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Molecular Determination of Toll-Like Receptors 2 and 4 among Asthmatic Patients in Basrah, South Iraq

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ABSTRACT

Aims Bronchial asthma is a life-threatening disease with a multifactorial etiology. It was shown that immunological dysregulations play an essential role in the exacerbation of the patient's attacks. Toll-like receptors act as a bridge in the transmission of different signals associated with the regulation of immune responses. According to numerous research, TLR2 causes different outcomes in asthma. TLR2 and TLR4 overexpression seems to predispose to an increase in the frequency of dyspneic attacks. Therefore, this study aimed to learn more about the function of TLR2 and TLR4 and investigate their underlying association with allergic airway inflammation in asthmatic patients.

Materials & Methods In this case-control study conducted at outpatient clinics in a Specialized Allergy Center in Basrah City, South of Iraq, 70 asthmatic patients and 70 healthy individuals were investigated. 4ml of venous blood was drawn and divided into two parts: 2ml was used for RNA extraction and qRT-PCR, and another 2ml was used for the serological tests. Data analysis was done by SPSS 26 software.

Findings The mean serum levels of TLR2 and TLR4 were significantly higher in the asthmatic group (28.33±13.00ng/ml and 46.30±20.32ng/ml, respectively) than in the control group (7.33±3.73ng/ml and 26.28±14.32ng/ml, respectively). Also, the expression level of TLR2 and TLR4 was significantly higher in the asthmatic group ($p<0.05$).

Conclusion TLR2 and TLR4 are increased in patients with bronchial asthma compared to healthy individuals. Elevated levels of both biomarkers may act as a trigger for increased secretion of other cytokines, leading to exacerbation of asthmatic signs and symptoms.

Keywords Asthma; Allergens; Toll like Receptor 2; Toll-like Receptor 4; Quantitative Real-Time PCR

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Introduction

Asthma is the most common, long-lasting inflammatory airway disease that affects more than 10% of the world population [1] and is a major health problem in the world [2]. The global prevalence of asthma is anticipated to be approximately 4.5% [3, 4]. There are about 334 million patients with asthma affecting all age groups across the world [2]. The prevalence of asthma has increased over time, and an additional 100 million people worldwide are expected to develop asthma by the year 2025 [2].

Asthma is defined as a chronic inflammatory disease of the airways. The chronic inflammation is associated with airway hyperresponsiveness (an exaggerated airway-narrowing response to specific triggers such as viruses, allergens, and exercise) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity. Symptom episodes are generally associated with widespread but variable airflow obstruction within the lungs that is usually reversible either spontaneously or with appropriate asthma treatment, such as a fast-acting bronchodilator [5].

Long-lasting bronchial inflammation can cause pathological alterations, for example, the improved thickness of the bronchial epithelium and friability of airway epithelial cells, epithelium fibrosis, hyperplasia, and hypertrophy of airway smooth muscle, angiogenesis, and mucus gland hyperplasia. The stimulation of bronchial epithelial cell would result in the release of inflammatory cytokines and chemokines that attract inflammatory cells into bronchial airways and plays an important role in asthma [1].

Asthma is associated with T helper cell type-2 (Th2) immune responses, which are typical of other atopic conditions. Asthma triggers may include allergic (e.g., house dust mites, cockroach residue, animal dander, mould, and pollens) and non-allergic (e.g., viral infections, exposure to tobacco smoke, cold air, exercise) stimuli, which produce a cascade of events leading to chronic airway inflammation. Elevated levels of Th2 cells in the airways release specific cytokines, including Interleukin (IL)-4, IL-5, IL-9, and IL-13, and promote eosinophilic inflammation and Immunoglobulin E (IgE) production. IgE production, in turn, triggers the release of inflammatory mediators, such as histamine and cysteinyl leukotrienes, that cause bronchospasm (contraction of the smooth muscle in the airways), edema, and increased mucous secretion, which lead to the characteristic symptoms of asthma [5, 6].

Allergic asthma is usually defined as asthma associated with sensitization to aeroallergens. Sensitization to aeroallergens significantly contributes to asthma symptoms and airway inflammation [6]. Inhalation of allergen leads to acute bronchoconstriction, followed by inflammatory cell influx, which triggers a late asthmatic response [7]. Allergic asthma is the most common asthma phenotype [8]. It is estimated that up to 80% of childhood asthma and more than 50% of adult asthma cases may have an allergic component [9].

Allergic asthma is marked by eosinophilic cell infiltration, hyperresponsiveness of the air passages, and abundant secretion of mucus materials, as well as infiltration of T helper 2 cells in the lungs [10, 11].

The airway passage is frequently exposed to different types of microorganisms. Therefore, it is a challenge with different barriers of specific and acquired immunity to overcome these invaders [11, 12].

Emerging evidence suggests that Toll-Like Receptors (TLRs) may be associated with the aberrant stimulation of immune responses, possibly contributing to the chronic inflammation seen in asthma [13]. Toll-like receptors, a part of Pattern Recognition Receptors (PRRs), are the first to identify microbes in the airway, have undergone evolutionary conservation, and are essential for immune responses, particularly when the extracellular matrix is used to recognize pathogens [14].

TLRs are a family of cell surface proteins. Upon stimulation, TLRs can modulate subsequent adaptive immune responses. TLR2 and 4 are expressed on the cellular surface and migrate to phagosomes after activation on recognizing the ligand. Just because these receptors are expressed on the cellular surface makes them easy to measure [15].

There are two sub-families of these receptors: The purpose of the first group of TLRs, which are largely present on the surface of the body and include TLR1, 2, 4, 5, 6, and 11, is to identify the elements of microbial membranes [10, 14]. Those in the second subgroup are TLR3, 7, 8, and 9. They can target microbial nucleic acids and are found intracellularly in the endoplasmic reticulum and inside vesicles, such as endosomes and lysosomes [16].

Through the use of a Leucine-Rich Pattern Recognition Receptor (PRR) domain, toll-like receptors possess a significant function in the nonspecific immune reaction by identifying pathogen-associated molecular patterns [16].

By bridging the preliminary identification of pathogenic microorganisms by nonspecific immune cells and the stimulation of specific immune reactions, TLRs have transformed the discipline of immunology by controlling the triggering of the secretion of different cytokines and promoting the function of immune cells that presenting antigens, so these receptors have a fundamental function in connecting the two arms of the immune system [17].

When microbial invaders are recognized by the Toll-like receptor, a subsequent signaling cascade results in the secretion of interleukins and many chemokines that motivates both the specific and nonspecific immune reaction to treat infections [18, 19].

Additionally, recent research has demonstrated that

TLR signaling can directly control t lymphocyte formation, proliferation, differentiation, and function in a variety of physiological contexts [17, 19].

The continual exposure to breathed air makes the lung and respiratory system particularly vulnerable to microorganisms and allergies. TLRs also identify exogenous Pathogen-Associated Molecular Patterns (PAMPs) and host-derived Damage-Associated Molecular Patterns (DAMPs) [17, 19].

The environment and population in Basrah city have been destroyed by years of war, famine, and fossil fuel extraction. Exposure to dispersed particles causes abnormalities and mutations in the genetic levels. Induction of cytotoxicity of immune cells, along with the increase of pro-inflammatory cytokines with carcinogenic tendency, all lead to increased complications and mortality from many diseases, especially heart and respiratory problems such as bronchial asthma, especially in children and the elderly. It also has a significant negative impact on the population's life expectancy [20].

Therefore, this study aimed to learn more about the function of Toll-like receptors 2 and 4 and investigate their underlying association with allergic airway inflammation in asthmatic patients.

Materials and Methods

This is a case-control study conducted at outpatient clinics in a Specialized Allergy Center in Basrah City, South of Iraq, from April 2022 to December 2022.

There were 70 participants, aged 18 to 75, who were receiving maintenance medication for asthma. All participants in the study had been diagnosed with bronchial asthma by physicians and were non-smokers. The control group included 70 individuals with no history of asthma and were non-smokers, who were matched with the patients in terms of age and gender.

From each participant in the study, 4ml of venous blood was drawn using a clean, sterile disposable syringe, which was then divided into two parts: 2ml was poured into an EDTA (Ethylenediaminetetraacetic Acid) tube for RNA extraction using TriPure™ Isolation Reagent solution (Sigma-Aldrich; USA). cDNA was created using WizScript RT FDmix (WIZBIO; South Korea). Then the quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was performed by special sets of primers [21-23] and ABsolute QPCR Mix, SYBR Green, no ROX. The condition of the amplification was created [24, 25].

Another 2ml of blood was emptied into a disposable plain tube for the serological tests. A sample's serum was separated at 10000 g for 10min and stored at -70°C to applicate in the serological analysis of TLR2 and 4 using commercial ELISA kits (Invitrogen Human TLR-2 ELISA Kit Catalog # EH459RB, and Human TLR-4 ELISA Kit Catalog Number EH460RB), which utilized a sandwich method. The detection rate of the kits was 0.32-80ng/mL and 0.4-100ng/ml, respectively. Serum levels were evaluated using an ELISA plate reader according to the manufacturer's instructions. At the time of the test, all serum samples that had been frozen at -70°C were thawed once. Each sample was examined twice [26].

Statistical analysis

SPSS 26 statistical software was used for analysis. The qualitative variables were presented as frequency and percentage, and the quantitative variables were presented as mean and Standard Deviation (SD). The mean serum levels of TLR2 and TLR4 in the asthma group and the control group were compared using the independent t-test. Pearson's correlation test was used to investigate the correlation between TLR2 and TLR4 serum levels with age and sex, and with each other.

Finding

A total of 140 people were included in the study, whose ages ranged from 18 to 75 years. The mean age of the case group and the control group was 44.33±16.81 and 44.34±16.84, respectively, which there was no significant difference between them (p>0.05).

Also, no significant difference was observed between the two groups in terms of age groups, gender, and place of residence (p>0.05; Table 1).

Table 1) Frequency distribution of demographic characteristics in the case (n=70) and control (n=70) groups

Variables	Case group	Control group
Age (years)		
Less than 20	7 (10.0%)	5 (7.1%)
20-39	19 (27.1%)	21 (30.0%)
40-59	28 (40.0%)	32 (45.7%)
60 and more	16 (22.9%)	12 (17.1%)
Gender		
Male	27 (38.6%)	34 (48.6%)
Female	43 (61.4%)	36 (51.4%)
Residence		
Center	39 (55.7%)	44 (62.9%)
Periphery	31 (44.3%)	26 (37.1%)

The mean serum levels of TLR2 and TLR4 in the asthmatic group were significantly higher than the control group (p=0.0001; Table 2).

Table 2) The mean serum levels of TLR2 and TLR4 (ng/ml) in the case and the control groups

Variable	Case group	Control group
TLR2	28.33±13.00	7.33±3.73
TLR4	46.30±20.32	26.28±14.32

There was a highly significant positive correlation between the mean serum levels of TLR2 and TLR4 (r=0.314; p=0.0001). No significant correlation was observed between age and TLR2 and TLR4 serum levels (p>0.05). However, there was a positive correlation between gender and TLR2 serum level (r=0.270; p=0.001), but no such correlation was observed between gender and TLR4 serum level (p>0.05; Table 3).

Table 3) Correlation between TLR2, TLR4, age and gender of the study population

Variable	TLR2	TLR4
Age	r=0.102; p=0.229	r=0.063; p=0.462
Gender	r=0.270; p=0.001	r=0.94; p=0.462
TLR2	1	r= 0.314; p=0.0001

The expression level of TLR2 and TLR4 was significantly higher in the asthmatic group compared to the control group (p<0.05; Table 4).

Table 4) Quantitative real-time PCR analysis (ΔCT) for TLRs gene expression in the case and control group (p<0.05)

Variable	Case group	Control group
TLR2	13.38±1.75	4.31±0.71
TLR4	16.15±1.62	5.36±0.86

CT: Cycle Threshold

Discussion

Asthma is a chronic inflammatory disease of the respiratory tract characterized by unstable obstruction of the airflow and severe response of the airways [27]. Asthma is a multifactorial disease in which family as well as infectious, allergic, socioeconomic, psychological, and environmental factors play a role [28]. Environmental factors such as exposure to various allergens, irritants, industrial pollutants, and particulate matter are involved in developing countries [29, 30]. For developed countries, outdoor pollutants, such as benzene, particulate matter, and irritant gases, including nitrogen dioxide (NO_2), ozone (O_3), and sulfur dioxide (SO_2), increase the incidence of respiratory diseases, especially asthma [31, 32].

The link between asthma and innate immunity has been previously shown by many studies, with evidence that the response to antivirals decreases bronchial cells in asthma patients, as well as increases the expression of receptors in severe asthma. This indicates that innate immunity has active participation in the exacerbation of asthma, and innate immunity represents the first line of defense against microbes [33]. The Toll-like receptor (TLR) family comprises receptors belonging to innate immunity with the mission of identifying microbes and activating immunity with both fungal and adaptive types by different cells in the airway, including fat cells, macrophages, and epithelial cells [34, 35].

The present study aimed to learn more about the function of toll-like receptors 2 and 4 and investigate their underlying association with allergic airway inflammation in asthmatic patients.

In this study, most of the patients with asthma were in the age group of 40-59 years (40.0%), and the frequency of females (61.4%) was more than males (38.6%). These results are similar to the study of Ahmad & Ibrahim in Sulaimani City [36], with the difference that in their study, the frequency of asthmatic men was higher.

In the present report, the mean serum concentration of TLR2 and TLR4 was significantly higher in the asthmatic group (28.33±13.00ng/ml and 46.30±20.32ng/ml, respectively) than in the control group (7.33±3.73ng/ml and 26.28±14.32ng/ml, respectively). This may prove the idea of the role of TLR 2 and 4 in mediating the pathogenesis of allergy and asthma. Interestingly, most natural allergens, such as house dust mites, share structural similarities with TLR4 co-receptors [37].

The ability of Pattern Recognition Receptors (PRRs) to detect infection at first and trigger a sequence of signaling within cells for the final eradication of the microorganisms and infected cells makes them a crucial part of innate immunity [38]. Innate immune cells that have been signaled by TLR4 typically produce type 1 cytokines, which leads to the establishment of Th1/Th17 cell immunity [39].

It was demonstrated that pre-exposure to some environmental stimuli shaped the body's defenses in the direction of t helper I cell through interleukin 12 synthesis and traditional triggering of macrophages within the alveoli while suppressing the invasion of eosinophilic cells and secretion of interleukin 4 [40].

Th2 cells release a variety of mediators, including interleukin 13, 9, 5, and 4, in response to environmental antigens. Through the release of the II interferon-gamma (IFNγ) and interleukin 10 as anti-inflammatories, which are released by Th1 cells, such responses can be inhibited by Treg cells [10].

Many studies suggest that toll-like receptors (TLRs) may be linked to immune responses that are abnormally stimulated, which may be a factor in the persistent inflammation seen in asthma [41]. Chun *et al*. found that the TLR1 and TLR2 expression in asthmatic patients is significantly higher, while TLR6 expression is lower compared to healthy subjects [42]. Although the role of TLR4 in asthma is still controversial, it is becoming clear that several factors should be considered in the TLR4-mediated development of allergen-induced Th2 responses, including polymorphism of CD14, the cell type in which TLR4 is engaged, the dose of stimuli, and the timing of exposure [43].

Shukur *et al*. found that in patients with severe asthma, hypersensitivity is always accompanied by elevated levels of innate cytokines in the blood, which stimulates the expression of TLR4 in human monocytes. Activated TLR4 mediates the inflammatory response (as manifested by an increased level of IL-8, IL-1, as well as TNF). This response is associated with TLR-4 gene polymorphisms [33]. In a meta-analysis, Zhao *et al*. extracted data from a combined sample of 7628 individuals, and the results showed that rs3804099 in TLR2 and rs4986791 in TLR4 contribute to the increased risk of asthma [44].

Epithelial cells and Fibroblasts that express TLR4 have been identified to be involved in localized inflammatory reactions. These cells provide an anchor-like role by attracting and immobilizing leukocytes by releasing a variety of chemokines and

cellular adhesion molecules. Chronic inflammation is a likely result when leukocytes remain in the respiratory tract [45].

Fang *et al*. found that mice treated with Ovalbumin-immunized allergic airway challenge had increased TLR2 expression and that TLR2 deficiency was associated with reduced allergic airway inflammation. The lack of TLR2, however, made autophagy activation less effective [46].

On the other hand, TLR2 can be activated by lipopeptides, which encourages naive T cells to differentiate into IL-10 and IFN-producing T cells in vitro and inhibits the generation of IL-4 by Th2 cells. However, under specific circumstances, some TLR2 ligands activate DC to cause a Th2 response or to release large amounts of IL-23, which then encourage the growth of Th17 cells [47].

Through the c-Jun N-terminal kinase (JNK) signaling cascade and the induction of autophagy, TLR2 may help to maintain allergic airway inflammation. These results might provide a brand-new signal target for reducing allergic airway inflammation [46].

In this study, we found a highly statistically significant positive correlation between TLR2 and TLR4 as both markers were found to be increased in asthmatic patients as compared to the control non-asthmatic group. Also, we found that there is no significant correlation between the age of the participants and the level of the selected markers, which requires further research in a large sample size.

Wang & McCusker stated that age is an important factor affecting TLR4-mediated Th2 response by Lipopolysaccharide (LPS) exposure; thus, age and dose of LPS could be key factors in determining protection against asthma [48].

Some research supported the idea that gene polymorphism in receptor-related genes, including CD14, TLR2, TLR4, and others, may affect allergy-induced inflammation and the regulation of the production of IgE [49].

The expression and operation of TLRs may be influenced by sex hormones because the menstrual cycle's hormonal fluctuations affect how the TLRs are activated. The results of the present study showed a higher level of TLR2 in women with asthma. Further, regardless of whether no female sex hormones are present, estrogen receptors have the ability to trigger TLR signaling [50].

According to The International Study of Asthma and Allergies in Childhood (ISAAC), 16.3% of primary school children in Iraq have been clinically diagnosed with childhood asthma [51]. In several parts of Iraq, the frequency of childhood asthma has been investigated; the largest one was carried out by Al-Thamiri *et al*. in Baghdad, which included 3360 primary school students [52].

Oleś & Szczepankiewicz reported that due to their function in immunological processes, Toll-like receptors have been demonstrated in animal models to potentially be a key therapeutic target in the treatment of asthma [53]. In addition, Zakeri & Russo reported that TLRs could suppress, exacerbate, or contribute to asthma pathogenesis. Therefore, TLRs agonists can use for the treatment of asthma [54].

Finally, another member of TLRs is TLR7 which may have a controversial effect on TLR2 and 4 by acting on smooth muscle in the airway passages, thus inhibiting the occurrence of hyperresponsiveness [55].

Conclusion

Toll-like receptors 2 and 4 are increased in patients with bronchial asthma compared to healthy individuals. Elevated levels of both biomarkers may act as a trigger for increased secretion of other cytokines, leading to exacerbation of asthmatic signs and symptoms.

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Ethical Permissions: Before conducting this study at the Specialized Allergy Center in Basrah, the approval of the Ethical Committee of the College of Medicine/University of Basrah and the Basrah Health Authority was obtained. Written consent was taken from the patients to participate in the study.

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References

1- Mandlik DS, Mandlik SK. New perspectives in bronchial asthma: pathological, immunological alterations, biological targets, and pharmacotherapy. Immunopharmacol Immunotoxicol. 2020;42(6):521-44.

2- Behera D, Sehgal IS. Bronchial asthma - Issues for the developing world. Indian J Med Res. 2015;141(4):380-2.

3- Masoli M, Fabian D, Holt S, Beasley R, Global Initiative for Asthma (GINA) Programme. The global burden of asthma: executive summary of the GINA Dissemination Committee report. Allergy. 2004;59(5):469-78.

4- To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012;12:204.

5- Quirt J, Hildebrand KJ, Mazza J, Noya F, Kim H. Practical guide for allergy and immunology in Canada 2018. Allergy Asthma Clin Immunol. 2018;14(Suppl 2):50.

6- Lemanske RF, Busse WW. Asthma: clinical expression and molecular mechanisms. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S95-102.

7- Lotvall J, Akdis CA, Bacharier LB, Bjermer L, Casale TB, Custovic A, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. J Allergy Clin Immunol. 2011;127(2):355-60.

8- Schatz M, Rosenwasser L. The allergic asthma phenotype. J Allergy Clin Immunol Pract 2014;2(6):645-8.

9- Johansson SG, Hourihane JO, Bousquet J, Bruijnzeel-Koomen C, Dreborg S, Haahtela T, et al. A revised

nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. Allergy 2001;56(9):813-24.
10- Haapakoski R, Karisola P, Fyhrquist N, Savinko T, Lehtimäki S, Wolff H, et al. Toll-like receptor activation during cutaneous allergen sensitization blocks development of asthma through IFN-gamma-dependent mechanisms. J Invest Dermatol. 2013;133(4):964-72.
11- Peebles RS Jr, Aronica MA. Proinflammatory pathways in the pathogenesis of asthma. Clin Chest Med. 2019;40(1):29-50.
12- Mak TW, Saunders ME. Immunity to pathogens. Immune Resp. 2006:641-94.
13- Phipps S, Lam CE, Foster PS, Matthaei KI. The contribution of toll-like receptors to the pathogenesis of asthma. Immunol Cell Biol. 2007;85(6):463-70.
14- Lebold KM, Jacoby DB, Drake MG. Toll-like receptor 7-targeted therapy in respiratory disease. Transfus Med Hemother. 2016;43(2):114-9.
15- Crespo-Lessmann A, Juárez-Rubio C, Plaza-Moral V. Role of toll-like receptors in respiratory diseases. Arch Bronconeumol. 2010;46(3):135-42. [Spanish]
16- Behzadi P, García-Perdomo HA, Karpiński TM. Toll-like receptors: general molecular and structural biology. J Immunol Res. 2021;2021:9914854.
17- Duan T, Du Y, Xing C, Wang HY, Wang RF. Toll-like receptor signaling and its role in cell-mediated immunity. Front Immunol. 2022;13:812774.
18- Farooq M, Batool M, Kim MS, Choi S. Toll-like receptors as a therapeutic target in the era of immunotherapies. Front Cell Dev Biol. 2021;9:756315.
19- Heine H, Lien E. Toll-like receptors and their function in innate and adaptive immunity. Int Arch Allergy Immunol. 2003;130(3):180-92.
20- Al-Kasser MK. Air pollution in Iraq sources and effects. IOP Conf Ser Earth Environ Sci. 2021;790:012014.
21- Wrensch M, Minn Y, Chew T, Bondy M, Berger MS. Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro Oncol. 2002;4(4):278-99.
22- Spandidos A, Wang X, Wang H, Seed B. PrimerBank: a resource of human and mouse PCR primer pairs for gene expression detection and quantification. Nucleic Acids Res. 2010;38(Database issue):D792-9.
23- Pombo-Suarez M, Calaza M, Gomez-Reino JJ, Gonzalez A. Reference genes for normalization of gene expression studies in human osteoarthritic articular cartilage. BMC Mol Biol. 2008;9:17.
24- Malvandi AM, Mehrzad J, Moghaddam MS. Gene expression quantification of toll like receptors 2, 4 and co-molecules in human glioblastoma cell line (U87-MG): toward a new in vitro model of inflammation. Iran J Basic Med Sci. 2011;14(5):428-35.
25- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc. 2008;3(6):1101-8.
26- Badr Z, AL-Moosawi W, Ali S. Evaluation of VEGF-A in relation to childhood acute lymphoblastic leukemia (in Basrah, Iraq): Evaluation of VEGF-A in relation to childhood acute lymphoblastic leukemia (in Basrah, Iraq). Iraqi Nation J Med. 2021;3(1):39-44.
27- National Heart Lung, and Blood Institute. National asthma education program expert panel report. Guidelines for the diagnosis and management of asthma. J Allergy Clin Immunol. 1991;88(3 Pt 2):425-534.
28- Weiss KB, Gergen PJ, Hodgson TA. An economic evaluation of asthma in the United States. N Engl J Med. 1992;326(13):862-6.
29- Obel KB, Ntumba KJM, Kalambayi KP, Zalagile AP, Munogolo KZ. Prevalence and determinants of asthma in adults in Kinshasa. PLoS One. 2017;12(5):e0176875.
30- Lajoie P, Dagenais G, Ernst P, Neukirch F, Gérin M, Gosselin P, et al. Systèmes respiratoire et cardio-vasculaire. Environnement et Santé Publique—Fondements et Pratiques. 2003:713-45.
31- Trasande L, Thurston GD. The role of air pollution in asthma and other pediatric morbidities. J Allergy Clin Immunol. 2005;115(4):689-99.
32- Carrillo G, Patron MJP, Johnson N, Zhong Y, Lucio R, Xu X. Asthma prevalence and school-related hazardous air pollutants in the US-Mexico border area. Environ Res. 2018;162:41-8.
33- Shukur W, Alyaqubi K, Dosh R, Al-Ameri A, Al-Aubaidy H, Al-Maliki R. Association of Toll-like receptors 4 (TLR-4) gene expression and polymorphisms in patients with severe asthma. J Med Life. 2021;14(4):544-8.
34- Wu X, Wang P, Zhang Y, Gao L, Zheng B, Xu Y, et al. Toll-like receptor characterization correlates with asthma and is predictive of diagnosis. DNA Cell Biol. 2020;39(7):1313-21.
35- Lee JE, Kim YH, Rhee CS, Kim DY. Synergistic effect of dermatophagoidesfarinae and lipopolysaccharide in human middle ear epithelial cells. Allergy Asthma Immunol Res. 2016;8(5):445-56.
36- Ahmad SK, Ibrahim HA. Some risk factors of asthma in bronchial asthma patient in Sulaimani City- Iraq. Mosul J Nurs. 2022;10(2):226-42.
37- Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5:461.
38- Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, functions, signaling, and role of their polymorphisms in colorectal cancer susceptibility. Biomed Res Int. 2021;2021:1157023.
39- Kim S, Becker J, Bechheim M, Kaiser V, Noursadeghi M, Fricker N, et al. Characterizing the genetic basis of innate immune response in TLR4-activated human monocytes. Nature Communications. 2014;5:5236.
40- Liu J, Cao S, Kim S, Chung EY, Homma Y, Guan X, et al. Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression. Curr Immunol Rev. 2005;1(2):119-37.
41- Duan T, Du Y, Xing C, Wang HY, Wang RF. Toll-like receptor signaling and its role in cell-mediated immunity. Front Immunol. 2022;13:812774.
42- Chun E, Lee SH, Lee SY, Shim EJ, Cho SH, Min KU, et al. Toll-like receptor expression on peripheral blood mononuclear cells in asthmatics; implications for asthma management. J Clin Immunol. 2010;30(3):459-64.
43- Wills-Karp M. Allergen-specific pattern recognition receptor pathways. Curr Opin Immunol. 2010;22(6):777-82.
44- Zhao J, Shang H, Cao X, Huang Y, Fang X, Zhang S, et al. Association of polymorphisms in TLR2 and TLR4 with asthma risk. Medicine (Baltimore). 2017;96(35):e7909.
45- Zuo L, Lucas K, Fortuna CA, Chuang CC, Best TM. Molecular regulation of toll-like receptors in asthma and COPD. Front Physiol. 2015;6:312.
46- Fang L, Shen Q, Wu H, He F, Ding P, Xu K, et al. TLR2 favors OVA-induced allergic airway inflammation in mice through JNK signaling pathway with activation of

autophagy. Life Sci. 2020;256:117896.
47- Muzio M, Fonte E, Caligaris-Cappio F. Toll-like receptors in chronic lymphocytic leukemia. Mediterr J Hematol Infect Dis. 2012;4(1):e2012055.
48- Wang Y, McCusker C. Neonatal exposure with LPS and/or allergen prevents experimental allergic airways disease: development of tolerance using environmental antigens. J Allergy Clin Immunol. 2006;118(1):143-51.
49- Klaassen EM, Thönissen BE, van Eys G, Dompeling E, Jöbsis Q. A systematic review of CD14 and toll-like receptors in relation to asthma in Caucasian children. Allergy Asthma Clin Immunol. 2013;9(1):10.
50- Echem C, Akamine EH. Toll-like receptors represent an important link for sex differences in cardiovascular aging and diseases. Front Aging. 2021;2:709914.
51- Salem MB, Al-Sadoon IO, Hassan MK. Prevalence of wheeze among preschool children in Basra governonate, southern Iraq. East Mediterr Health J. 2002;8(4-5):503-8.
52- Al-Thamiri D, Al-Kubaisy W, Ali SH. Asthma prevalence and severity among primary-school children in Baghdad. 2005;11(1-2):79-86.
53- Oleś D, Szczepankiewicz A. Role of Toll-like receptors in the development of allergic inflammation in asthma. Adv Dermatol Allergol. 2012;29(4):275-8.
54- Zakeri A, Russo M. Dual role of Toll-like receptors in human and experimental asthma models. Front Immunol. 2018;9:1027.
55- Ekman AK, Adner M, Cardell LO. Toll-like receptor 7 activation reduces the contractile response of airway smooth muscle. Eur J Pharmacol. 2011;652(1-3):145-51.