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Antibiotic Therapy in Asthma as One of First Step Treatment, a Phase 3 Randomized Clinical Trial

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ABSTRACT

Background: Focus of this study was on subjects suffering from new onset asthma, but actually, they may involve with an infection. Objectives: The target of this study was to determine the frequency of total control of asthma in new onset asthma.

Methods: this is a phase 3 randomized clinical trial. The participants were 50 new onset asthma, who feel suffering from respiratory infection, however the physician diagnosis was asthma according to wheezy chest, spirometry and clear chest roentgenogram. Age less than 18 years and pregnant women were excluded. The subjects were randomly divided into two groups, doxycycline hyclate and the placebo arms. The drugs were repacked in similar in shape container. Primary endpoint was time to first recurrence. Secondary endpoints were frequency of total control of asthma, dyspnea, cough, asthma control test (ACT) score, FEV1, FEV1/FVC, eosinophil and neutrophil in blood and sputum.

Results: The difference of clinical findings between two groups before the trial were not significant. Ten percent reported acute attack of asthma during last two months. Treatment with Doxycyline revealed significant improvement of clinical findings, asthma attack and ACT score after two months of treatment, however, complete resolution of clinical findings were significantly higher in Doxycyline group compare to placebo. Clinical findings and FEV1 percent predicted in Doxycyline group T2low asthma group showed significant improvement which were not depicted in T2high group. Eighteen months follow up of the subjects showed complete resolution of asthma symptoms in 12 (48%) on Doxycyline group, which was significantly higher than control group (5 (20%). Time to first exacerbation in doxycycline group was 13.6±5.64 months which was significantly longer than control group (7.49±6.95, P=0.002). Time to first exacerbation in CRP positive was significantly longer (13.64±5.35 months) than CRP negative subjects (9.67±7.76 months, P=0.02).

Conclusion: Doxycycline is an effective antibiotic for preventing recurrence of asthma, in selected new subjects who suspicious for respiratory infection.

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Introduction

Traditionally, infection was not considered a differential diagnosis for asthma, as it was viewed as a companion condition (1). However, acute cough, a cardinal sign of respiratory infections (2), is also a common symptom in asthma, particularly during the initial onset of the disease. While airway hyperresponsiveness can help diagnose asthma, this symptom and wheezing on chest auscultation have been reported in various respiratory infections as well (3). Consequently, the role of infections caused by organisms such as Mycoplasma pneumoniae and Chlamydophila pneumoniae has been recognized in chronic asthma (4), and respiratory infections are now known to be able to mimic asthma (5).

It is believed that infections may influence the pathophysiology of asthma (6). Disruption of epithelial barrier with infection is a proposed mechanism (7), that can explain the occurrence of new-onset asthma after an infection (8, 9). The infection can be viral (2), bacterial (10), fungal (11) or protozoal (12), and many of them these infections have effective treatments. The airways are not a sterile environment and the residential bacterial population has been reported (13). Disruption of epithelial barrier may lead to entrance of bacterial component into the bronchial mucosa, which may lead to asthma induction, inducing asthma through mechanisms involving flagelin (14) and endotoxin (15). Infection may reside in or around epithelial cells and exert epimmunome from epithelial cells, exerting an effect on the epimmunome from these cells, which can lead to the activation of T helper 2 and ILC2 lymphocytes, thereby inducing new-onset asthma (16). Previous studies have detected distinct microbial composition in neutrophilic asthma and have recommended new treatments focusing on the airway microbiota (17). Accordingly, using suitable antibiotics to eradicate bacteria that contribute to the induction and exacerbation of asthma may be an effective strategy (18). However, administration of inappropriate antibiotics that might potentially disturb the useful preventive microbiota should be avoided (19). In this regard, the eradication of pathogenic bacteria with more correlation with asthma such as *Haemophilus* spp. and *Moraxella* spp., which have been shown to be prevalent in neutrophilic asthma (20), could be a potential target for treatment of asthma . Azithromycin, a macrolide antibiotic, has shown promising results in the treatment of resistant asthma (21, 22). Additionally, doxycycline, a widely available antibiotic, has been recommended for the treatment of respiratory pathogens, including bacterial and atypical respiratory pathogens (23, 24).

The aim of this study is to evaluate the efficacy of doxycycline in eradicating respiratory pathogens, including resistant intracellular germs, and to determine the frequency of complete remission of asthma after treatment, particularly in individuals experiencing asthma for the first time. This approach may differentiate these individuals from those with allergic asthma.

Materials and Methods

Setting

The intention of the present study was to conduct a phase 3 randomized clinical trial on fifty newly diagnosed asthmatic subjects. The place of research was a sub-specialty respiratory clinic in Mashhad, north east of Iran. The study started in June 2020 and concluded in November 2021.

Participants

The subjects were selected from new onset asthmatic patients who strongly asked their physicians to administer antibiotic therapy. However, the inclusion criteria were age more than 18 years, apparent new onset respiratory symptoms, particularly cough with or without dyspnea for more than 6 weeks, confirmed history

of airway hyper-responsiveness, a vax and vane course and clear chest rhontgenogram. Positive spirometry findings included low FEV1<80% predicted, FEV1/FVC< 75% and more than 12% response to 200 microgram inhaled salbutamol. Exclusion criteria included pregnancy, bronchiectasis, COPD, exclusive clinical findings of pneumonia, liver disease, dysphagia or gastero-esophageal reflux and hypoxemia.

Experimental procedures

Randomization was performed by the aid of the randomizer website (https://www.randomizer.org) which gave us permuted block randomization and the random table including the unique code for the drug package. All subjects received standard treatment of asthma (inhaled corticosteroid plus long acting beta2 agonist) and in the following they were divided into two groups of doxycycline hyclate and the placebo arms. The drugs were repacked in similar containers, which looked like the that of the original doxycycline, and the only difference was the unique code, that was not recognizable to the staff of the clinic.

While the primary endpoint was determined to be the time of first recurrence, secondary endpoints were frequency of total control of asthma, dyspnea, cough, asthma control test (ACT) score, FEV1, FEV1/FVC, serum CRP, eosinophil and neutrophil in blood and sputum. These parameters have been evaluated after three months and 80 weeks after the starting time point (Figure 1). Subgroup endotype classification was done in which subjects who had blood eosinophil count more than 300/mm3 were considered as T2 high and the remainder were considered as T2 low.

Ethical considerations

The study was performed for subjects who asked the physician to start antibiotic therapy. However, the study aim and protocol were discussed to the patients and the subjects have signed written informed consent. This clinical trial has been approved by the Iranian Registry of Clinical Trials (IRCT20220412054507N1) and also National Committee for Ethics in Biomedical Research (IR.IAU.MSHD.REC.1400.036).

Statistical analysis

The estimated sample size was 50 subjects who were divided into two groups. All data analyses are considered explorative due to the absence of a statistical power calculation because of the pilot nature of this clinical trial. Parametric data were compared between two groups by paired t-test. Paired t-test and McNemar test were used to compare data before and after the trial. IBM SPSS Statistics (IBM Corporation, version 19.0) were used to perform data analysis. P values below 0.05 were considered statistically significant.

Results

Fifty asthmatic subjects (25 subjects in doxycyline and placebo each group) have finished the two weeks course of this study. Mean of age was 42.86±13.49 years (21-73) and sex distribution was 23(46%) male and 27 (54%) female. Table 1 shows the basic demographic data in studied groups, mean age was 42.86±13.49 years. Age and sex distribution showed no significant differences. Mean of blood and sputum eosinophil was high (more than 3%) but the there was no significant difference between the groups.

Basal clinical findings showed cough and dyspnea in 96% and 72% of subjects respectively with ACT score of 21.26±1.24 (Table1). The difference of clinical findings between two groups before the trial were not significant. Ten percent reported acute attack of asthma during last two months.

Treatment with Doxycyline revealed significant improvement of cough, dyspnea, wheezing, asthma attack and ACT score after two months of

Table 1. Baseline characteristics of the asthma subjects enrolled into two weeks trial of doxycycline and placebo.

Variable	Categories	Abnormal Count (percent) Range	Total	Doxycycline N=25	Placebo N=25	P value
Age (years)		-	42.86±13.49 (21-73)	40.68±11.20	45.04±15.37	0.25
Sex, N(%)	Male	-	23 (46%)	12 (48)	11 (44)	0.77
	Female	-	27 (54%)	13 (52)	14 (56)	
Eosinophil count (cells/mcL)		8/64 (12%) 20-2500	4.71±1.79	5.64±1.23	3.87±2.36	0.07‡
White blood cell count (cells/L)		14/64 (19%) 6-15	8.93±2.87 **(cells/L)**	9.34±2.28 **(cells/L)**	8.53±3.47 **(cells/L)**	0.09‡
Hemoglobin (G/dl)		14/64 (22%) 9-1747/64	13.5±1.91 **(cells/L)**	13.44±1.70 **(cells/L)**	13.6±2.11 **(cells/L)**	0.47
Sputum eosinophil (>3%)		49/70 (70%) 2-14	6.75±3.92 **(cells/L)**	6.87±4.20 **(cells/L)**	6.64±3.67 **(cells/L)**	0.80
Sputum neutrophil (>67%)		50/70 (71%) 62-86	71.77±6.50 **(cells/L)**	70.68±6.77 **(cells/L)**	72.86±6.13 **(cells/L)**	0.16

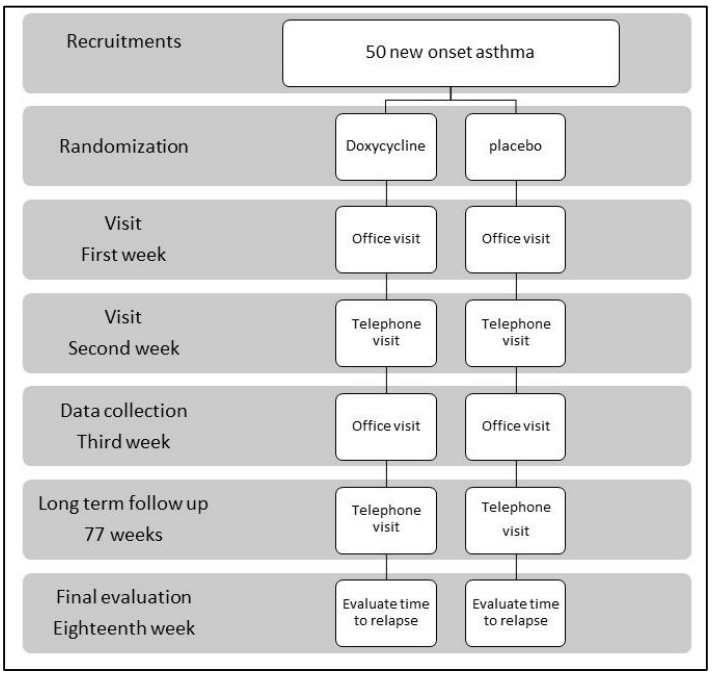

Fig 1. Overview of treatment plan for comparison of doxycycline and placebo in addition to standard asthma treatment.

Table 2. Comparison of clinical findings between Doxycycline and Placebo groups, after 2 months of treatment, in asthma-like infection.

Findings			Before		After	
	score	Total	doxycycline	placebo	doxycycline	Placebo
Cough severity	No	2(4%)	2(8%)	0(0%)	15(60%)*	9(36%)‡
	Mild not daily	5(10%)	3(12%)	2(8%)	9(36%)*	10(40%)‡
	Moderate daily	31(62%)	15(60%)	16(64%)	1(4%)*	11(44%)‡
	Severe	9(18%)	4(16%)	5(20%)	0(0%)*	2(8%)‡
	Continuous	3(6%)	1(4%)	2(8%)	0(0%)*	1(8%)‡
Dyspnea	No	19(38%)	8(32%)	11(44%)	20(80%)*	18(72%)‡
	Mild not daily	14(28%)	6(24%)	8(32%)	4(16%)*	2(8%)‡
	Moderate daily	13(26%)	9(60%)	4(16%)	1(4%)*	5(20%)
	severe	2(4%)	2(8%)	0(0%)	0(0%)*	0(0%)‡
Sputum	-	50(100%)	25(100%)	25 (100%)	20(80%)	18(72%)
Nasal grip	-	31(62%)	13(52%)	18(72%)	9(36%)	7(28%)‡
ACT score		21.26±1.24	21.32±1.21	21.2 ±1.38	24.12±0.92*	21.96±1.67‡
Have asthma attack	During 2months	5 (10%)	3(12%)	4(16%)	0(0%)* ł	6(27.27%)‡
wheezing	During 2 months	50(100%)	18(72%)	14(56%)	5(20%) * ł	21(84%) ‡

*= significance difference between before and after the trial in Doxycycline group

ł = Significant difference between Doxycycline and placebo groups

‡ = Significance differences between before and after the trial in Placebo group

Table 3. Comparison of selected asthma parameters and symptoms, between sputum cytology pattern after 2 months.

		Before		After	
Parameters	groups	T2high	T2low	T2high	T2low
Cough	Dox	8(32%)	15(60%)	5(20%)	4(16%)*
	Placebo	7(28%)	10(40%)	8(32%)	8(32%)
Dyspnea	Dox	6(24%)	11(44%)	4(16%)	3(12%)*
	Placebo	5(20%)	9(36%)	3(12%)	5(20%)*
Wheezing	Dox	7(28%)	11(44%)	0(0%)*	1(4%)*
	Placebo	6(24%)	8(32%)	10(40%)	7(28%)
ACT (Score)	Dox	21.1±4.9	21.6±5.1	23.1±7.1 *	24.2±9.0*
	Placebo	20.3±4.6	21.2±7.5	21.2±5.3	21.4±4.5
FEV1	Dox	69.1 ±19.1	68.4 ±11.9	77.5±18.5 *	76.0±15.22*
	Placebo	60.6±11.7	58.8±7.98	61.8±14.6	60.0±11.7
FEV1/FVC	Dox	81.91±16.1	82.05±11.2	84.5±19. 3*	84.18±12.9
	Placebo	76.41±18.26	77.98±17.19	75.95±14.3	78.04±8.91

treatment (Table 2), however, complete resolution of cough (15(60%) compare to 9(36%), X2= 4.3, P=0.03), asthma attack (zero in Case group compare to 6(27.27%) in control) and wheezing (20 (80%) compare to 4 (16%), X2= 9.2, P=0.001) were significantly higher in Doxycyline group compare to placebo. According to sputum inflammatory cell distribution, the subjects were divided to T2high and T2low asthma. Comparison of Doxycyline and control groups showed significant improvement of cough, dyspnea, wheezing and FEV1 percent predicted in T2low asthma group compare to T2high group of Doxycyline group and all control group (Table 3).

Eighteen months follow up of the subjects showed complete resolution of asthma symptoms in 12 (48%) on Doxycyline group, which was significantly higher than control group (5 (20%), P=0.03). Time to first exacerbation in doxycycline group was 13.6±5.64 months which was significantly longer than control group (7.49±6.95, P=0.002). (Figure 1)

The serum CRP level and neutrophil count were compared in Doxycycline group. Relapse in CRP positive subjects was 10 (40%) subjects which was not statistically different from non-relapsing subjects (12 (48%), P=0.76), however, time to first exacerbation in CRP positive was significantly longer (13.64±5.35 months) than CRP negative subjects (9.67±7.76 months, P=0.02).

Baseline neutrophil count did not show significant correlation between relapse rate and duration to first exacerbation, in Doxycycline group.

Discussion

Result of this clinical trial showed that adding Doxycycline to inhaled corticosteroid plus long acting beta2 agonist have suppressed clinical findings of new onset asthma better than the placebo. The aim of this study was eradication of possible respiratory pathogen with Doxycycline, and put the disease to complete remission. This target was achieved in 48% of Doxycycline treated group, which is great success in treatment of asthma. Considering the remaining subjects who experienced the recurrence of asthma, we noticed longer duration of remission (13 months compare to 7 months in control group) with Doxycycline. CRP level was more informative than neutrophil count at the beginning of treatment, as the subjects with positive CRP experienced longer time of free of symptoms (13 months compare to 9 months). The result of this study confirm the possible origin of respiratory pathogen in asthma.

When cough, wheeze and shortness of breath don't equal asthma? (25). There are many differential diagnosis for asthma (1, 3, 5), however some of them are very similar to asthma that we can call them Pseudoasthma (26). Many disease may introduce in this condition, which although are able to differentiated from asthma with precision attention, but practically some of them are very difficult to diagnose in clinic.

In this study, we focused to acute infection, that presented as psudo-asthma. The clinical landmark for us was to select them were clinical findings resembling typical asthma, with one exception “no past history of respiratory symptoms”. By this method, we tried to separate the infection that mimicking asthma (pseudo-asthma) from usual asthma superimposed by infection. The former needs antibiotic, which we showed a promising results from doxycycline, and the later usually does not need antibiotics, due to prevalence of viral infections.

Infection may cause true asthma; translocation of bacteria to inter- and sub-epithelial areas (27) and the development of epithelial cell disruption, barrier dysfunction and dysregulation in mediators such as Il-33, Il-25 and TSLP is an interesting hypothesis (28), that can discuss many aspect of asthma and bacterial infection association. Furthermore, intermittent exacerbations caused by the same strain suggest that, in COPD, airway epithelial cells might provide a protected reservoir of infectious agents (28); however, apoptosis only occurs at later stages of infection thereby promoting the deepest possible tissue penetration and bacterial access to the host’s lymphatic and circulatory systems and affording protection of the

pathogen from antibiotics and bactericidal antibody (29). According to this hypothesis infection has a crucial role in asthma pathogenesis and anti- bacterial therapy is worth trying (4).

However studies (like the present study) which follow the subjects for determination of long term benefit of these treatment are lacking. The subjects suffered from either of these mechanisms were the target of this study, which were successful in 48% of subjects with complete recovery during 18 months of study duration. We believe that the patient suggestion about suffering from infection or allergy is worth to take consideration, and We believe that, when a patient asks her/his physician: why I acquired the asthma at an exact time? The subject is informing his/her doctor about acquiring an infection at that time.

Why doxycycline? Although experimental studies showed better action of azithromycin rather than doxycycline in asthma , however, we noticed that Doxycycline (30) is a highly lipophilic drug, which is able to crosses multiple membranes of target molecules (31). Doxycycline hyclate shows favorable intra-cellular penetration, and along with its many bacterial effects, it also exhibits antiprotozoal properties (10, 32). Therefore, this ability make doxycycline superior to azithromycin, which does not any activity against Lophomonas blattarum, the protozoa that recently proved to have a role in asthma (33). Some mechanism of action other than its antibiotic mechanism have been introduced for doxycycline in asthma and COPD (34, 35), mechanism such as inhibition of matrix metalloproteinases (36), inhibition of TLR4/NLRP3/caspase-1/GSDMD pathway (37). In a recent study, Doxycyclin showed IgE and Il-4 suppression of lymphocyte that infected with chlamydia pneumonia (38); the author of this study claimed that this effect did not related to its antimicrobial activity, and it may related to an immunomodulatory anti-allergic effect.

However we believe that doxycycline exerts its effect by antimicrobial activity into living organisms inside the cytoplasmic bacteria, including airway epithelial cells (39), which have escaped from host defense (40). By eradication of infected micro-organism inside the effector cells such as epithelial cells, the cascade of asthma and other allergic disease may be stopped. The authors of this study recommend to continue the study about in term of finding more objective finding for using doxycycline in asthma, such as CRP, neutrophilia or sputum IL-8, and we recommend further research about other wide spectrum antibiotics with good penetration into cytoplasmic vacuoles.

Conclusion

In conclusion, doxycycline may be used in new onset asthma, and subjects who`s feeling is suggestive for an infectious problem. The aim of this treatment is to eradicate the current respiratory disease. However, we need diagnostic tools for selecting the subjects who suffer from hidden infection, in or around epithelial cells; a marker in the sputum or blood, more sensitive than CRP or neutrophilia (for example IL1-β or IL-8).

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Ethics approval and consent to participate

The article has accepted by the Ethics Committee of the Central Organization of Islamic Azad University of Mashhad (IR.IAU.MSHD. REC.1399.088) and in the Iranian Registry of Clinical Trials https://www.irct.ir/ under the registration number ((IRCT20220412054507N1).

Conflict of interest

The authors of the present study declare that there are no financial conflicts of interest to disclose.

References

1. Lillington G, Faul J. Differential diagnosis of asthma in adults. in: Gershwin, M.E., Albertson, T.E. (eds) Bronchial Asthma. Humana Press, 2001, Totowa, NJ. P. 251.
2. Torres A, Niederman M, Chastre J, et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia. *Euro Respir J* 2017; **50**(3) 1700582;
3. Johnson J, Abraham T, Sandhu M, et al. Differential Diagnosis of Asthma. *Allergy and Asthma* 2019; **23**:383-400.
4. Blasi F, Johnston SL. The role of antibiotics in asthma. *Int J Antimicrob Agents* 2007; **29**(5):485-93.
5. Milgrom H, Wood RP, Ingram D. Respiratory conditions that mimic asthma. *Immunology and Allergy Clinics of North America* 1998; **18**(1):113-32.
6. Schwarze J, Gelfand EW. Respiratory viral infections as promoters of allergic sensitization and asthma in animal models. *Euro Respir J* 2002; **19**:341.
7. Gon Y, Hashimoto S. Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol Int* 2018; **67**(1):12-7.
8. Mthembu N, Ikwegbue P, Brombacher F, et al. Respiratory viral and bacterial factors that influence early childhood asthma. *Front allergy* 2021; 22.
9. Yano T, Ichikawa Y, Komatu S. Association of *Mycoplasma pneumoniae* antigen with initial onset of bronchial asthma. *Am J Respir Crit Care Med* 1994; **149**:1348–1353.
10. Nagayama Y, Tsubaki T, Toba T, et al. Role of bacterial infection in the exacerbation of acute or prolonged asthma attack in children. *Allergology Int* 1999; **48**:137–44.
11. Shamim S, Agarwal A, Ghosh BK, et al. Fungal pneumonia in intensive care unit: when to suspect and decision to treatment: a critical review. *J Assoc Chest Phys* 2015; **3**(2):41.
12. Mirzazadeh F, Berenji F, Amini M, et al. *Lophomonas blattarum* in asthmatic patients and control group. *J Res Med Dent Sci* 2017; **5**(5):1-5.
13. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS ONE* 2010; **5**:e8578.
14. Hajam IA, Dar PA, Shahnawaz I, et al. Bacterial flagellin—a potent immunomodulatory agent. *Exp Mol Med* 2017; **49**:e373.
15. Williams LK, Ownby DR, Maliarik MJ, et al. The role of endotoxin and its receptors in allergic disease. *Ann Allergy Asthma Immunol* 2005; **94**:323–32.
16. Swamy M., Jamora C., Havran W., et al. Epithelial decision makers: in search of the epimmunome. *Nat Immunol* 2010; **11**:656–665.
17. Yang X, Li H, Ma Q, et al. Neutrophilic asthma is associated with increased airway bacterial burden and disordered community composition. *Biomed Res Int* 2018; **9**:9230234.
18. Ahmadizar F, Vijverberg SJH, Arets HGM, et al. Early life antibiotic use and the risk of asthma and asthma exacerbations in children. *Pediatr Allergy Immunol* 2017; **28**(5):430-7.
19. Taylor SL, Leong LEX, Choo JM, et al. Inflammatory phenotypes in patients with severe asthma are associated with distinct airway microbiology. *J Allergy Clin Immunol* 2018; **141**(1):94-103.e15.
20. Fabbrizzi A, Amedei A, Lavorini F, et al. The lung microbiome: clinical and therapeutic implications. *Intern Emerg Med* 2019; **14**(8):1241-50.
21. Brusselle GG, VanderStichele C, Jordens P, et al. Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-

controlled trial. *Thorax* 2013; **68**(4):322-9.
22. Sadeghdoust M, Mirsadraee M, Aligolighasemabadi F, et al. Effect of azithromycin on bronchial wall thickness in severe persistent asthma: A double-blind placebo-controlled randomized clinical trial. *Respir Med* 2021; **185**:106494.
23. Pestel M. Doxycycline in the treatment of respiratory tract infections. results of a pan-European multi-centre trial. *Chemotherapy* 1975; **21** Suppl 1:91-108.
24. Maesen FP, Davies BI, van Noord JA. Doxycycline in respiratory infections: a re-assessment after 17 years. *J Antimicrob Chemother* 1986; **18**(4):531-6.
25. Malka J, Spahn JD. When Cough Wheeze and Shortness of Breath Don't Equal Asthma. *J Allergy Clin Immunol Pract* 2016; **4**(1):179-81.
26. Weinberger M, Abu-Hasan M. Pseudo-asthma: when cough, wheezing, and dyspnea are not asthma. *Pediatrics* 2007; **120**(4):855-64.
27. Sajjan U, Corey M, Humar A et al. Immunolocalisation of Burkholderia cepacia in the lungs of cystic fibrosis patients. *J Med Microbiol* 2001; **50**:535-46.
28. Dong X, Ding M, Zhang J, et al. Involvement and therapeutic implications of airway epithelial barrier dysfunction in type 2 inflammation of asthma. *Chin Med J* 2022; **135**:519-31.
29. van Schilfgaarde M, Eijk P, Regelink A, et al. *Haemophilus influenzae* localized in epithelial cell layers is shielded from antibiotics and antibody-mediated bactericidal activity. *Microb Pathog* 1999; **26**:249-62.
30. Wang Z, Xin L, Zhang W. Study effect of azithromycin and doxycycline in mucus producing and inflammatory signaling pathways of allergic asthma. *Iran J Allergy Asthma Immunol* 2022; **21**(2):119-27.
31. Patel RS, Parmar M. Doxycycline Hyclate. [Updated 2023 May 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. (Available from: https://www.ncbi.nlm.nih.gov/books/NBK555888/)
32. Bonnetblanc JM. Doxycycline [Doxycycline]. Ann Dermatol Venereol. 2002 Jun-Jul;129(6-7):874-82. French.
33. Ghaderi Y, Mirsadraee Y, Berenji F, et al. Frequency of *Lophomonas blattarum* in asthmatic subjects and effect of anti-lophomonas treatment on asthma, A phase 3 clinical trial. Ubpublished data.
34. Lee KS, Jin SM, Kim SS, Lee YC. Doxycycline reduces airway inflammation and hyperresponsiveness in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 2004; **113**(5):902-9.
35. Von Hertzen L, Töyrylä M, Gimishanov A, et al. Asthma, atopy and *Chlamydia pneumoniae* antibodies in adults. *Clin Exp Allergy* 1999; **29**(4):522-8.
36. Bhattacharyya P, Paul R, Bhattacharjee P, et al. Long-term use of doxycycline can improve chronic asthma and possibly remodeling: the result of a pilot observation. *J Asthma Allergy* 2012; **5**:33-7.
37. Feng Y, Li M, Yangzhong X, et al. Pyroptosis in inflammation-related respiratory disease. *J Physiol Biochem* 2022; **78**(4):721-37.
38. Dzhindzhikhashvili MS, Joks R, Smith-Norowitz T, et al. Doxycycline suppresses *Chlamydia pneumoniae*-mediated increases in ongoing immunoglobulin E and interleukin-4 responses by peripheral blood mononuclear cells of patients with allergic asthma. *J Antimicrob Chemother* 2013; **68**(10):2363-8.
39. Bertuzzi M, Hayes G, Bignell E. Microbial uptake by the respiratory epithelium: outcomes for host and pathogen. *FEMS Microbiol* Rev 2019; **43**(2):145-61.
40. Murphy TF, Brauer AL, Schiffmacher AT, et al. Persistent colonization by *Haemophilus influenzae* in chronic obstructive pulmonary disease. *Am J Resp Crit Care* 2004; **170**:266-72.