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Effect of Asthma on Erectile Dysfunction in Rats as Determined by Biological Network Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: We explored the effect of asthma on erectile dysfunction (ED) and the effects of the expression of related proteins.

Material/Methods: We used a bioinformatics database to predict the targets and pathways associated with asthma and ED. The rat model of asthma was caused by an ovalbumin solution. The number of erections in 30 min was observed by injecting apomorphine into the neck at a dose of 100 μg/kg. Rats with no erection were regarded as the model group (group B), and the previous random 6 normal rats were regarded as the control group (group A). We used hematoxylin and eosin (HE) to compare the tissue structure of the cavernous body of the penis. Real-time quantitative polymerase chain reaction (RT-qPCR) and western blotting were used to determine the expression levels of insulin (INS), interleukin 6 (IL6), albumin (ALB), tumor necrosis factor (TNF), and vascular endothelial growth factor A (VEGFA) at both the protein and messenger ribonucleic acid (mRNA) levels.

Results: HE staining results show that compared with group A, the blood sinus distribution of the cavernous body in group B was disordered, and the density of endothelial cells and smooth muscle cells decreased significantly. Western blotting and RT-qPCR showed that the levels of IL6, TNF, and VEGFA protein and mRNA in group B were significantly higher ($P<0.05$) than those in group A. The levels of INS and ALB were not significantly different between the 2 groups.

Conclusions: On the basis of the results, we found that asthma caused pathological changes in the penises of rats and led to reduced erectile function via changes in the expression of IL6, TNF, and VEGFA proteins.

MeSH Keywords: Asthma • Biological Assay • Erectile Dysfunction • Models, Biological

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Background

Erectile dysfunction (ED) refers to the inability of a male to achieve or maintain a complete erection that is sufficient to achieve satisfactory sexual intercourse [1]. Globally, ED affects approximately 53% of men between 40 and 70 years of age and currently affects around 150 million people [2]. Asthma is a chronic airway inflammatory disease that involves a variety of cells and cellular components [3]. The global prevalence of asthma ranges from 1% to 18% and it is currently estimated that there are approximately 300 million asthma patients worldwide [4]. A 2011 epidemiological survey carried out in Taiwan showed that the prevalence of ED in asthmatic patients (0.98%) was higher than that in nonasthmatic patients and that the severity of ED was positively correlated with the severity of asthma [5]. Another study showed that subjects with asthma experienced a 1.909-fold (95% confidence interval 1.276–2.856; P=0.002) increase in incident ED independent of age, the number of clinical visits to a urologist, and other comorbidities [6]. However, the specific mechanism underlying the relationship between asthma and ED remains unclear. At the same time, we found through a literature search that although there are related reports on the association between asthma and ED, most of them are based on population studies and epidemiological surveys. Therefore, the aim of the present study was to use bioinformatic analysis to predict the molecular pathways and targets of asthma and ED, and then verify them through *in vitro* experiments. Our working hypothesis was that asthma exerts effects over related proteins to induce systemic inflammation and extensive vascular endothelial damage, which then leads to ED.

Material and Methods

Ethical approval of the study protocol

The experimental protocol was approved by the Experimental Animal Ethics Committee of Beijing University of Chinese Medicine (number: BUCM-4-2019091105-3064).

Experimental animals

Twenty Sprague-Dawley rats (specific pathogen free [SPF], ages 4–5 weeks, weight range 120–130 g) were purchased from Speyford (Beijing) Biotechnology Co., Ltd. (license number SCXK [Beijing] 2016-0002). Rats were maintained in an SPF-level animal room at Beijing University of Chinese Medicine; the photoperiod was 12 h of illumination and 12 h of dark; humidity was 55–60%; temperature was 22–26°C. All rats were acclimatized in these conditions for 7 days with deionized water and solid feed. Before experiments, we confirmed that the rats exhibited normal sexual function by a mating behavior test and a noncontact penile erection test [7].

Drugs and reagents

Ovalbumin (OVA) was purchased from Sigma (A5503-10G, SJ, CN) and aluminum hydroxide gel was obtained from Pierce (no.77161, SJ, CN). Apomorphine (APO) was purchased from Sinopharm Group Chemical Reagent Co., Ltd. (A4393, SJ, CN). All primers were acquired from Invitrogen Biotechnology Co., Ltd (SJ, CN). We also purchased a range of assay kits from Bioss (Woburn, MA, USA), including an insulin gene (INS) kit (bs-0862R), tumor necrosis factor (TNF) kit (Bs-10802R), interleukin 6 (IL6) kit (bs-0379R), albumin gene (ALB) kit (bs-0945R), vascular endothelial growth factor A gene (VEGFA) kit (Bs-20393R), and a β-actin kit (bs-0061R).

Identification of molecular targets

We identified the molecular targets of asthma and ED via the GeneCards database (*https://www.genecards.org/*) and the OMIM database (*https://omim.org/*). The separate targets of asthma and ED were then intersected and considered as potential targets for asthma and the development of ED. We used these targets for further network construction and analysis.

Protein–protein interaction (PPI) network analysis

The STRING database (*https://string-db.org/*) was used to identify potential PPIs. To improve the reliability of the data obtained, the PPIs were further filtered, the minimum interaction score was set to 0.40, and the remaining PPIs were used for network construction and analysis [8].

Network construction and analysis

Cytoscape software (version 3.7.1, *https://cytoscape.org/*) was used to construct an asthma-ED-target network and a PPI network. The Cytoscape plug-in 'cytohubba' was then used to further analyze the PPI network to identify essential targets.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The biological information annotation database (DAVID, *https://david.ncifcrf.gov/*, version 6.8) provides systematic and comprehensive biological annotation of function for genes or proteins on a large scale and can be used to identify the most significantly enriched biological annotations. We imported the most common targets for asthma and ED into the DAVID database, set the 'identifier' as 'official gene symbol' set the 'type list' to 'gene list' and limited the 'species setting' to 'human.' We then performed GO and KEGG pathway analyses for the common targets of asthma and ED [9].

Construction and verification of a rat model of asthma

All 20 rats were allocated a number and their individual weights were recorded. A random number table was then used to randomly select 14 rats for asthma modeling (the asthma modeling group), and the remaining 6 were considered the normal group. In the first 3 weeks of the experiment, the normal group of rats was injected (intraperitoneally) with 1 mL of sterile saline every day; on days 0, 7, and 14 the asthma modeling group was sensitized by injecting 1 mL of miscible liquid (intraperitoneally) containing 100 mg of OVA and 10 mg of aluminum hydroxide gel. From the 22nd day of the experiment, the normal group was given sterile saline through a compression atomizer (NE-C900, DL, CN) at a rate of 0.2 mL/min for 30 min; the asthma modeling group was given 1% OVA (atomized) at a rate of 0.2 mL/min over the course of 30 min; the 2 groups received this treatment once a day for 3 weeks. After 3 weeks, we evaluated the asthma modeling group in terms of behavior, the presence of upright fur, shortness of breath, nose scratching, incontinence, and abdominal breathing, as described previously [10].

Screening of the ED model

Rats in the asthmatic model group were weighed and placed in a quiet observation box for 10 min with dim lighting. Each rat then received a subcutaneous injection of APO into the neck at a dose of 100 μg/kg. The status of each rat was recorded by video camera for 30 min after the injection to assess whether erection occurred. Erection was defined as the protrusion, swelling, or growth of the penis [11]. Rats that did not experience erection were considered to be experiencing asthma-induced ED and were included in the model group. Among 14 rats in the asthmatic model group, a total of 6 rats failed to achieve an erection and was identified as model rats with asthma-induced ED (group B); the previous 6 normal group rats were regarded as the control group (group A).

Staining using hematoxylin and eosin (HE)

All rats in groups A and B were weighed and anesthetized by intraperitoneal injection with 3% pentobarbital sodium solution (0.1 mL/100 g). After the pain reaction of the rat disappeared, the ventral foreskin of the penis was cut with tissue scissors, the penis was separated, the glans (including penile cartilage) was removed, and 50-100 mg of the middle penile tissue was cut for subsequent staining and observation. After collecting and washing all the penile tissue of rats in groups A and B, a section from each penis was embedded in paraffin, cut into thin slices (5 μm thick), and stained with HE. Each specimen was then observed and photographed using an optical microscope (L340099, Olympus, TKY, Japan) equipped with a digital camera [12].

Western blotting detection

To detect the expression levels of INS, ALB, TNF, IL6, and VEGFA proteins, we removed a portion of cavernous tissue from each rat. These tissues were placed into ice-cold tissue lysis buffer and homogenized. The homogenate was then centrifuged at 12,000 revolutions per minute for 10 min to allow collection of the supernatant. Protein concentration in each lysate was determined using a bicinchoninic acid protein assay kit and the concentrations adjusted with radioimmunoprecipitation assay buffer and boiled for 5 min. Each sample was then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membrane. We diluted the primary antibody, incubated at 4°C overnight, and washed with Tris-buffered saline with Tween (TBST) at room temperature on a decolorizing shaker 3 times for 5 min each time. Then the secondary antibody was diluted 1: 3000 with TBST. After incubating at room temperature for 30 min, the PVDF membrane was washed 3 times with TBST on a decolorizing shaker at room temperature for 5 min each time. The 2 electrochemiluminescence reagents A and B were mixed in a medium volume in a centrifuge tube, and after fully contacting the PVDF membrane, they were placed in a fully automated chemiluminescence imaging system to read the data [13].

Real-time quantitative polymerase chain reaction (RT-qPCR) detection

A PCR instrument (SLAN-96P, SJ, CN) was used for RT-qPCR. When extracting total ribonucleic acid (RNA), TRIzol reagent was added to homogenized tissue to maintain the integrity of RNA in the sample and to inhibit RNA degradation. The purity and concentration of total RNA was determined, and complementary deoxyribonucleic acid prepared by reverse transcription. The primer sequences are shown in Table 1. After adding samples to 96-well plates, fluorescent qPCR was undertaken for amplification. The number of cycles (Ct value) experienced by the fluorescence signals in each reaction tube when it reached the set threshold was recorded. The difference in gene expression was determined by the $2^{-Ct\Delta\Delta}$ method [14].

Statistical analysis

All analyses will be performed using SPSS software (version 26.0, Armonk, NY, USA), with a two-sided P value <0.05 considered significant. Continuous variables will be compared using the t test or Wilcoxon rank-sum test as appropriate. Since the indicators we collect are all measurement data, the experiment is randomly grouped. After testing, the normality test was $P>0.05$ and the variance homogeneity test $P>0.05$, so the independent sample t test was used.

Table 1. Specific primer information. Primers were purchased from Invitrogen Biotechnology Co. Ltd. (SJ, CN) and tested by Beijing Huaying Biotechnology Research Institute.

GeneBank	Primer name	Primer sequence (5'–3')	Fragment length (bp)	Annealing temperature (°C)
NM 031144.3	β-Actin 1F	CCTCACTGTCCACCTTCCA	120	66.0
	β-Actin 1R	GGGTGTAAAACGCAGCTCA		65.0
XM 021075147.1	INS 1F	TCTGTCAACTTTGCCGACT	114	64.3
	INS 1R	AACCGTGTCTTCGTCCAG		64.4
XM 021075147.1	IL6 1F	CTCACGCACCGATGTCT	109	64.2
	IL6 1R	AGGCTGTGGGCTCAATC		64.3
NM 134326.2	ALB 1F	GCTGCTGACTTTGTTGAGG	108	64
	ALB 1R	GTAATCGGGGTGCCTTCT		63.9
NM 012675.3	TNF 1F	CAGCCAGGAGGGAGAAC	93	63.9
	TNF 1R	GTATGAGAGGGACGGAACC		63.9
NM 00110333.2	VEGFA 1F	CATCAAGCTCTCTCCTCCA	91	63.2
	VEGFA 1R	GGCCTCTTCTTCCACCA		63.5

Results

Collection of targets

The flow chart for our study is shown as Figure 1. Genecard screening allowed us to identify 500 asthma targets and 500 ED targets. After intersection, 103 targets were identified that related both asthma and ED (Figure 2).

Construction and topological analysis of the asthma-ED network and the PPI network

The 103 overlapping targets for asthma and ED were analyzed by the STRING database. Cytoscape software was used to construct a PPI network for the 103 targets. The 'cytohubba' plug-in was then used to analyze the PPI network and identified the top 5 degrees as key targets: INS (ranked 1), IL6 (ranked 2), ALB (ranked 3), TNF (ranked 4), and VEGFA (ranked 5) (Figure 3, Table 2). Cytoscape software was then used to construct an asthma-ED network (Figure 4).

GO biological process enrichment analysis

Enrichment analysis of the 103 targets using DAVID version 6.8 further indicated the involvement of 157 cell biological processes, 12 cell components, 20 molecular functions, and 52 signaling pathways. The top 5 biological functions and signal pathways were then selected on the basis of P values (Figures 5, 6).

Verification of the asthma model

Rats in the asthma model group showed evidence of 6 different types of symptoms. They exhibited anxiety, upright fur, shortness of breath, obvious abdominal breathing, nose scratching, and incontinence; none of the control rats exhibited any symptoms. Twelve rats that had developed asthma were then used to investigate the ability to achieve erection (Table 3).

Erection analysis

Rats in group A (controls) achieved a mean of 2±0.82 erections. In contrast, no erections were observed in group B. Consequently, there was a statistically significant difference between groups A and B with respect to the number of erections achieved during the experimental period (P<0.05, Table 4).

Structural analysis of the corpus cavernosum

HE staining showed evidence of spongy trabeculae and an even distribution of blood sinus in the corpus cavernosum of rats in group A, with some red blood cells evident in the sinus cavity. The trabecular sinus contained a large amount of smooth muscle and some small blood vessels; there was no proliferation of the interstitial tissue. In contrast, the blood sinus distribution within the corpus cavernosum of rats from group B was disordered and there was a reduction in the density of endothelial cells and smooth muscle cells. The number of collagen fibers of group B was also increased compared with rats in group A (Figure 7).
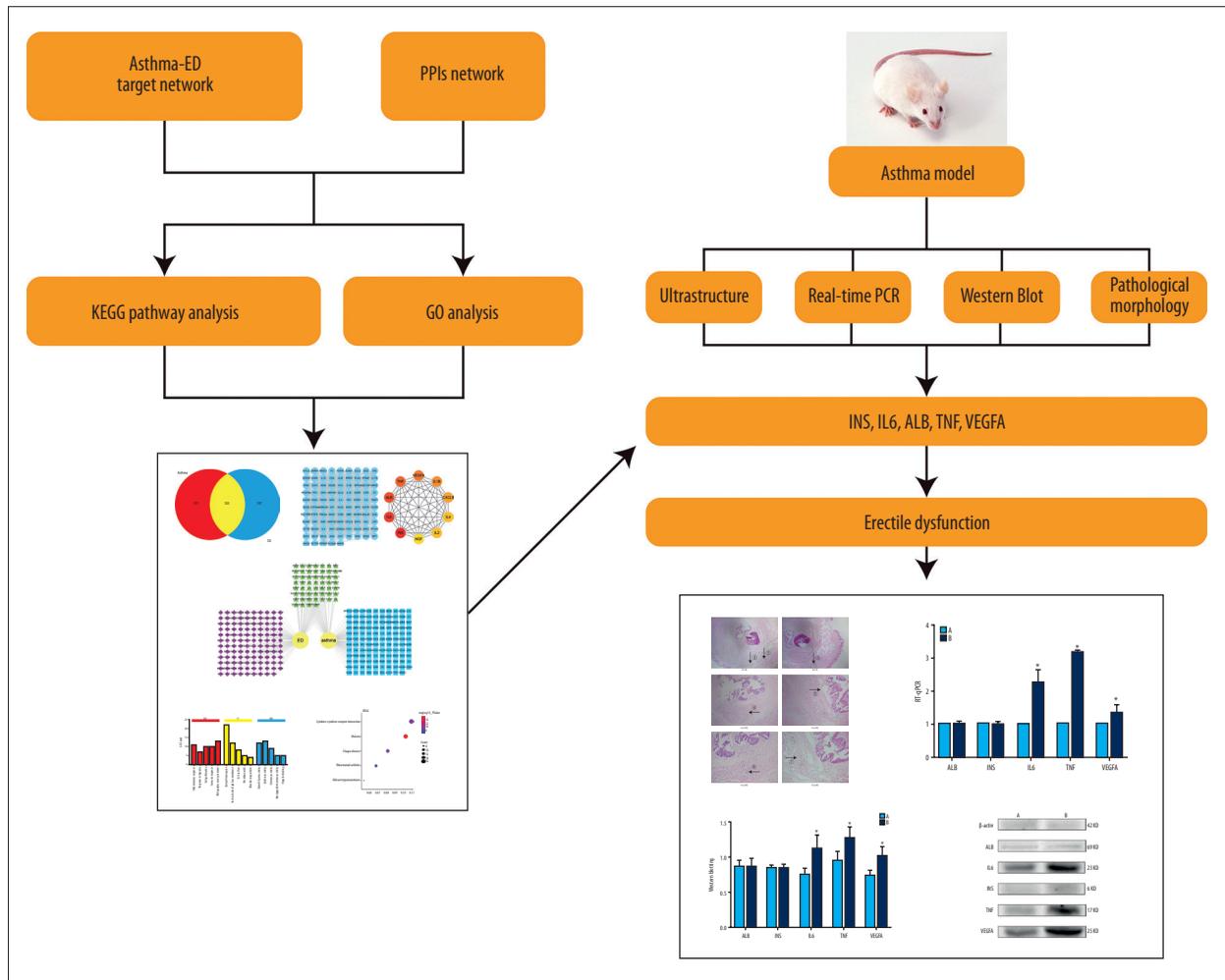
Figure 1. Study flow chart. On the basis of the DAVID database, we predicted the proteins and pathways associated with asthma and erectile dysfunction (ED). We used real-time quantitative polymerase chain reaction and western blotting to measure expression of insulin, albumin, vascular endothelial growth factor A, tumor necrosis factor, and interleukin 6 proteins and messenger ribonucleic acid in rat penis cells of each group. The results of *in vivo* experiments were consistent with the prediction results of bioinformatics analysis. Asthma can induce systemic inflammation and extensive vascular endothelial damage, which then leads to ED.
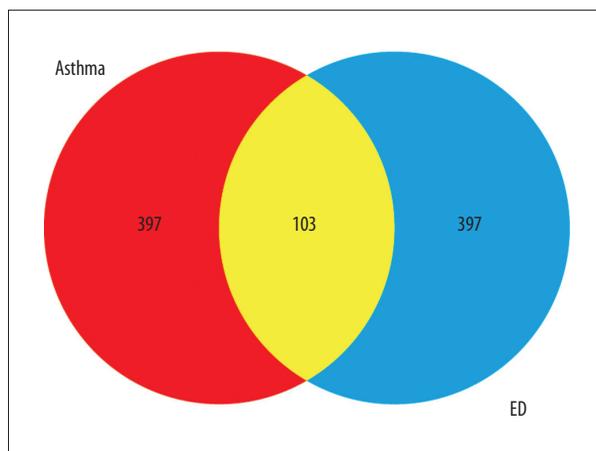

Figure 2. Venn diagram: intersection of targets for asthma and targets for erectile dysfunction (ED). On the basis of DAVID database, we predicted the intersection of targets for ED and targets for asthma. Individual targets for ED are shown as a blue circle. Individual targets for asthma are shown as a red circle. The intersection of these targets is shown as a yellow circle.

Expression of INS, ALB, TNF, IL6, and VEGFA proteins, as determined by Western blotting

Compared with group A, the levels of IL6, TNF, and VEGFA proteins were significantly increased in group B ($P<0.05$). There were also significant differences in the levels of INS and ALB protein when comparing the 2 groups (Figure 8).
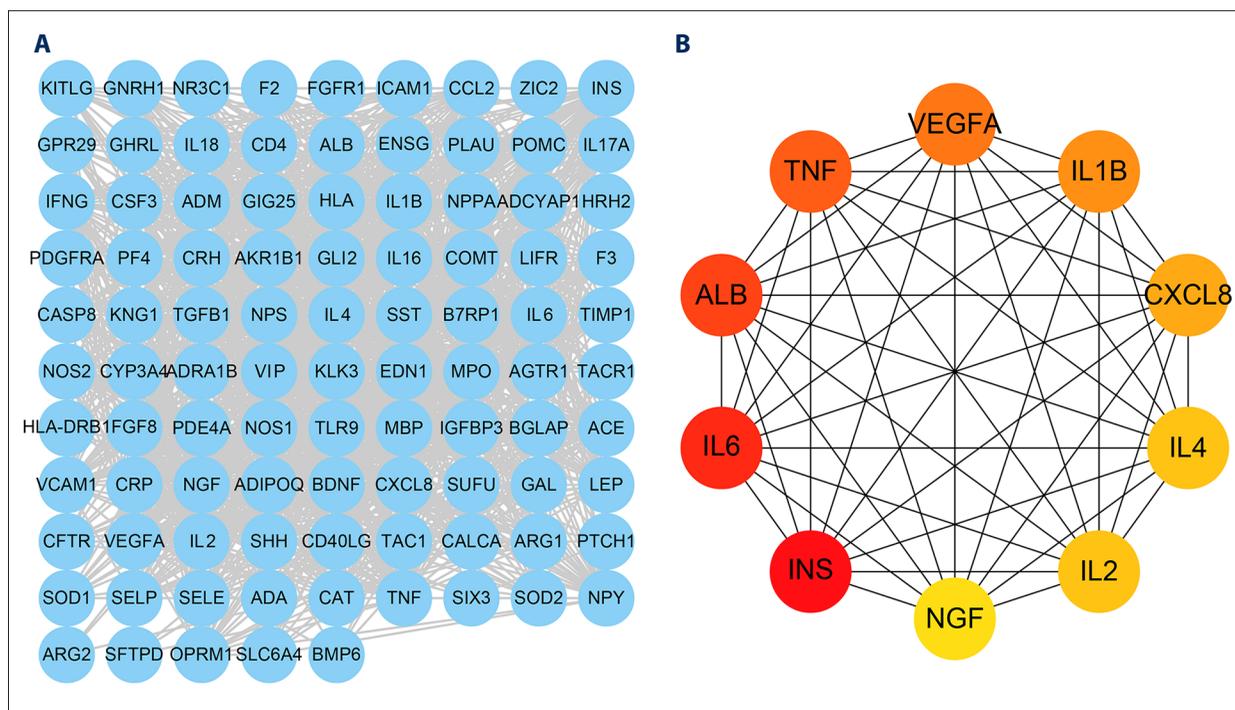
Figure 3. Protein–protein interaction (PPI) network. (**A**) PPI network built by Cytoscape v3.7.1. (**B**) PPI network processed by a Cytoscape v3.7.1 plug-in called cytoHubba. The node color is proportional to the degree of PPI.

Table 2. Information relating to the 5 key target genes, as determined by the cytohubba plug-in in Cytoscape software.

Rank	Gene name	Score (degree)
1	IL6	79
2	INS	75
3	ALB	72
4	TNF	71
5	VEGFA	65

Expression of INS, ALB, TNF, IL6, and VEGFA messenger (m) RNA, as determined by qPCR

Compared with group A, the levels of IL6, TNF, and VEGFA mRNA were significantly increased in group B ($P<0.05$). There were no significant differences in the levels of INS and ALB mRNA when comparing the 2 groups (Figure 9).

Discussion

In this study, we combined data from bioinformatics analysis and animal experiments to test a hypothesis and identify a specific mechanism for the association between asthma and ED for future research. Our experimental results are consistent with our original hypothesis, although the specific mechanism underlying the relationship between asthma and ED remains unclear. To the best of our knowledge, this is the first study to demonstrate a link between asthma and ED using an experimental rat model.

Increased levels of the TNF cytokine over an extended period of time in the plasma of asthmatic rats could act as a ubiquitous inflammatory factor. The role of TNF has already been demonstrated in a range of pathophysiological mechanisms associated with asthma, including inflammation and responsiveness of the airways [15]. Previous studies have found that the expression levels of TNF in the peripheral blood of patients with asthma are higher than those without asthma and that the expression levels of TNF are positively correlated with the severity of asthma. TNF is also known to directly inhibit the expression of endogenous nitric oxide synthase (eNOS) mRNA and thereby reduce the activity of the eNOS protein [16]. TNF can also inhibit the activity of eNOS by blocking the degradation of asymmetric dimethylarginine, thus reducing the production of NO [17]. Researchers have also found that TNF-α knockout rats show increased expression levels of neuronal NOS (nNOS) in cavernous tissues, thus indicating that TNF-α suppresses the expression of nNOS in cavernous tissues; this mechanism will reduce the level of NO, the main messenger induced by penile erection, thus resulting in ED. The expression and activity of eNOS and nNOS both play an essential role in regulating penile erection [18].
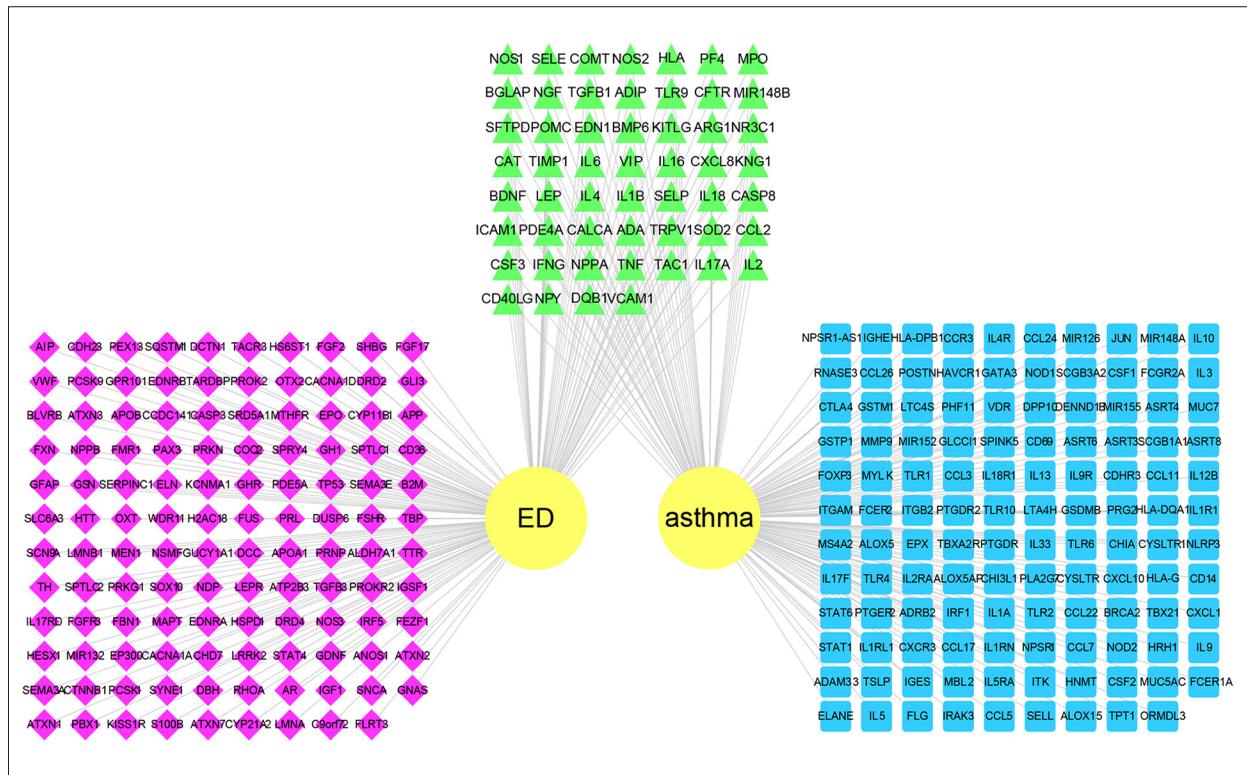
Figure 4. Network construction for asthma-erectile dysfunction (ED). Asthma (yellow circle)-ED (yellow circle)-asthma individual targets (blue square)-ED individual targets (purple diamond)-intersecting targets (green triangle).
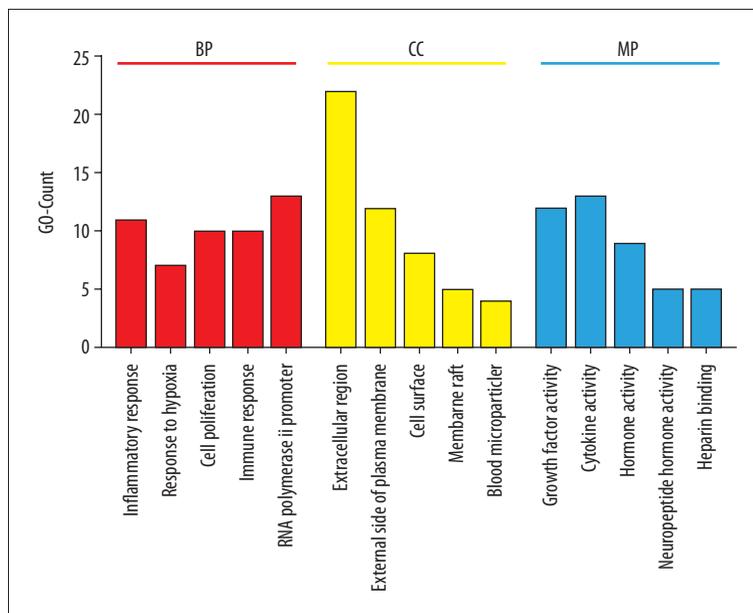

Figure 5. Gene ontology enrichment analysis. The *x* axis is the top 5 results of biological processes (BP), cell components (CC), and molecular function (MF) *P* values. The *y* axis is the number of enriched targets (count). The red bar represents BP, the yellow bar represents CC, and the blue bar represents MF.

In addition, IL6 is a versatile cytokine that participates in immune responses and inflammatory processes and is known to promote the proliferation and differentiation of various cells; it can also induce T cells to express IL2 receptors. Both immune and inflammatory responses will also have an influence on erectile function [19]. Under pathological conditions, high expression of VEGF binds to its receptors, which can cause structural changes in the extravascular matrix and interstitial edema, induce airway remodeling, and aggravate the symptoms of asthma [20]. At the same time, VEGFA overexpression can increase vascular permeability, aggravate the inflammatory response, and further aggravate the occurrence of ED [21].
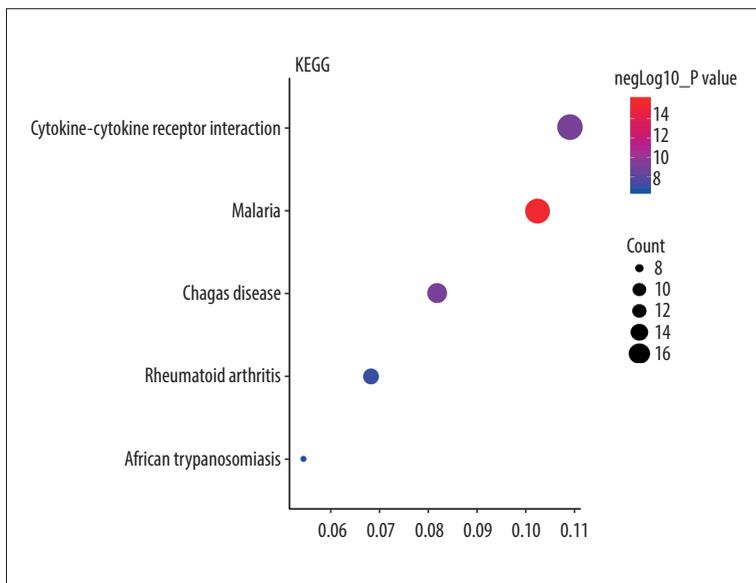
Figure 6. Kyoto Encyclopedia of Genes and Genomes enrichment analysis. *y* axis is the name. *x* axis is the richness factor. The size of the node is proportional to the number of genes. The node color is proportional to the *P* value.

Table 3. Observation index. + Denotes the degree: the more +, the greater the degree.

	Number	Anxiety	Upright fur	Shortness of breath	Obvious abdominal breathing	Nose scratching	Incontinence
Group A	6	–	–	–	–	–	–
Group B	6	++	++	+++	+++	+++	++

Table 4. Number of erections ($x \pm s$, $n=6$) of rats in each group. Number of erections in 30 min was observed by injecting apomorphine (APO) into the neck at a dose of 100 µg/kg. Group B was compared with group A, * $P < 0.05$.

Group	Number of erections
A	2±0.82
B	0*

Of interest, the levels of INS and ALB proteins obtained from our experiments are not consistent with our predictions. Western blot showed that there were no significant differences in the expression of INS and ALB protein levels when groups A and B were compared. However, previous research has shown that INS is related to lung function and bronchial hyperresponsiveness and can regulate endocrine function by lowering blood sugar, thus improving ED [22]. ALB is also regarded as a diagnostic indicator for the damage to glomerular and renal tubular function caused by asthma attacks [23]. Therefore, we believe that the observed inconsistencies with regard to INS and ALB may be related to different modeling methods.

In summary, we found that asthma can cause ED in rats via a mechanism that involves TNF, IL6, and VEGFA proteins. Only a small number of reports has been published that relate to the mechanisms underlying the association between asthma and ED. Furthermore, it is evident from the literature that treatment options for patients with this condition are not sufficient. Our current research combined bioinformatics and experimental research in an innovative manner such that we could provide an initial explanation of the mechanisms underlying ED in asthmatics. Our findings will be useful in developing new treatments.

Our work had some limitations that need to be considered. For example, our predictions did not include indicators related to sex hormones; thus, we did not assay for these hormones in our animal experiments. We believe that the hypoxic mechanisms associated with asthma can also lead to endocrine dysfunction. Our work is therefore limited because hormone levels are closely related to erectile function. Further research should now be carried out and include the detection of hormone levels. Such experiments will provide more rigorous guidance for the clinical treatment of ED caused by asthma. In addition, the sample size of our experiment is small, and the results are only preliminary and should be used with caution. We will further verify the relationship between asthma
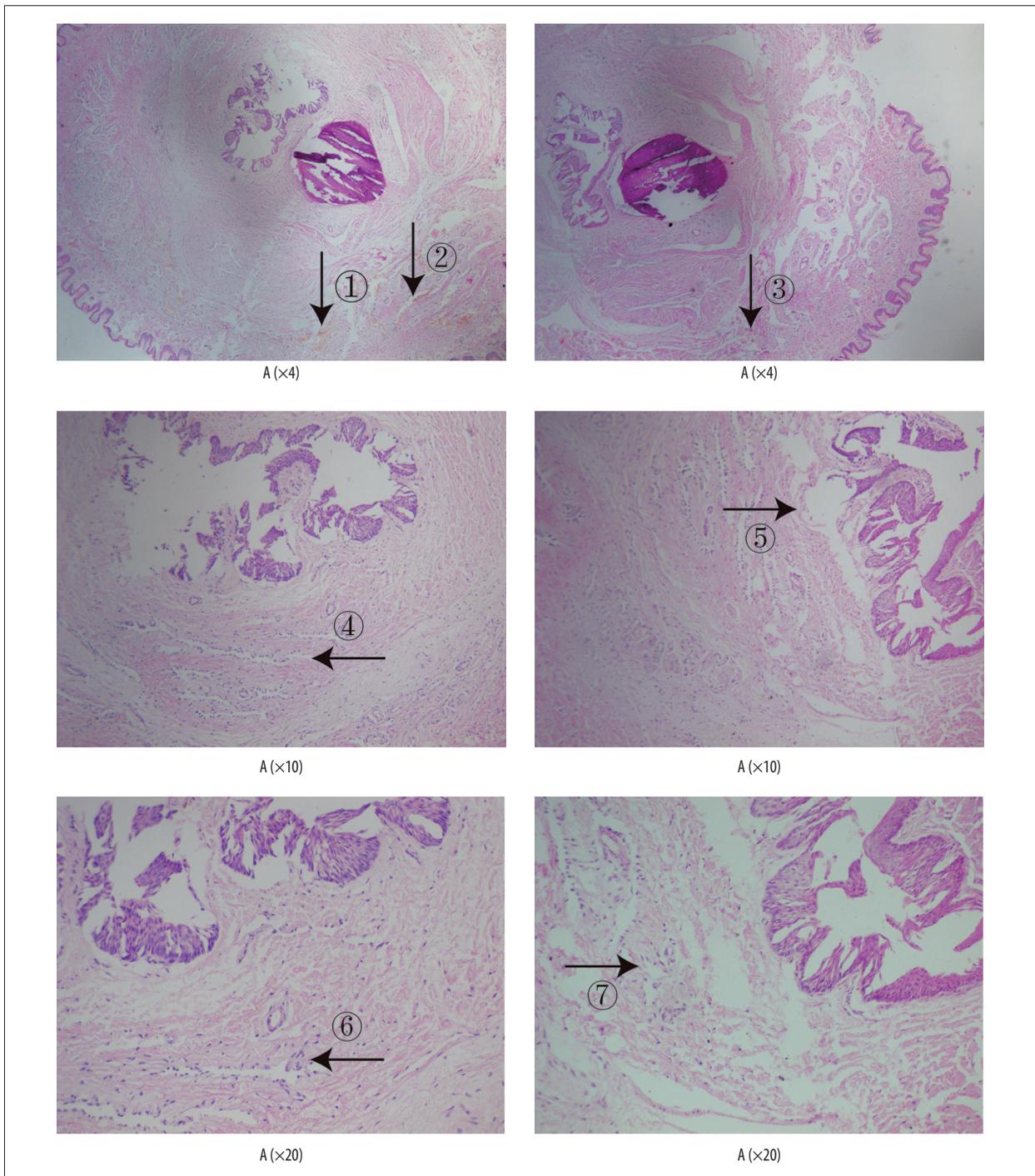
Figure 7. Analyses of penis tissue in rats using hematoxylin and eosin (HE) staining. The penis tissue of rats was stained with HE to observe pathologic changes in the penis under electron microscopy (*n*=6 animals per group). In group A: (i) the blood sinuses are abundant and neatly arranged (arrows ① ②); (ii) tissue gap is small (arrows ④); (iii) nucleus is abundant (arrows ⑥). In group B: (i) the blood sinuses are significantly reduced (arrows ③); (ii) smooth muscle fibers are disorderly arranged and tissue gaps increase (arrows ⑤); (iii) the nucleus is reduced (arrows ⑦).
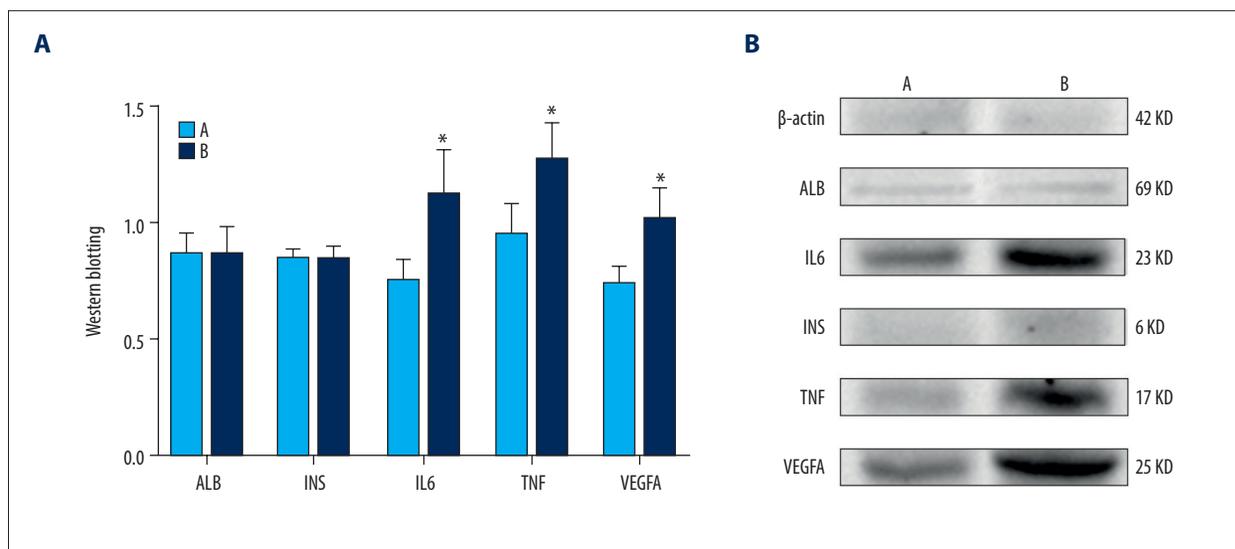
Figure 8. Expression of insulin (INS), albumin (ALB), tumor necrosis factor (TNF), interleukin 6 (IL6), and vascular endothelial growth factor A (VEGFA) in rat penis. (**A**) The red bar chart represents expression of INS, ALB, TNF, IL6, and VEGFA in group A; the blue bar chart represents expression of these substances in group B. Values are mean±SEM (*n*=6 animals per group). The *t* test was used. Group B was compared with group A, * *P*<0.05. (**B**) Western blotting showing expression of INS, ALB, TNF, IL6, and VEGFA proteins. β-Actin is a loading control.
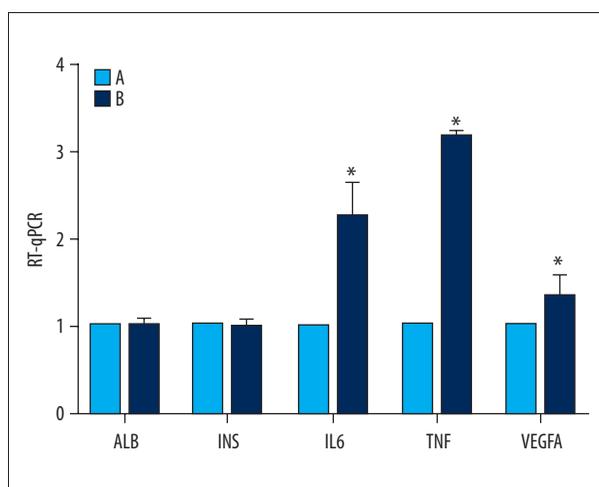

Figure 9. Expression of the messenger ribonucleic acid (mRNA) of insulin (INS), albumin (ALB), tumor necrosis factor (TNF), interleukin 6 (IL6), and vascular endothelial growth factor A (VEGFA) in rat penis. The red bar chart represents expression of the mRNA of INS, ALB, TNF, IL6, and VEGFA in group A; the blue bar chart represents expression of the mRNA of these substances in group B. Values are mean±SEM (*n*=6 animals per group). The *t* test was used. Group B was compared with group A, * *P*<0.05.

and erection by expanding the sample size. We suggest that it can be improved in terms of sample size and the detection of various hormones.

Conclusions

Asthma causes the body to be in a state of prolonged chronic inflammation by causing the increase of IL6 and TNF. In the case of systemic inflammation, inflammatory factors can damage the human vascular endothelial cells and reduce the level of VEGFA and other proteins. Therefore, the decline of erectile function in asthmatic rats may be caused by damage to corpus cavernosum vascular endothelial cells and the decrease of VEGFA expression or activity in plasma with high concentrations of IL6 and TNF.

Conflict of interest

None.

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References:

1. Thompson IM, Tangen CM, Goodman PJ et al: Erectile dysfunction and subsequent cardiovascular disease. JAMA, 2005; 294(23): 2996–3002

2. Wang W, Fan J, Huang G et al: Meta-analysis of prevalence of erectile dysfunction in mainland China: Evidence based on epidemiological surveys. Sex Med, 2017; 5(1): e19–e30

3. Chung KF: Precision medicine in asthma: Linking phenotypes to targeted treatments. Curr Opin Pulm Med, 2018; 24(1): 4–10

4. Wang D, Xiao W, Ma D et al: Cross-sectional epidemiological survey of asthma in Jinan, China. Respirology, 2013; 18(2): 313–22

5. Lu CC: Asthma and risk of erectile dysfunction in Taiwan – patient-based or population-based? J Sex Med, 2012; 9(3): 943

6. Chou KT, Huang CC, Chen YM et al: Asthma and risk of erectile dysfunction – a nationwide population-based study. J Sex Med, 2011; 8(6): 1754–60

7. Itoga A, Zha X, Nagase K et al: Correcting imbalance of sex hormones by a phosphodiesterase 5 inhibitor improves copulatory dysfunction in male rats with type 2 diabetes. BMJ Open Diabetes Res Care, 2020; 8(1): e001111

8. Tao W, Xu X, Wang X et al: Network pharmacology-based prediction of the active ingredients and potential targets of Chinese herbal Radix curcumae formula for application to cardiovascular disease. J Ethnopharmacol, 2013; 145(1): 1–10

9. Zhao RL, He YM: Network pharmacology analysis of the anti-cancer pharmacological mechanisms of *Ganoderma lucidum* extract with experimental support using Hepa1-6-bearing C57 BL/6 mice. J Ethnopharmacol, 2018; 210: 287–95

10. Xiong Y, Hu S, Zhou H et al: High-throughput 16S rDNA sequencing of the pulmonary microbiome of rats with allergic asthma. Genes Dis, 2019; 7(2): 272–82

11. Park J, Chai JS, Kim SW et al: Inhibition of Jun N-terminal kinase improves erectile function by alleviation of cavernosal apoptosis in a rat model of cavernous nerve injury. Urology, 2018; 113: 253.e9–e16

12. Appleton CT, McErlain DD, Henry JL et al: Molecular and histological analysis of a new rat model of experimental knee osteoarthritis. Ann NY Acad Sci, 2007; 1117: 165–74

13. Huang YT, van der Hoorn D, Ledahawsky LM et al: Robust comparison of protein levels across tissues and throughout development using standardized quantitative western blotting. J Vis Exp, 2019; (146): 59438

14. Hong KM, Najjar H, Hawley M, Press RD: Quantitative real-time PCR with automated sample preparation for diagnosis and monitoring of cytomegalovirus infection in bone marrow transplant patients. Clin Chem, 2004; 50(5): 846–56

15. Bazan-Socha S, Mastalerz L, Cybulska A et al.: Prothrombotic state in asthma is related to increased levels of inflammatory cytokines, IL-6 and TNFα, in peripheral blood. Inflammation, 2017; 40(4): 1225–35

16. Long T, Liu G, Wang Y et al: TNF-α, erectile dysfunction, and NADPH oxidase-mediated ROS generation in corpus cavernosum in high-fat diet/streptozotocin-induced diabetic rats. J Sex Med, 2012; 9(7): 1801–14

17. Carneiro FS, Webb RC, Tostes RC: Emerging role for TNF-α in erectile dysfunction. J Sex Med, 2010; 7(12): 3823–34

18. Demirtaş Şahin T, Yazir Y et al: TNF-α antagonism with etanercept enhances penile NOS expression, cavernosal reactivity, and testosterone levels in aged rats. Can J Physiol Pharmacol, 2018; 96(2): 200–7

19. Proboszcz M, Paplińska-Goryca M, Nejman-Gryz P et al: A comparative study of sTREM-1, IL-6 and IL-13 concentration in bronchoalveolar lavage fluid in asthma and COPD: A preliminary study. Adv Clin Exp Med, 2017; 26(2): 231–36

20. Zhao X, Yu FQ, Huang XJ et al: Azithromycin influences airway remodeling in asthma via the PI3K/Akt/MTOR/HIF-1α/VEGF pathway. J Biol Regul Homeost Agents, 2018; 32(5): 1079–88

21. Urios A, Ordoño F, García-García R et al: Tadalafil treatment improves inflammation, cognitive function, and mismatch negativity of patients with low urinary tract symptoms and erectile dysfunction. Sci Rep, 2019; 9(1): 17119

22. Husemoen LL, Glümer C, Lau C et al: Association of obesity and insulin resistance with asthma and aeroallergen sensitization. Allergy, 2008; 63(5): 575–82

23. AbdulWahab A, Zeidan A, Avades T et al: Serum zinc level in asthmatic and non-asthmatic school children. Children (Basel), 2018; 5(3): 42