54±4.5</td>
<td>28.64±1.85**</td>
<td>29.45±2.09**</td>
</tr>
</tbody>
</table>

Values are represent as mean±SD. C+ND: control with normal diet, S+ND: OVA-sensitized with normal diet, C+HFD: control with high-fat diet, S+HFD: OVA-sensitized with high-fat diet. Differences between the results of C+ND with those of other groups: \( p<0.05, \) \( ** p<0.01, \) *** \( p<0.001\). Differences between the results of S+ND with those of control high fat diet and sensitized high fat diet: \( * p<0.05, \) ** \( p<0.01, \) *** \( p<0.001\). For each group, n=5.
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Figure 1. The serum levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low density lipoprotein (LDL) in experimental groups. Values are depicted as mean±SD. C+ND; control with normal diet, S+ND; OVA-sensitized with normal diet, C+HFD; control with high-fat diet, S+HFD; OVA-sensitized with high-fat diet. Statistical differences between the control and other groups; *: p<0.05, **: p<0.01, ***: p<0.001. Statistical differences between the sensitized normal diet with obese groups; +: p<0.05, ++: p<0.01, +++: p<0.001. For each group, n=5.

Serum Lipid Profile

Figure 1 presented the lipid profile (TC, TG, HDL and LDL) in all groups. Lipid profile in obese groups (C+HFD and S+HFD) were significantly higher than the control groups (p<0.05 to p<0.001). With the exception of differences in HDL between C+ND and S+ND (p<0.05), the significant differences between the control groups (C+ND versus S+ND) and obese groups (C+HFD versus S+HFD) were not observed.

Lung Histopathology

With regard to our scoring described in materials and methods section, OVA-sensitization caused a significant elevation in lymphoid hypertrophy, airway smooth muscle hyperplasia and emphysema pathological changes in sensitized groups (S+ND and S+HFD) compared to non-sensitized groups (C+ND and C+HFD), (p<0.05 to p<0.001, Figure 2 and 3b to d). Also, after diet-induced obesity, lipid aggregation in lung tissue significantly enhanced in obese groups (C+HFD and S+HFD) in comparison with normal diet groups (C+ND and S+ND), (p<0.05 to p<0.001, Figure 2 and 3a). The edematous pathological changes in C+HFD, S+ND and S+HFD were significantly higher than those in C+ND group (p<0.001 for all cases, Figure 2 and 3e), but there were no significant differences between other groups. On the other hand, the airway smooth muscle pathological changes in S+HFD significantly were increased compared to S+ND group (p<0.001, Figure 2 and 3c), and the emphysema pathological change in S+HFD was non-significant higher than S+ND (p=0.093, Figure 2 and 3d). Although the lymphoid hypertrophy pathological change was higher in S+HFD group than S+ND group, but this difference was not statistically significant.

IL-1β mRNA Expression in Lung Tissue

As seen in Figure 4, there was a significant increase in rat lung of IL-1β mRNA expression of S+HFD group compared with other groups (p<0.05 to p<0.001). The level of rat lung IL-1β mRNA expression in S+ND group was also significantly higher than C+ND (p<0.05). There was no significant difference between C+ND and C+HFD groups.

Relationship between IL-1β mRNA Expression and Obesity Indexes

A significant positive correlation was found
between IL-1β mRNA expression and percentage of weight changes ($r=0.547$, $p<0.05$; Figure 5). This correlation was not significant in other obesity indexes, Lee index ($r=0.259$, $p=0.271$) and percentage of body fat ($r=0.259$, $p=0.271$).

**IRAK-1 mRNA Expression in Lung Tissue**

In Figure 6, IRAK-1 mRNA expressions in lung tissue of different groups are shown. The IRAK-1 mRNA expressions in group S+HFD increased significantly compared to other groups ($p<0.01$ to $p<0.001$). There was not any significant difference among other groups.

**Relationship between IRAK-1 mRNA Expression and Obesity Indexes**

There was significant positive correlation between IRAK-1 mRNA expression with obesity indexes, percentage of weight changes ($r=0.658$, $p=0.002$), Lee index ($r=0.578$, $p=0.008$) and percentage of body fat ($r=0.578$, $p=0.008$; Figure 7).

**TRAF-6 mRNA Expression in Lung Tissue**

Figure 8 shows that TRAF-6 mRNA expressions in group S+HFD increased significantly compared to C+ND, C+HFD, and S+ND groups ($p<0.001$). There was not any significant difference among other groups.

**Relationship between TRAF-6 mRNA Expression and Obesity Indexes**

Significant positive correlations between TRAF-6 mRNA expression level and percentage of weight changes were observed in lung tissue ($r=0.455$, $p=0.044$; Figure 9). But these correlations were not significant when compared with Lee index ($r=0.412$, $p=0.071$) and percentage of body fat ($r=0.412$, $p=0.71$).
Figure 3. The lipid aggregation (a), lymphoid hypertrophy (b), airway smooth muscle hyperplasia (c), emphysema (d), and edematous changes (e) of lungs in control with normal diet (C+ND), OVA-sensitized with normal diet (S+ND), control with high-fat diet (C+HFD), and OVA-sensitized with high-fat diet (S+HFD), (for each group, n=5). Differences between the results of C+ND with those of other groups; **; \( p < 0.01 \), ***; \( p < 0.001 \). Differences between the results of S+ND with those of control high fat diet and sensitized high fat diet; +; \( p < 0.05 \), ++; \( p < 0.01 \), +++; \( p < 0.001 \). Difference between the results of C+HFD with S+HFD; ###; \( p < 0.001 \).

**IL-1β Protein Levels in Lung Tissue**

The data presented in Figure 10, demonstrates that OVA-sensitization and challenging caused significant increase in IL-1β protein levels in sensitized groups (S+ND and S+HFD) compared with non-sensitized groups (C+ND and C+HFD), \( p < 0.05 \) to \( p < 0.001 \). Also, diet-induced obesity, resulted in a significant increase in the level of IL-1β protein in S+HFD group when compared with S+ND group \( (p < 0.01) \). There was not significant difference between C+ND and C+HFD group.
Figure 4. Expression level of IL-1β mRNA in the lung tissue of experimental groups. Values are depicted as mean±SD. C+ND; control with normal diet, S+ND; OVA-sensitized with normal diet, C+HFD; control with high-fat diet, S+HFD; OVA-sensitized with high-fat diet. Statistical differences between the C+ND and other groups; ***; p<0.001. Statistical differences between the S+ND vs obese groups; +; p<0.05. Statistical difference between C+HFD vs S+HFD; ###; p<0.001. For each group, n=5.

Figure 5. Pearson correlation analysis of IL-1β mRNA with percentage of body weight changes in the study groups. There was a significant positive correlation between IL-1 mRNA expression and percentage of body weight changes (correlation coefficient=0.547, p<0.05).
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Figure 6. Expression level of IRAK-1 mRNA in the lung tissue of experimental groups. Values are depicted as mean±SD. C+ND; control with normal diet, S+ND; OVA-sensitized with normal diet, C+HFD; control with high-fat diet, S+HFD; OVA-sensitized with high-fat diet, TRAF-6; TNF receptor associated factor-6. Differences between the results of normal diet with those of other groups; ***; \( p < 0.001 \). Differences between the results of S+ND with those of high fat diet groups; +++; \( p < 0.001 \). Difference between the results of C+HFD with S+HFD; ##; \( p < 0.01 \). For each group, \( n = 5 \).

Figure 7. Pearson correlation analysis of IRAK-1 mRNA with percentage of body weight changes in the study groups. There was a significant positive correlation between IRAK-1 mRNA expression and percentage of body weight changes (correlation coefficient=0.658, \( p < 0.01 \)).
TRAF-6: β-glucuronidase
(Fold change)
0
1
2
3
4
5
***
+++
###

Figure 8. Expression level of TRAF-6 mRNA in the lung tissue of experimental groups. Values are depicted as mean±SD. C+ND; control with normal diet, S+ND; OVA-sensitized with normal diet, C+HFD; control with high-fat diet, S+HFD; OVA-sensitized with high-fat diet, TRAF-6; TNF receptor associated factor-6. Differences between the results of C+ND with those of other groups; ***; *p* < 0.001. Differences between the results of S+ND with those of control high fat diet and sensitized high fat diet; ++; *p* < 0.001. Difference between the results of C+HFD with S+HFD; ###; *p* < 0.001. For each group, n=5.

TRAF-6: β-glucuronidase
(Fold change)

Figure 9. Pearson correlation analysis of TRAF-6 mRNA with percentage of body weight changes in the study groups. There was a significant positive correlation between IL-1 mRNA expression and percentage of body weight changes (correlation coefficient=0.455, *p* < 0.05).
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<table>
<thead>
<tr>
<th>C+ND</th>
<th>S+ND</th>
<th>C+HFD</th>
<th>S+HFD</th>
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<tbody>
<tr>
<td><img src="chart.png" alt="" /></td>
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<td><img src="chart.png" alt="" /></td>
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</tbody>
</table>

Figure 10. Protein levels of IL-1β in the lung tissue of experimental groups. Values are depicted as mean±SD. C+ND; control with normal diet, S+ND; OVA-sensitized with normal diet, C+HFD; control with high-fat diet, S+HFD; OVA-sensitized with high-fat diet. Differences between the results of C+ND and those of other groups; **; $p<0.01$, ***; $p<0.001$. Differences between the results of S+ND and those of control high fat diet and sensitized high fat diet; +; $p<0.05$, ++; $p<0.01$. Difference between the results of C+HFD and S+HFD; ###; $p<0.001$. For each group, n=5.

**DISCUSSION**

It is well known that obesity is a low-grade chronic pro-inflammatory condition.\textsuperscript{28} It is unknown that how these conditions can lead to the development of airway inflammation and asthma in humans. Most experimental studies which have focused on the relationship between obesity, airway inflammation, and asthma, mainly used genetically engineered animals to show the possible mechanisms between obesity and asthma. But these genetically obese mice, such as leptin deficiency (ob/ob mouse) and leptin receptor deficiency mice (db/db mouse), have some complications including comorbid conditions affecting the immune responses in airways.\textsuperscript{29} In this study we used the high fat diet to induce obesity in Wistar rats. Our results showed that diet-induced obesity caused increase in body weight and obesity indexes in rodent (percentage of weight changes, Lee index and fat percent) which was in accordance with the previous studies.\textsuperscript{21} On the other hand, as a result of diet-induced obesity, lipid profile (total cholesterol, triglyceride, HDL-C and LDL-C) increased significantly in obese groups.

Airway hyperresponsiveness (AHR), BAL fluid eosinophilia and smooth muscle hypertrophy are some characterize of human asthma. Airway remodeling is a pathophysiological characteristic of asthma, which is identified by goblet cell hyperplasia, increasing in thickness of basement membrane, sub-epithelial fibrosis, collagen deposition, angiogenesis and airway smooth muscle hypertrophy and hyperplasia.\textsuperscript{30} In our present study, OVA-sensitization showed some aspects of human asthma include lymphoid hypertrophy, airways smooth muscle hyperplasia, edema, and emphysema in lean and obese groups; with marked augmentation in obese sensitized rat. Previous studies have shown that some of histological changes in the lungs in obesity associate with asthma. Camergo and colleagues indicated that obesity caused decrease in airway caliber and increase in bronchial hyper reactivity in obese rats.\textsuperscript{31}

Obesity is a risk factor for asthma and causes an increase in the prevalence of asthma.\textsuperscript{32} Human and animal studies supported the relationship between obesity and asthma.\textsuperscript{33-35} In obese human and mice, chronic systemic inflammation characterized by elevation of circulating leukocytes and serum concentrations of cytokines, chemokine and acute phase proteins.\textsuperscript{36,37} Studies have shown that systemic inflammatory markers are associated with diseases...
associated with obesity, such as type II diabetics and artherosclerosis. These findings suggest that the inflammation functionally is very important in the development of these diseases. A variety of inflammatory moieties which are increased in obese individual blood, IL-1β and TNF-α are of particular interest. Specific cytokines, particularly IL-1β, have been shown to have a special role in altering the airway responsiveness in asthma. Hakonarson and his colleagues have revealed that airway smooth muscle could synthesize and release IL-1β in asthmatic patients and this indicated that IL-1β acts in an autocrine manner in airway smooth muscles.

Our results in the current study showed that sensitization with OVA caused to increase the expression level of IL-1β in male Wistar lung, which was in consistent with the previous findings. Also, in our study design, diet-induced obesity led to overexpression of IL-1β in lung compared to other groups. Another study has also shown such increase in IL-1β in lung tissue. Additionally, in current study, we showed that post translational changes in obese asthmatic condition resulted in production of IL-1β in the lung.

Overexpression and enhanced production of IL-1β in obese and sensitized rat’s lung indicated the functional modification of lung tissue and led to autologous expression of IL-1β. On the other hand, studies reported that increase in the synthesis and release of IL-1β, result in the synthesis of other inflammatory mediators that may effect lung function leading to exacerbation of lung inflammation in asthma. Up regulation of IL-1β in lung in this study showed the autocrine mechanisms may involve in pathophysiology in obesity-asthmatic model. There are more evidences indicating the potential role of IL-1β in triggering the expression and release of a variety of interleukins, such as IL-1, IL-6, IL-8, IL-11 and IL-15, which can exacerbate the asthma condition in turn.

Also, it has been shown that the stimulatory effect of β-arrestin on IL-17 in a murine asthma. Furthermore, the link between obesity with asthma, a number of hypotheses has been proposed which can be pointed to the role of obesity on immune responses. Adipose tissue in addition to the production of adipokines, such as leptin, is an important source of cytokines, such as IL-1β and TNF-α. These cytokines have an effect on the various processes in asthma, such as release of and IL-9, damag bronchial epithelial cells, recruitment and adhesion of eosinophil to airway epithelial cells, and bronchoconstriction. On the other hand, our results of histological examinations showed that fat tissue mass increased in the lungs after diet-induced obesity. It is inferred that the increase in fat mass in the lungs, as well as in visceral adipose tissue, can lead to an increase of adipokines in circulation and in the lungs tissue. Enhanced adipokines in lungs may change the airway responsiveness in obese asthmatic statue. The findings of the current study, such as histological changes and up regulation of IL-1β in obese asthmatic condition support the hypothesis that not only systemic but also local inflammation may be involved in pathophysiologic changes in obesity associated asthma statue.

IL-1β exerts its signal transduction pathways through interaction with IRAK-1 after band with its receptors. Then IRAK-1 interacts with TRAF adaptor proteins, especially TRAF-6, which leading to activation of kind of transcription factors, such as NF-kB and active protein (AP)-1. In the current study, the IRAK-1 and TRAF-6 expressions levels were examined in lung to better understanding of IL-1β roles in relationship between obesity and asthma. Diet-induced obesity caused markedly overexpression of IRAK-1 and TRAF-6 mRNA in obese sensitized group. This elevated expression, at least in part, might explain the possible role of IL-1β in this condition and activation of its signal transduction pathways. It is noteworthy that other factors, such as Toll-like receptors and free fatty acids, are able to activate this signaling pathway and the authors cannot rule out the role of these factors in the activation of IL-1β signaling pathway, it needs further studies.

It should be noted that the complexity of the mechanisms responsible for exert IL-1β autocrine effects is difficult to explain in obesity with asthma. In fact, other members of IL-1 axis should be considered for better understanding of the role of IL-1β. Studies have shown that IL-1 exerts its effects through the IL-1 type I receptor (IL-1R1), but type II receptors; both IL-1RII and solution form of it (sIL-1RII) and solution form of IL-1RI (sIL-1RI), act as decay receptors that are involved as damping effect for IL-1. Also, IL-1ra, an endogen IL-1 antagonist, should be considered in this axis. Based on available evidence, it seems that under normal conditions, IL-1β and other family members of IL-1 are involved to prevent excessive immune response in all cell type. On the other hand, in
certain disease conditions, there is an imbalance in the production of the IL-1β and other molecules in IL-1 axis, this phenomenon might occur in obese with asthma. Although the expression of IL-1β mRNA increased in the current study, the role of other molecules involved in IL-1 axis cannot be ruled out. Perhaps the expression or activation of other molecules reduced or activated in obesity associated with asthma which further studies. So, the ratio of active (IL-1RI) to inactive (sIL-1RI, IL-1RII, and sIL-1RII) receptors may determine the amount of inflammation.

We also examined the correlation between the expression of IL-1β, IRAK-1 and TRAF-6 mRNA with obesity indexes in rodent. Our result indicated that there was a weak positive correlation between the expression of IL-1β and TRAF-6 mRNA with percentage of weight changes in study groups. However, there was not any significant correlation between IL-1β and TRAF-6 mRNA with Lee index and percentage of body fat. This study showed that in asthmatic condition, increase in weight changes enhances expression of pro-inflammatory cytokine, IL-1β. Studies have shown that asthma is a chronic inflammatory state which increases conventional biomarkers, such as eosinophil and eNO. But, in the obesity associated with asthma, studies reported that these biomarkers do not increase substantially with increasing body mass index (BMI). The authors suggested that other mechanisms may involve in its pathophysiology in the obesity associated with asthma. Based on the above observation, it is conceivable that asthma phenotype might change in obesity condition. In conclusion, our results showed that in the lung of male OVA-sensitized rats, diet-induced obesity lead to an increased expression of IL-1β, IRAK-1 and TRAF-6 mRNA. IL-1β is a key cytokine which has pro-inflammatory effects in all cell types. Many animal and human studies indicated that different kinds of cytokines increase in obesity with asthma condition. Up regulation of IL-1β in obesity state may result in exacerbation of inflammation of airways in asthma. Enhanced circulating IL-1β with its autocrine effect may involve in synergistically increase of inflammation in obesity associated with asthma in airways. Furthermore, signaling pathway molecules of IL-1β are IRAK-1 and TRAF-6. Increased expressions of IRAK-1 and TRAF-6 mRNA in this study, at least in part, can show activation of IL-1β signaling pathways.

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