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Experimental Research Article

Increased inflammation in the lungs of asthmatic pregnant mice was associated with elevated leptin levels

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ABSTRACT

Introduction: Many studies have shown that asthma is characterized by inflammation of the airway and infiltration of eosinophil cells (EOSCs). It has also shown that during pregnancy, the level of leptin, as a regulator of immune responses, increases with the progression of the pregnancy process. In this study, the effect of asthma on inflammatory factors was evaluated in the lung and uterine tissues of asthmatic pregnant or non-pregnant mice.

Methods: In this experimental study, 40 female Balb/c mice (8 weeks old) were classified into 4 groups, and asthma by ovalbumin (OVA) at a concentration of 20 µg/100µl was induced. Lung and uterus tissues were histopathologically evaluated for the presence of inflammation. The level of leptin hormone in blood serum was investigated using an indirect enzyme-linked immunosorbent assay (ELISA). Also, Interleukin-8 (*IL-8*), forkhead box protein 3 (*Foxp3*), eosinophil chemotactic protein (*eotaxin*), and mucin 5AC (*Muc5ac*) gene expression were measured in respiratory and uterine cells by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR) assay ($P<0.05$, $P<0.01$ and $P<0.001$).

Results: Morphological assessment of inflammation in lung tissue showed a significant increase in asthmatic groups compared to healthy groups. Hormone measurement revealed a significant rise in leptin levels in pregnant groups compared to non-pregnant groups. Also, the expression level of *IL-8*, *Foxp3*, *eotaxin*, and *Muc5ac* genes increased in pregnancy compared to negative control.

Conclusion: In asthma, inflammation rate increases at the cellular and molecular levels, and the leptin increment might have an influence on the inflammation.

Keywords:
Asthma
Pregnancy
Eosinophil cells
Leptin
Inflammatory factors

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Introduction

Pregnancy is one of the most complicated bio-phenomena and the embryo is considered an auto-transplant element that should be tolerated during pregnancy (Roy et al., 2009; Wang et al., 2023). Researchers believe that the implantation of the embryo is the most important stage in maintaining the pregnancy (Mirzaeian et al., 2020). Nearly half of human embryo implantation cases are unsuccessful (Van Hoogenhuijze et al., 2021; Wang et al., 2023). Many unsuccessful pregnancies are caused by the absence of adequate preparation of uterine tissue to receive the embryo (Lédée et al., 2020). Therefore, the research has been directed toward the usage of preparation methods of the uterus to receive the embryo as best as possible. For this purpose, maintaining an appropriate inflammatory status is essential for successful implantation. Overall, it is clear that changes in the immune system, including pathological states such as asthma, may affect uterine tissue and possibly pregnancy.

Asthma is characterized by chronic inflammation of the airways and the infiltration of inflammatory cells such as EOSCs and T cells. It has been shown asthma in pregnant women is a typical and possibly significant medical issue (Tan and Thomson 2000). Up to 13% of pregnancies worldwide are affected by asthma, and its clinical course changes and is unpredictable while pregnant (Robijn et al., 2019). On the other hand, many studies showed the amount of leptin hormone in the serum of pregnant women increases progressively which adequately regulates signaling pathways in the maternal immune system and asthma throughout pregnancy.

Leptin is a structural member of a pro-inflammatory cytokines-like family that is encoded by the LEP gene and is mainly secreted by adipocytes (Pérez-Pérez et al., 2020). Several studies have shown that leptin acts as a regulator in both innate and adaptive immunity (Kiernan and MacIver 2021). Signaling pathways of leptin are damaged due to a rise in body mass (Zonneveld et al., 2021). Raising amounts of leptin cause low-grade inflammation, obesity, cardiovascular diseases, diabetes, asthma, malignancy, and autoimmune disease. Conversely, decreased leptin concentrations are associated with an enhanced risk of infection and decreased cellular immune responses (Kiernan and MacIver 2021). Some researchers indicated leptin can enhance cytokine response, especially levels of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), lipopolysaccharide, and IL-1, 6, 17 (Cordero-Barreal et al., 2021; Fatel et al., 2019).

Other cytokines and chemokines involved in immune system response during asthma inflammation include IL-8, Foxp3, Eotaxin, and Muc5ac. IL-8 is produced by macrophages and other cell types such as epithelial cells, airway smooth muscle cells, and endothelial cells (Brat et al., 2005). This protein as a chemotactic factor, induces chemotaxis in neutrophils and other granulocytes and causes these cells to migrate to the site of infection. (Modi et al., 1990). It has been assessed leptin with increased expression of IL-8 stimulates phagocytosis (Dixit et al., 2003). FOXP3 is a member of the FOX family that binds to target DNA and is involved in the production of immunosuppressive T cells. FOXP3 decreases the expression of IL-2 but increases the expression of CD25 while leptin does exactly the opposite. Also, in an asthmatic state, Eotaxin in line with leptin activates EOSCs, basophils, and T-helper lymphocytes that produce IL-4 and IL-5 cytokines (Zeibecoglou et al., 1999). On the other hand mucins including Muc5ac, are a family of high molecular weight proteins produced by epithelial tissue in most animals. The main characteristic of mucins is their ability to form a gel and their functions range from lubrication to cell signaling to create chemical barriers. They often have an inhibitory role and bind to pathogens as part of the immune system. Mucins are used as markers for the diagnosis of malignancies (Marin et al., 2007).

The research question is whether leptin hormone affects the expression of inflammatory cytokines in asthmatic pregnant. Therefore, the aim of this project investigate of serum level of leptin and the gene expression levels of inflammatory cytokine and chemokines *IL-8*, *Foxp3*, *Eotaxin*, and *Muc5ac* in asthmatic pregnant and non-pregnant mice. Also, the degree of inflammation in lung and uterine tissues has been examined.

Materials and Methods

Animal and group design

In this experimental study, female Balb/c white 8-weeks-old mice (20-25 g) were purchased from the Pasteur Institute of Tehran (Karaj-Iran) and kept under suitable conditions in terms of food and water. The animals were housed at 18-24 °C, 30-60% humidity, and the regular 12 h dark/light cycle was kept persistent. The ventilation system of the animal house is equipped with

equipment to keep the animals in an allergen-pathogen-free environment. In this research, 40 mice were casually distributed into four groups (n = 10). In two groups, airway inflammation was confirmed by ovalbumin (Sigma-Aldrich, USA) (OVA-sensitized). Group I Healthy and non-pregnant (Negative Control) was the non-sensitized and received phosphate buffer saline (PBS), group II Allergic asthma and non-pregnant (Positive Control) received OVA, group III Healthy and pregnant was the non-sensitized, and group IV Allergic asthma and pregnant received OVA.

Asthma induction

To induce asthma in mice, according to a previously described protocol airway inflammation was made in 2 of the groups using OVA (Athari et al., 2016). Briefly, first on days 0 and 14, 20 µg/100 µl OVA with aluminum hydroxide (Sigma-Aldrich, Netherlands) was intra-peritoneal injected. Subsequently10 ml of 1% OVA solution on days 24, 26, 28, and 30 was nebulized into the trachea using an ultrasonic nebulizer (NE-U07, Omrom, Japan) for 30 min per day (Fig. 1). All mice were kept in a place free of allergens and pathogens. The nebulizer device is the NEUO7 model which has no sound due to the use of an ultrasound system to produce particles and can produce particles smaller than 3 µm. Particles of this size will have the ability to penetrate even small airways. Finally on day 31, the mice in groups I and II were euthanatized by CO2, and blood samples and related tissues were stored at -70°C for subsequent analyses. On the other hand, in groups III and IV animals got pregnant after the completion of the asthmatic period on day 31 and they were also sampled.

Histology assessment

In all groups, the left lower lobe of the lung is carefully separated and each lobe is divided into three parts. Also, the uterine tissue was removed and all samples were placed in Bowen's fixative solution for 24 h. After placing the tissue in 10% formalin, embedded in paraffin wax (Hamedi et al., 2023). To stain, the tissue sections with a thickness of 6 µm were placed in Hematoxylin/Eosin (H&E) dye. Finally, the slides were prepared for an Olympus B × 50 microscopic furnished with a Leica DFC 320 Digital Camera (Sarabadani et al., 2021). For quantification of histological sections, EOSCs were evaluated at 1000x magnification in five repeats by two pathologists. Nonexistence or very little existence of EOSCs was scored 0, partial layer of EOSCs was scored 0.1-1, one comprehensive layer of EOSCs was scored 1, two comprehensive layers of EOSCs were scored 2, three comprehensive layers of EOSCs were scored 3 and extra three comprehensive layers of EOSCs were scored 4.

Leptin hormone level assay in the blood serum

After the completion of the treatment period, blood was collected from the hearts of mice anesthetized with ether. The blood sample was poured into sterile microtubes and centrifuged at 2000 rpm for 5 minutes. Subsequently, the supernatant solution, which is the serum transferred to a new microtube and then transferred to a -20°C freezer. Leptin hormone level measured by ELISA kit (Zellbio, Germany). To perform this assay, 96-well plates coated with antibodies against leptin were utilized. Then the mouse serum sample was added, which binds with the antibody coated on the bottom of the plate. Also, standard and control samples were added to other wells. Then the secondary anti-leptin antibody that is attached to the labeled enzyme was added. The plate was incubated for 2 h at 37°C so that the antigen in the mouse serum binds to the added antibodies. Then the wells were washed with a special buffer 5 times and the dye solution was added to the wells. The resulting color is formed by the immune complex and is relational to the concentration of leptin in the sample. Finally, the color was created and therefore the amount of leptin hormone was measured with an ELISA reader.

QRT-PCR analysis

To check the expression level of *IL-8*, *Foxp3*, *Eotaxin*, and *Muc5ac* genes in lung and uterine tissues, tissue samples were first isolated and homogenized. RNA was then extracted from the homogenized tissues and used for Real-Time PCR assays.

Trizol solution was added to each microtube and the resulting mixture was transferred to RNase-free microtubes. For every 1 ml of Trizol solution, 200 µl of chloroform was added to each microtube (to separate protein, RNR, and DNA) and then a vortex was performed. The microtubes were placed on a shaker for 10 min and centrifuged (1200 rcf, 4 °C, 15 min) (Ghorbanian et al., 2023; Ghorbanian et al., 2011). From the three phases formed, the supernatant phase containing RNA was sep-

TABLE 1: Primers applied for qRT-PCR

primers	5'-3'	Sequence (5'-3')
Gapdh	Forward	TGTTCCTACCCCCAATGTGT
	Reverse	GGTCCTCAGTGTAGCCCAAG
IL-8	Forward	CACCTCAAGAACATCCAGAGCT
	Reverse	CAAGCAGAACTGAACTACCATCG
Foxp3	Forward	GGCCCTTCTCCAGGACAGA
	Reverse	GCTGATCATGGCTGGGTTGT
Eotaxin	Forward	CTGCTCACGGTCACTTCCTT
	Reverse	GGGGTCAGCACAGATCTCTT
Muc5ac	Forward	CAGGACTCTCTGAAATCGTACCA
	Reverse	AAGGCTCGTACCACAGGGA

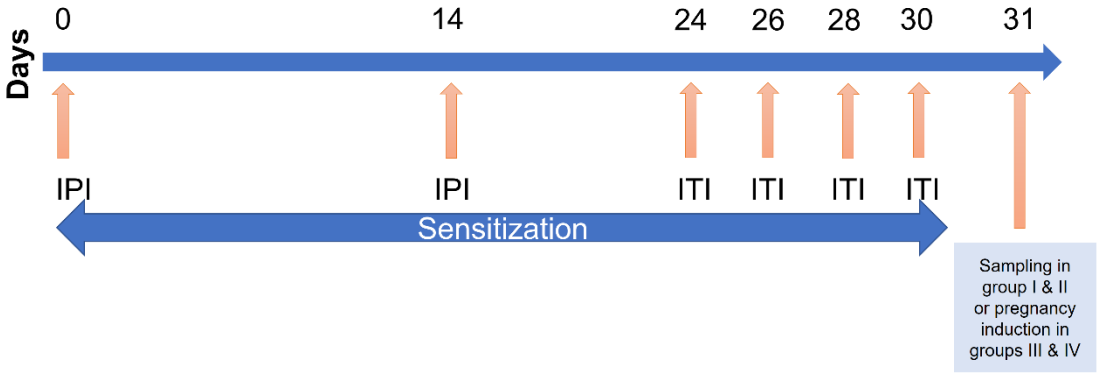

FIGURE 1. Animal sensitization by OVA. For making the asthma mice model or sensitization, OVA (20µg/100µl) was injected intra-peritoneal on days 0 and 14, and then 10 ml of 1% OVA solution was inhaled via intra-trachea on days 24, 26, 28, and 30. Finally, the sampling was process performed on day 31. (Intra-peritoneal Injection: IPI, Intra Trachea Inhaled: ITI)

arated and transferred to a new microtube free of RNA. Then 500 µl of isopropanol solution was added to the microtube with RNase free filter for every 1 ml of Trizol solution. Afterward, 1 mL of 70% ethanol was added after centrifugation (1200 rcf, 4 ˚C, 15 min) and the mixture was spun again. In 20 ml of DEPC water, an RNA plate was solved. The cDNA Synthesis Kit was then utilized to create cDNA (TaKaRa, USA). Finally, 2 µl of cDNA were mixed with 10 µl of distilled water, 0.5 µl of forward and reverse primers, 2.5 µl of SYBR green, and 0.2 µl of ROX. The comparative threshold cycle number (2-Ct) technique was used to control the level of gene expression in whole cells (Mirzaeian et al., 2023). The data related to the primers is presented in Table 1.

Statistical analyses

The results were statistically analyzed using SPSS version 26 software. Experimental groups were evaluated by the One-Way or Two-Way ANOVA test and Tukey's multiple comparison test. The corresponding graphs were drawn with Microsoft Excel software. $P<0.05$, $P<0.01$, and $P<0.001$ were regarded as significant levels.

Results

Microscopic alterations in tissue morphological features induced with OVA

Morphological screening of lung tissue for assessment of inflammation revealed no significant differences in two asthmatic groups (OVA and OVA, pregnant), whereas, there was a significant increase in asthmatic groups from two healthy groups (negative control and pregnant) ($p<0.001$) (Fig. 2A, B). In screening for uterine tissue inflammation, there was no significant differ-

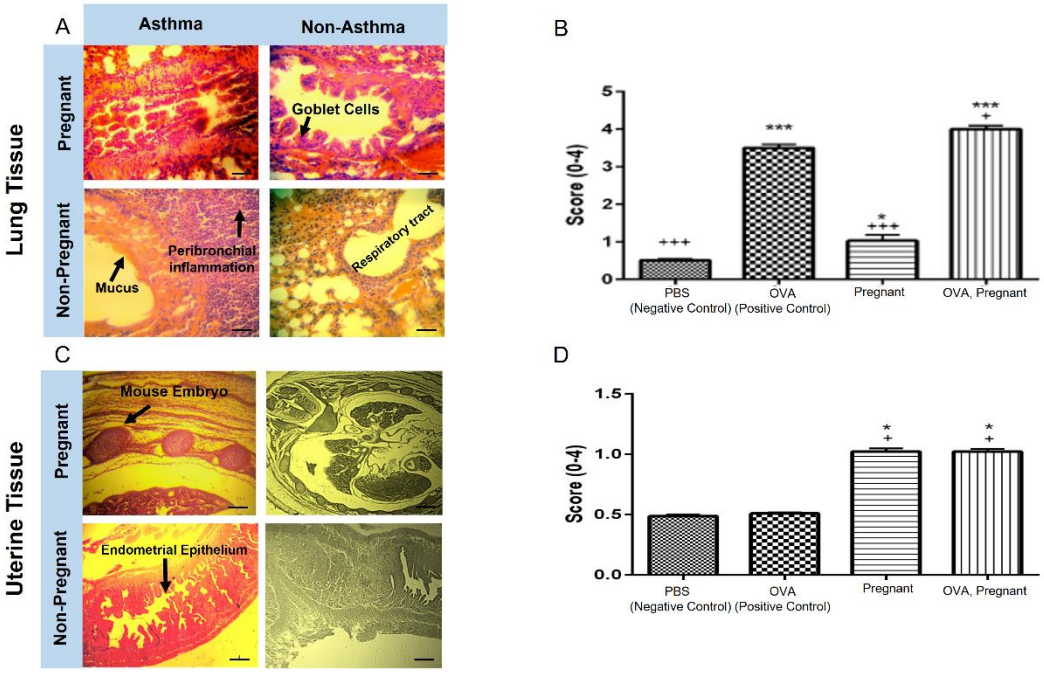

FIGURE 2. Qualitative and quantitative examination of tissue inflammation by H & E staining. A, C. Histological images of lung and uterine tissues inflammation (magnification = 400X, Scale bar = 50µm). B, D. Quantitative graphs related to lung and uterine tissue inflammation. Comparison of the healthy group and other groups (*p<0.05, **P<0.01, ***p<0.001). Comparing the asthmatic group and other groups (+p<0.05, +++p<0.001). This experiment was conducted in at least three independent statistical repeats. (n=10 mice/ group).

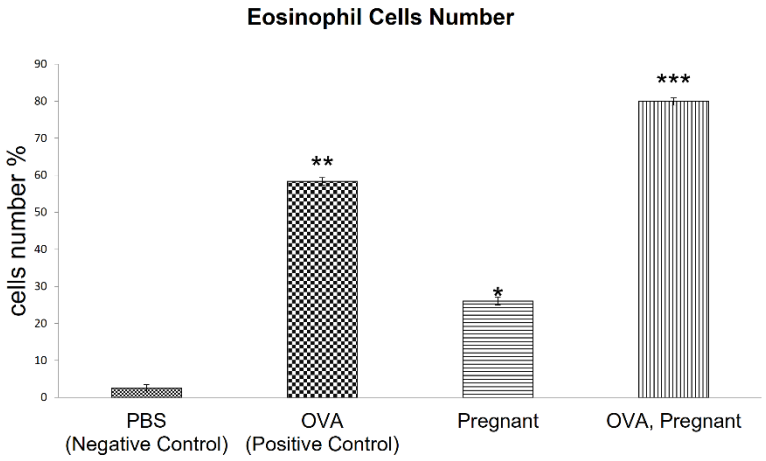

FIGURE 3. Examination of inflammatory eosinophil cell percentage. Comparative analysis of the percentage of EOSCs in the positive control, pregnant and OVA, pregnant groups compared to the negative control group (*P<0.05, **P<0.01, ***P<0.001). This experiment was conducted in at least three independent statistical repeats (n=10 mice/ group).

ence between the negative control and positive control groups, whereas a notable difference was observed in the comparison of these groups considering pregnant and OVA, pregnant (Fig. 2C, D).

Evaluation of inflammatory *eosinophil cells in lung tissue*

The results of the comparative analysis of the percentage of EOSCs in the positive control, pregnant, and OVA, pregnant groups compared to the negative control group showed that there is a significant increase in the percentage of EOSCs in experimental groups compared to the negative control group ($P<0.05$, $P<0.01$ and $P<0.001$) (Fig. 3).

Leptin Hormone Level measurement in blood serum

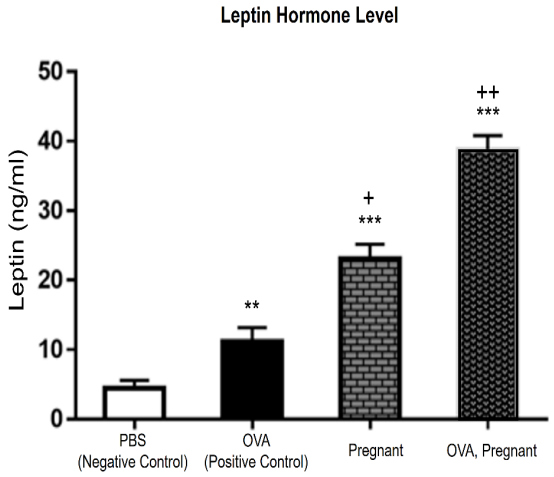

FIGURE 4. Measurement of Leptin hormone level in blood serum. Comparing the experimental groups with the negative control (*P<0.05, **P<0.01). Comparing the experimental groups with the positive control (+P<0.05, ++P<0.01). This experiment was conducted in at least three independent statistical repeats (n=10 mice/ group).

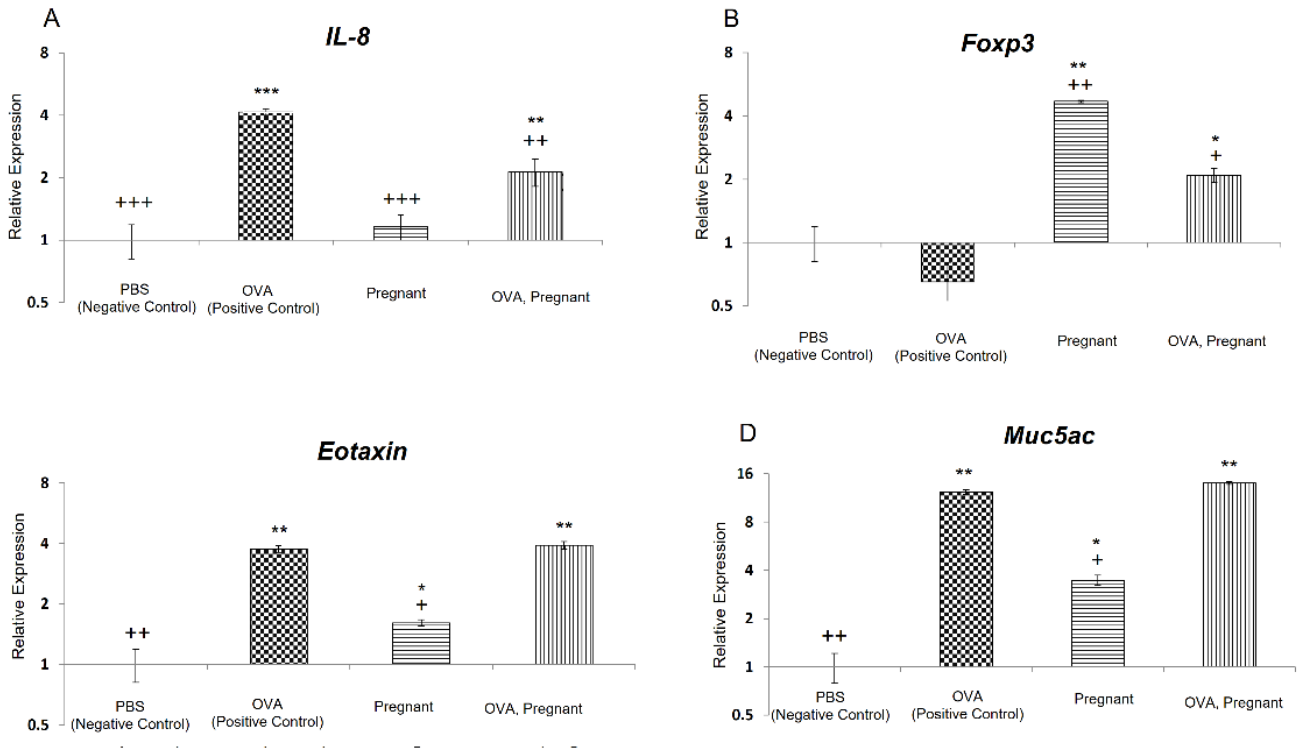

FIGURE 5. Analysis of gene expression level in lung tissue. A. IL-8 B. Foxp3 C. Eotaxin D. Muc5ac. Comparing the experimental groups with the negative control (*P<0.05, **P<0.01). Comparing the experimental groups with the positive control (+P<0.05, ++P<0.01). This experiment was conducted in at least three independent statistical repeats (n=10 mice/ group).

The results of the comparative analysis in the lung tissue showed a significant increase in the leptin hormone in three experimental groups compared to the negative control group. Also, there was a significant rise in leptin levels in pregnant groups compared to non-pregnant groups (P<0.05, P<0.01, and P<0.001) (Fig. 4).

Evaluation of inflammation gene expression in lung tissue

Evaluation of *IL-8* gene expression in lung tissues revealed an increase in the experimental groups compared to the negative control group, however, this dominance was statistically significant only in asthmatic groups.

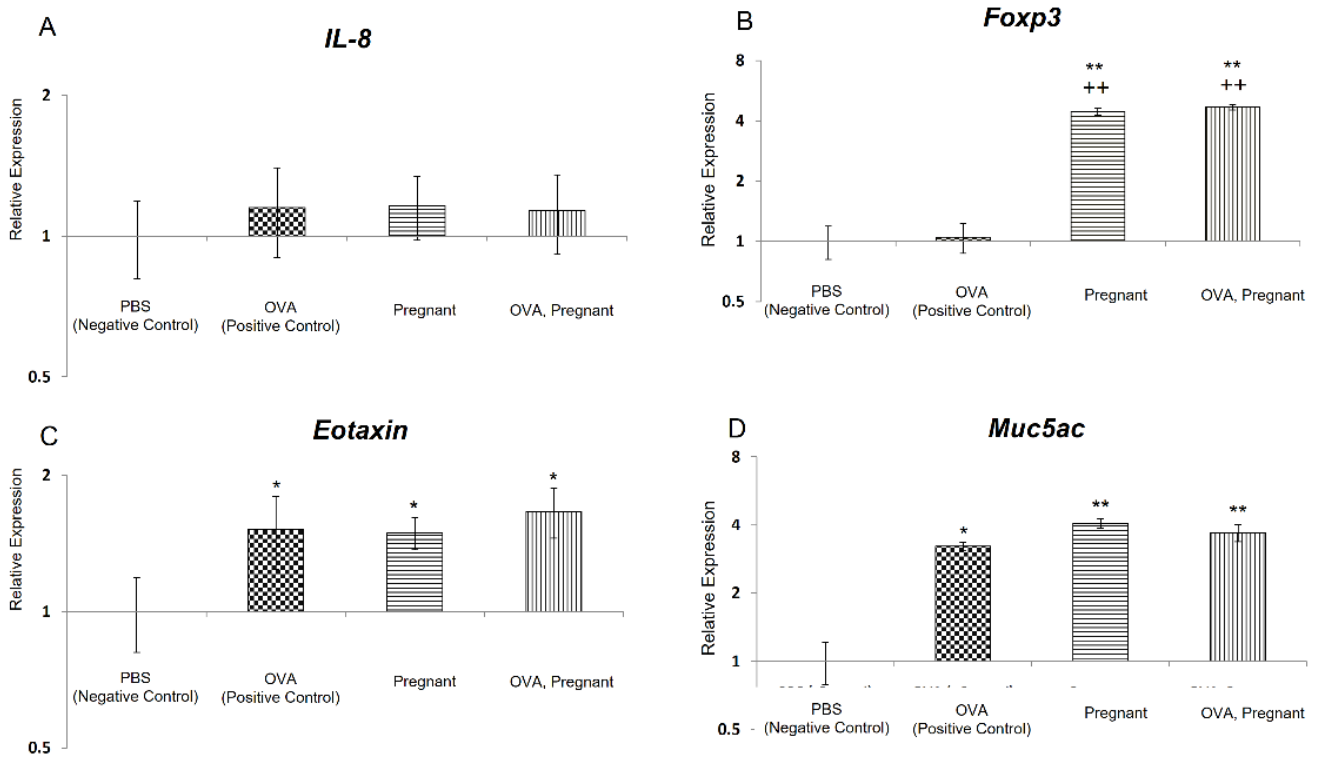

FIGURE 6. Analysis of gene expression level in uterine tissue. A. *IL-8* B. *Foxp3* C. *Eotaxin* D. *Muc5ac*. Comparing the experimental groups with the negative control (*P<0.05, **P<0.01). Comparing the experimental groups with positive control (+P<0.05, ++P<0.01). This experiment was conducted in at least three independent statistical repeats (n=10 mice/ group).

Also, in the comparison of the positive control group to the other groups a significant increase was seen in the level of *IL-8* expression (Fig. 5A). Comparing the negative control group with pregnant groups and positive control showed a significant increase and non-significant decrease, respectively in the expression of the *Foxp3* gene. In addition, in the pregnant groups compared to the positive control group, this expression was significantly increased (Fig. 5B). In experimental groups compared to the negative control group, *Eotaxin* expression improved statistically significantly. Also, asthmatic groups revealed a notable increase than non-asthmatic groups (Fig. 5C). Similarly, the expression level of *Muc5ac* experimental groups significantly increased compared to the negative control group. Additionally, the asthmatic groups revealed a notable increase compared to the non-asthmatic groups (Fig. 5D).

Evaluation of inflammation gene expression in uterine tissue

In uterine tissue, the level of *IL-8* gene expression in the experimental groups increased compared to the negative control group, but this increase was not significant (Fig. 6A). On the other hand, expression of the *Foxp3* gene in pregnant groups significantly increased relative to control groups (Fig. 6B). Nonetheless, none of the differences between the pregnant vs. OVA, pregnant and negative control vs. positive control groups were statistically significant. Furthermore, evaluation of the *Eotaxin* gene indicated that the experimental groups were significantly greater than the negative control group, but no statistically significant differences were observed between the experimental groups (Fig. 6C). Finally, the *Muc5ac* expression assessment found a significant increase from the experimental groups relative to the negative control group, (Fig. 6D).

Discussion

Asthma is an inflammatory disease that causes an immune response to antigens by the production of pro-inflammatory cytokines, and activation of B cells and mast cells in airway tract (Dantzer 2004). In this study, to induce asthma in mice, the OVA was injected intra-peritoneally and then inhaled at specific time intervals. Serra et al used OVA to persuade asthma in Balb/c mice (Serra et al., 2019).

In our examination, it was found that in the OVA, Pregnant group, the percentage of EOSCs significantly increased compared to the OVA group (Fig. 3). Therefore, we confirmed that the percentage of EOSCs increases in asthma state. Much research regarding the increase in the percentage of EOSCs in asthmatic mice is in line with the results of this research. Research conducted by Kaveh et al. which supports our finding, observed that the percentage of EOSCs in asthmatic rats increased significantly compared to the control group (Kave H. et al., 2013). Also, Chan et al. displayed a significant increase in EOSCs in patients affected by asthma compared to healthy persons (Chan and Lipworth 2023).

Another important marker in the immune system is the leptin hormone of the cytokine family. Our findings for serum leptin hormone indicated an increase significantly in the OVA, Pregnant group compared to the OVA group (Fig. 4). Sood et al. showed that increased serum leptin hormone was associated with asthma (Sood et al., 2006). It is believed that leptin plays an important role in the uterus, embryonic, and postnatal lung development in mice. In addition, some data suggest that leptin concentrations increase dramatically during inflammation, which in turn increases inflammation (Sumayya et al., 2021). The findings of Stephanie et al. on the mice model indicated that serum leptin levels rise during asthma (Shore et al., 2005). In support of this topic, experiments showed leptin levels are rapidly increased in inflammatory conditions by the pro-inflammatory cytokines such as tumor necrosis factor (TNFα), IL1, IL1β and IL6. Then leptin regulates the production of these cytokines and causes a pro-inflammatory state and phagocytosis by macrophages is also increased. Injection of lipopolysaccharide, which is commonly used for examination of systemic inflammation, leads to an increase in plasma leptin.

Several studies demonstrated that lymphoid cells, including EOSCs, increase in blood, during asthma. Winkler et al. demonstrated that in the allergic inflammatory regions of the lung, there is an accumulation of EOSCs which is migrated from the blood to the site of inflammation, and these cells increase the expression and release IL-5, 8, 13 for the induction of inflammation (Winkler et al., 2019). since there was a direct relationship between the increase of EOSCs and elevation of IL-8 expression in the lung in OVA, pregnant group, our result is confirmed (Fig. 5A). Also, as we detected in the histological analysis in Figure 2 the groups which have increased in the percentage of EOSCs had more severe inflammation and damage, could be due to the presence of EOSCs.

Janulaityte et al. described the role of allergic EOSCs in pulmonary cells following asthma development. They reported that EOSCs change the structure of respiratory cells in the lung by inflammatory mediators. The findings of this research show that EOSCs in the lung tissue of asthmatic patients play a major role in aggravating the disease and causing damage to lung cells (Janulaityte et al., 2019). On the other hand, Yuliani et al. revealed that IL-8 is overexpressed in the lung tissue of OVA-induced asthmatic mice (Yuliani et al., 2022). We also observed in this research that the level of expression of *IL-8* in the OVA group increased significantly in expression compared to the OVA, Pregnant group and negative control. Also, Groot et al. demonstrated that EOSCs induced oxidative stress in asthma. This research indicates that EOSCs cause releasing of reactive oxygen species (ROS), impairment of lung tissue, and disruption of the balance of antioxidants (de Groot et al., 2019; Mirzaeiyan et al., 2014).

The expression level of the *Foxp3* gene in the OVA, pregnant group was found to be higher than the OVA group and negative control (Fig 5B). Provost et al. consistent with the results of our study have reported that patients with asthma have decreased Foxp3 protein expression in T regulatory cells (Provoost et al., 2009). Therefore the use of drugs that increase *Foxp3* expression, such as glucocorticosteroids, increases the activity of T regulatory cells. In a research performed by Polanczyk et al. an increase in *Foxp3* expression and T regulatory cell function were observed in pregnant mice compared to non-pregnant mice (Polanczyk et al., 2005). Therefore, it can be concluded that the increased expression level of *Foxp3* increases the tolerance of the mother to the embryo.

According to the findings of Bejeshk et al. there has been a significant increase in the expression level of *Eotaxin* in asthmatic rats (Bejeshk et al., 2022). In this research, we observed that the level of expression of *Eotaxin* in experimental groups has increased significantly compared to negative control (Fig. 5, 6C). On the other hand, there was no observed significant difference in the OVA, Pregnant group compared to the OVA group. Mattoli et al. also showed that *Eotaxin* expression is

increased in asthma and induces EOSCs contribution (Mattoli et al., 1997). Because Eotaxin induces EOSCs to be secreted at the site of inflammation, as a result, the direct relation between the quantity of *Eotaxin* gene and EOSCs may make sense (Fig. 3 and Fig. 5C). Also, Robertson et al. showed that during the estrous cycle and pregnancy in mice, the level of expression of *Eotaxin* gene increases and causes the inflammatory cells to recruit to the uterine tissue, which the estrous phase in mice is characterized by the presence of mononuclear white blood cells (Robertson et al., 1998).

Moreover, our study showed that the expression of the *Muc5ac* gene also increased significantly in the lung tissue in asthmatic groups (Fig. 5D). But was not observed significant difference in asthmatic pregnant compared to asthmatic non-pregnant mice. Similar findings by Evans et al. reported mucin to be more secreted in the lungs of asthmatic patients, indicating an increase in its expression (Evans et al., 2009). Also, it has been reported by Nguyen et al. that asthmatic mice demonstrated an increase in *Muc5ac* gene expression that leads to an increase in inflammation of the lung tissue (Nguyen et al., 2008). Several studies have confirmed that synthesis and secretion of the *Muc5ac* gene in uterine tissue increases at the early gestational stage (Braga and Gendler 1993). Furthermore, Xu et al. revealed that gene expression in the mucin synthesis pathway increases in the uterine tissue of pregnant mice (Xu et al., 2012). Our finding also revealed that *Muc5ac* gene expression increased significantly in the pregnant group compared to the negative control (Fig. 5, 6D)

Overall, it appears that during pregnancy in asthmatic mice, inflammatory factors such as IL-8, Eotaxin, and Muc5ac are synthesized and secreted relatively more in lung and uterine tissues. Accordingly, the anti-asthmatic medicines that inhibit the synthesis or activation of these factors can prevent or minimize the complications of this disease. Also, Foxp3 can activate T regulatory cells which leads to a reduction in inflammatory cells and inflammation.

However, numerous limitations in our research should be considered for future investigations in asthmatic patients. First, the mortality rate was high when mice were made asthmatic. Next, we just examined four markers and did not assess the expression level of other inflammatory markers. It would be much better if the inflammatory factors were also investigated at the protein level. Another limitation of this project is the lack of investigation of leptin pathways and targeting leptin or its receptors. Lastly, we also could evaluate all the factors after asthma treatment. For example, adipose-derived mesenchymal stem cells (Ramezani et al., 2023) can be used in the treatment of asthma. All these limitations should be addressed in future investigations.

Conclusion

In this research, it was observed that leptin hormone increased significantly in asthmatic pregnant mice compared to asthmatic non-pregnant mice, which is in line with the expression levels of inflammatory genes in lung tissue. Therefore, the use of drugs that inhibit the expression of studied inflammatory factors will reduce the complications of the disease. On the other hand, EOSCs have shown a significant increase in asthmatic pregnant mice.

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Statement of Ethics

All experiments of this study were permitted by the ethical committee of the Science Faculty at the Islamic Azad University Science and Research Branch (Ethical code: 176947).

Conflict of Interest Statement

The authors declare no conflicts of interest relevant to this research.

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Author Contributions Statement

G.B.O.; Investigation, Data Curation. L.M.; Writing-Original draft preparation, Resources. S.M.H.; Formal analysis, Validation. M.S.; Software. P.Y.; Methodology. S.F.G.; Visualization. P.M.; Reviewing and Editing. E.E.; and S.S.A.; Supervision, Conceptualization, Project administration. All authors have edited and approved the final draft of this manuscript.

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