Original Article

ISSN: 1735-0344 *Tanaffos 2025; 24(2): 181-186*

TANAFFOS

Effect of Antibiotic Therapy against *Lophomonas blattarum* in Asthmatic Subjects: A Phase 3 Clinical Trial

Younos Ghaderi [1], Majid Mirsadraee [2], Fariba Berenji [3], Zahra Naseri Anhaei [4], Shadi Ghafari [5]

[1] Department of Cardiology, Imam Reza hospital, Mashhad University of medical sciences, Mashhad, Iran, [2] Department of Internal Medicine, Islamic Azad University- Mashhad Medical Sciences Branch, Mashhad, Iran, [3] Department of Medical Parasitology, Imam Reza Hospital, Central Lab, Parasitology & Mycology Lab, Mashhad University of Medical Sciences, Mashhad, Iran, [4] Department of Gynecology and Obstetrics, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran, [5] Department of Physiology, Faculty of Biology, Islamic Azad University-Damghan Branch, Damghan, Iran

Received: 20 February 2024
Accepted: 19 January 2025

Correspondence to: Mirsadraee M
Address: Department of Internal Medicine, Islamic Azad University- Mashhad Medical Sciences Branch, Mashhad, Iran
Email address: majidmirsadraee@iau.ac.ir
majidmirsadraee@yahoo.com

Background: *Lophomonas blattarum* causes chronic non-specific respiratory symptoms, which can easily mask asthma symptoms. The study aimed to predict the benefits of eradicating Lophomonas on the course of asthma.

Materials and Methods: Fifty subjects resistant to high-dose inhaled corticosteroid/long-acting beta 2 agonist and other controllers such as montelukast or tiotropium were enrolled in this phase 3 clinical trial. Lophomonas was evaluated using a direct wet smear of bronchial lavage or induced sputum. Random allocation was performed by drawing lots from identical envelopes, and concealment was achieved by repackaging Tinidazole and placebo (vitamin B1) tablets in identical bottles. The clinic staff were blind to the study, and the code was opened by a pharmacist. The primary outcome was the asthma control test (ACT) score.

Results: Direct smear showed Lophomonas in 52% of the Tinidazole group and 28% of the control group. Other baseline parameters were not significantly different between the Tinidazole and control groups. Cough disappeared in 12 out of 25 (48%) in the Tinidazole group (P=0.001), and the scoring of cough and dyspnea showed significant improvement in the Tinidazole group. Post-nasal drip, sputum, wheezing, and airway hyper-responsiveness were other secondary parameters that showed significant improvement. The ACT score improved from 16.84±2.65 at baseline to 23.11±2.58, and FENO and FEV1 were other objective outcomes that showed significant improvement.

Conclusion: *Lophomnas blattarum* was detected in a significant number of severe asthmatics, and treatment directed at this protozoan will cause a significant improvement in asthmatic subjects.

Keywords: Asthma, *Lophomonas blattarum*, Protozoa, Tinidazole, Imidazole

INTRODUCTION

Lophomonas blattarum is a flagellated protozoan parasite primarily found in the gut of cockroaches and other insects. It is classified within the domain Eukaryota, phylum Metamonada, and family Lophomonadidae. This organism has gained attention as an emerging pathogen responsible for bronchopulmonary infections in both immunocompromised and immunocompetent individuals (1).

On the other hand, clinical findings of asthma are well described by the GINA strategy and many textbooks. Airway hyper-responsiveness (AHR) and an intermittent-relapsing course gain particular attention for the diagnosis of asthma. Therefore, the clinical diagnosis of asthma typically suggests a history of lung symptoms, including cough and/or dyspnea, associated with an intermittent course and AHR. In 40% of cases, normal physical examinations, normal lung imaging, and normal

spirometry have been reported, indicating that challenge tests may be needed as confirmatory diagnostic tests. This scenario is very similar to *Lophomonas*, including lung disease (2). Therefore, *Lophomonas blattarum* infections can be mistaken for asthma in individuals with long-lasting respiratory symptoms, including patients who may exhibit normal lung imaging and an ill-defined history of intermittent courses and AHR (3).

After our first study about Lophomonas (4), we detected some subjects who represented symptoms like asthma. Similarly, the association between *Lophomonas* infestation and chronic allergy was reported as a case report and a small case series (5, 6). Therefore, due to some common symptoms between asthma and Lophomonas infections, as well as the appropriate response to therapy with metronidazole, we decided to determine the frequency of hidden Lophomonas infections in asthmatic subjects and evaluate the response to a new imidazole, tinidazole, in asthma.

The question of this study was: Can *Lophomonas blattarum* be overlooked in individuals suffering from asthma, and will the treatment of Lophomonas provide additional benefits to asthma therapy? The study aimed to predict the frequency of *Lophomonas blattarum* in asthmatic subjects and to determine the impact of eradicating Lophomonas on the course of asthma treatment in conjunction with standard asthma therapy.

MATERIALS AND METHODS

Pretrial phase

All asthmatic patients referred to a private pulmonary clinic in Mashhad, Iran, from 2019 to 2020, were diagnosed and managed according to the guidelines of the GINA strategy. Subjects who were resistant to the combination of high-dose inhaled corticosteroids/long-acting beta 2 agonist and a second controller (montelukast or tiotropium) were enrolled in this study.

Participants

Fifty subjects aged over 15 years were enrolled in this active comparator, intention-to-treat, phase 3 clinical trial. Cough or dyspnea and AHR were mandatory criteria for enrollment of all subjects, while wheezing on physical examination, a history of similar disease, and typical spirometry findings, including high FENO, were optional. The subjects were randomly divided into two groups: one group received Tinidazole as the case group, while the comparison group received vitamin B1 as a placebo. All subjects continued their previous medications and were allowed to use a salbutamol inhaler in the event of an asthma attack.

Methods

After the enrollment, clinical findings, spirometry, FENO, and asthma control test (ACT) were evaluated for all subjects. The change in the ACT score was considered the primary endpoint. Cough and dyspnea severity were graded using methods described in a previous study (7). *Lophomonas blattarum* was evaluated using two methods: 1) Bronchial lavage obtained during bronchoscopy, 2) Sputum analysis obtained through spontaneous expulsion of sputum or the induced sputum method, as discussed previously (8). The bronchoscope was rinsed with purified water using an automatic machine to avoid external contamination, and bronchial lavage/sputum samples were passed to the parasitology lab within two hours. Microscopic evaluation of the specimens was performed as a wet preparation by the most experienced technician in the lab. Randomization was conducted with the aid of the randomizer website (https://www.randomizer.org), which gave us the permuted block randomized table, including the unique code for the drug package. The code was inserted into identical pockets for each subject, and the codes were revealed after the sampling. The required drug was administered to the subject, irrespective of the lab result. The rationale behind this strategy was the low sensitivity of direct smear, and therefore, we consider the response to treatment as indirect evidence of Lophomonas.

The case group received 500 mg of Tinidazole every 12 hours for 14 days. Vitamin B1 was chosen as the placebo in the control group due to its similar shape to the tablets. All

the drugs were extracted from their original placed in the new bottles, which were completely identical to each other.

Ethical considerations

This study was conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice Guidelines. The study was approved by the ethical committee of our local university (IR.IAU.MSHD.REC.1399.006). The study was discussed with all subjects, including the type of drug and placebo. All subjects signed a written consent form. The study was registered with the Iranian clinical trial registry (IRCT20091111002695N10).

Statistical analysis

The sample size was 50 subjects, which was calculated based on the frequency of *Lophomonas blattarum* in a previous study conducted in our local region (4). T-test, Mann-Whitney U test, and chi-square were used to evaluate the difference between the two groups, and NcNemar, paired t-test, and Wilcoxon rank test were used to assess the difference between before and after the trial. A p-value less than 5% was considered statistically significant.

RESULTS

Baseline data

Fifty subjects entered this clinical trial and finished the study. Table 1 shows the demographic data, which indicates no significant difference between groups (although the job variable appeared to differ, the statistical difference was not significant). All subjects had a chronic course (lasting more than three months), and chest x-rays were normal for all subjects. Workers expressed concern about air pollution, but none of the subjects reported a noteworthy change in symptoms in their workplace.

Direct smear showed *Lophomonas* in 52% of the Tinidazole group and 28% of the control group, which was statistically significant.

Clinical findings

Cough disappeared in 12 out of 25 (48%) in the Tinidazole group (P=0.001), and overall grading of cough showed significant improvement (Table 1). Complete resolution of dyspnea was detected in 4 (16%) subjects in the Tinidazole group, which was not significant; however, the Wilcoxon rank test showed a significant difference in the grading of dyspnea before and after the trial (Table 1). Tinidazole showed significant improvement in sputum production after the trial, regarding both amount and color (Figure 1).

Table1. Demographic data of the patients

	Unit - score	Total	Tinidazole	Placebo
Age	Years	4714.3	49.04±3.08	45.12±2.62
Male/female	ratio	1/1	11/14	14/11
Residency	Rural/urban	39/11	22/3	17/8
Job	Worker/employee	20/29	8/17	12.13

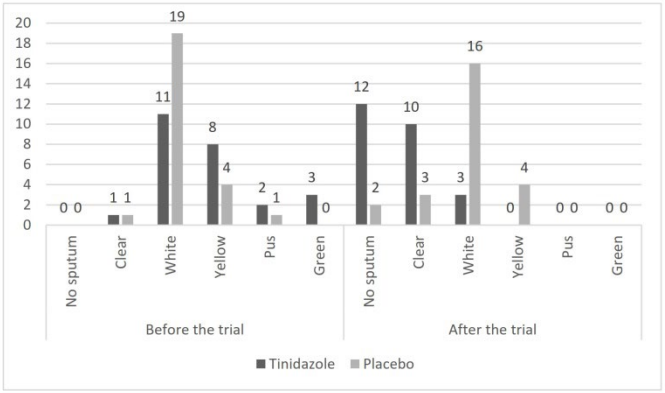

Figure 1. Comparison of the quality of sputum before and after the trial in the Tinidazole and control groups

The ACT score, as a primary outcome, showed a significant improvement after the trial in the Tinidazole group, which was not detected in the control group. Secondary outcomes, including postnasal drip (PND), wheezing, and airway hypersensitivity, showed significant improvement after the trial in the Tinidazole group, and the differences between the Tinidazole and placebo groups after the trial were significant (Table 1).

Objective paraclinical outcomes, including spirometry (FEV1) and FENO, showed significant improvement after the trial in the Tinidazole group. Specifically, FEV1 in the

Tinidazole group was significantly higher than in the control group, and FENO was significantly lower than in the control group. After the trial, direct smear for *Lophomonas* showed a positive result in one subject, which was significantly lower than before the trial (Tables 2,3).

Course

The course of treatment, side effects, and missing: Most of the Tinidazole group (16 out of 25) reported partial improvement in their disease, while eight subjects (32%) reported complete control of their chronic disease. The placebo group reported improvement in 5 subjects (20%), which was significantly lower than the Tinidazole group. Side effects were reported in two subjects who received Tinidazole, including bitter taste, tingling, and nausea. The control group reported no new side effects. All subjects completed the course of treatment and participated in the final visit after two weeks.

Table 2. Comparison of clinical findings between asthmatic subjects treated with tinidazole for *Lophomonas blattarum*, compared to placebo

Clinical findings			Before		After	
Temperature	**(°C)**	36.97±0.4	37.7±0.4	36.8±0.38	36.9 ±0.29	36.9 ± 0.35
Cough	Frequency	60 (100%)	30 (100%)	30 (100%)	18 (60%)*‡	28 (94%)
	No	0 (0%)	0 (0%)	0 (0.0%)	5 (20%)‡	0 (0%)
	Mild	5 (10%)	1 (4%)	4 (8%)	13 (52%)	5 (20%)
Cough Severity	Moderate	14 (28%)	6 (24%)	8 (32%)	5 (20%)	8 (32%)
	Severe	29(68%)	16 (64%)	13 (52%)	2 (8%)	12 (48%)
	Very Severe	2 (4%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)
PND[1]	Frequency	28 (56%)	16 (64%)	12 (48%)	3 (12%)*‡	9 (36%)
Dyspnea	Frequency	50 (100%)	25 (100%)	25 (100%)	21 (84%)*‡	25 (100%)
	No	0 (0%)	0 (0%)	0 (0%)	4 (8%) ‡ 16	0 (0%)
Dyspnea Severity	Mild	3 (6%)	1 (4%)	2 (8%)	(64%)‡	3 (12%)
	Moderate	21 (42%)	10 (40%)	11 (44%)	5 (20%)‡	15 (60%)
	Severe	26 (52%)	14 (56%)	12 (48%)	0 (0%)‡	7 (28%)
Sputum	Frequency	50 (0%)	25 (100%)	25 (100%)	13 (52%)‡	23 (92%)
Wheeze	Frequency	23 (46%)	17 68%)	18 (72%)	6 24%)‡	14 (56%)
Airway Sensitivity	Frequency	37 (74%)	19 (76%)	18 (72%)	6 (24%)‡	14 (60%)

*= significant difference between Tinidazole and placebo group

‡= significant difference between before and after the trial

1= post nasal drip, 2= Asthma control test, 3= Fraction of exhaled nitric oxide, 4= FEV1/ FEV1 predicted, 5=Bronchial lavage fluid

Table 3. Comparison of para-clinical findings between asthmatic subjects treated with tinidazole compared to placebo

			Before		After	
	Unit – score	**Total**	**Tinidazole**	**Placebo**	**Tinidazole**	**Placebo**
ACT[1] **score**	Score	16.7±2.73	16.84±2.65	16.56±2.85	23.11±2.58*‡	17.84±2.40
FENO[2]	PPM	34.44±10.34	35.32±10.11	33.56±10.71	22.72±9.59*‡	30.40±8.06‡
FEV1 %pred[3]	%	70.72±7.95	69.04±7.90	72.4±7.80	77.08±6.37*‡	72.60±6.58
***Lophomonas* in Sputum or BLF**[4]	Frequency	20 (40%)	13 (52%)	7 (28%)	1 (4%)	5 (20%)
Inflammatory cells	Eosinophil	17 (28%)	9 (30.0%)	8 (26.7%)	8(26.7%)	9 (30%)
	Neutrophil	23 (38%)	10 (33.3%)	13 (43.3%)	12(40.0%)	12 (40%)
	Mixed	7 (12%)	2 (6.7%)	5 (16.7%)	0 (0.0%)	3 (5%)
	Paucigraneulocytic	13 (22%)	9 (30.0%)	4 (13.3%)	10(33.3%)	16 (26.7)
Overall recovery	Complete improvement				8(32%)	4 (16%)
	Incomplete improvement				16(64%)	1 (4%)
	No change				1 (4%)	16 (64%)
	Worsened				0 (0.0%)	4 (16%)

*= significant difference between the Tinidazole and placebo groups.

‡= significant difference between before and after the trial

1= ACT= asthma control test, 2= FENO= Fraction of expiratory nitric oxide, 3= Forced expiratory volume in one second percent predicted, 4= Bronchial lavage fluid

DISCUSSION

This study aimed to elucidate the importance of *Lophomonas blattarum* in subjects whose clinical diagnosis was asthma. The participants were asthmatic individuals who were resistant to standard therapy outlined in the phase 4 GINA strategy (9). The study has two objectives: first determine the frequency of the *Lophomonas blattarum* in bronchial specimens, including lavage fluid or sputum, and second, to assess the effect of anti-*Lophomonas* treatment on the course of asthma, irrespective of laboratory result. Direct smear for *Lophomonas* showed the frequencies of 28% in the control group and 52% in the case group. This may be related to considering a positive result in case of a good response to treatment, which was evident in the Tinidazole group. However, the frequency was high in both the case and control groups, exceeding the researcher's expectation at that time. This high frequency of *Lophomonas* in asthmatic subjects was later reported in a similar study conducted at an experienced research center (45%) (10). *Lophomonas* respiratory disease has been reported in both immunocompetent and immunodeficient subjects (11). Inhaled corticosteroids, which are commonly used for the treatment of asthma, may cause immune deficiency in the bronchial mucosa. If this is the case, the low immune state at the surface of the bronchi can explain the high frequency of Lophomonas. Ding et al. reported the clinical findings of 57 subjects suffering from respiratory symptoms in China (12). They ruled out infectious causes and identified asthmatic symptoms in 32 subjects, including blood eosinophilia and increased CD8/CD4 ratio. We believe that *Lophomonas* is capable of disrupting the epithelial barrier through enzymatic reaction, similar to host dust mite (13) and cockroach-induced asthma (14).

Treatment of *Lophomonas* by Tinidazole showed promising results, as cough, dyspnea, airway hyper-reactivity, and wheezing improved. Approximately 30% of patients considered it a complete resolution of the disease. Nevertheless, the clinician of the present study advised patients to continue the routine asthma therapy. Post-nasal drip also improved significantly, highlighting the role of *Lophomonas* in upper airway diseases, including chronic rhino-sinusitis, as previously reported (15).

Lophomonas blattarum presented with chronic respiratory symptoms, typical spirometry findings, high FENO, and a normal chest X-ray, making it very similar to asthma. Therefore, this condition can be very confusing in clinical practice, and these diagnostic tests may not effectively differentiate asthma from *Lophomonas* infestation.

In this study, the main limitation was the low sample size. Given this sample size, we consider this study a pilot study. The most significant barrier to obtaining a larger sample size was the collection of bronchial samples. Bronchoscopy is an invasive procedure that can be done only in research projects for asthmatic subjects, and while induced sputum can be obtained with premedication, it may inadvertently trigger asthma attacks. PCR, as a new diagnostic technique, has improved the accuracy of detecting Lophomonas (10,16). However, it still requires bronchial secretions. Therefore, in the future, an accurate, practical, and safe diagnostic test for detecting Lophomonas in the sputum of asthmatic subjects is needed.

In this study, a direct smear of the wet specimens was used for the diagnosis of Lophomonas. Previous studies recommended trichrome staining as the best staining for the diagnosis of Lophomonas (17); however, our technician preferred to use a wet smear because it could detect the movement of flagella. In addition to smear, response to therapy with anti-protozoa (Tinidazole) was considered as indirect evidence of Lophomonas. Nonetheless, we should remember that these anti-protozoan agents can be effective on similar protozoa with pulmonary involvement, such as other protozoa in the superfamily of *hypermastigotes*, which are reported to improve with the Imidazole anti-protozoan group (18). Therefore, the good result of treatment with tinidazole, just shows a new treatment for persistent asthma, in the future.

CONCLUSION

In conclusion, *Lophomonas blattarum*, as a lung infestation, is a significant cause of asthma, including persistent asthma. In case of severe persistent asthma that requires initiation of phase 5 medications of the GINA

strategy, we recommend evaluating *Lophomonas* or considering a course of tinidazole or metronidazole.

Funding source

This study was financially supported by the Deputy of Research at Islamic Azad University, Mashhad Medical Science Branch, Iran.

Conflicts of Interest

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

Acknowledgment

The author of this study acknowledges Mr. Parian for the precise evaluation of the Lophomonas wet smear.

REFERENCES

1. Nakhaei M, Fakhar M, Sharifpour A, Ziaei Hezarjaribi H, Banimostafavi ES, Nazar E. Global Status of Emerging *Lophomonas* Infection: A Systematic Review of Reported Cases (1993-2020). ***Interdiscip Perspect Infect Dis*** 2022;2022:3155845.
2. Martinez-Girón R, Cornelis van Woerden H. Lophomonas blattarum and bronchopulmonary disease. ***J Med Microbiol*** 2013;62(Pt 11):1641-8.
3. Fakhar M, Sharifpour A, Nakhaei M, et al. Lophomonas and Lophomoniasis," Noorouzi Publisher. Gorgan, Iran: First edition; 2021. pp. 178. [In Persian]
4. Anhaee Nasseri Z, Mirsadraee M, Manafi Varkiani M, Ghaderi Y, Berenji F, Ghaffari S. Effective Treatment of Chronic Cough with Tinidazole as the Newest Antiprotozoa against *Lophomonas blattarum*. ***J Parasitol Res*** 2022; 2022:2413941.
5. Jorjani O, Bahlkeh A, Koohsar F, Talebi B, Bagheri A. Chronic Respiratory Allergy Caused by Lophomonas blattarum: A Case Report. ***Medical Laboratory Journal*** 2018;12(2): 44-6.
6. Mirzazadeh F, Berenji F, Amini M, Salehi M, Shamsian A, Fata A, et al. Lophomonas blattarum in asthmatic patients and control group. ***Journal of Research in Medical and Dental Science*** 2017;5(5):1-5.
7. Mirsadraee M, Dehghan S, Ghaffari S, Mirsadraee N. Long-term effect of antifungal therapy for the treatment of severe resistant asthma: an active comparator clinical trial. ***Curr Med Mycol*** 2019;5(4):1-7.
8. Mirsadraee M, Sabbagh Sajadieh Z, Ghafari S, Tavakoli A, Sabbagh Sajadieh S. Cromolyn, a New Hope for Limited Treatment of Neutrophilic Asthma: a Phase II Randomized Clinical Trial. ***Tanaffos*** 2019;18(3):208-214.
9. Global Initiative for Asthma. Global Strategy for Asthma Management and prevention, 2018. Available from: www.ginasthma.org.
10. Fakhar M, Nakhaei M, Sharifpour A, Safanavaei S, Abedi S, Tabaripour R, et al. Morphological and Molecular Identification of Emerged Lophomonas blattarum Infection in Mazandaran Province, Northern Iran: First Registry-Based Study. ***Acta Parasitol*** 2021;66(4):1510-6.
11. Ribas A, Martínez-Girón R, Ponte-Mittelbrum C, Alonso-Cuervo R, Iglesias-Llaca F. Immunosupression, flagellated protozoa in the human airways and metronidazole: observations on the state of the art. ***Transpl Int*** 2007;20(9):811-2.
12. Ding Q, Shen K. Pulmonary Infection with Lophomonas blattarum. ***Indian J Pediatr*** 2021;88(1):23-7.
13. Heijink IH, van Oosterhout A, Kapus A. Epidermal growth factor receptor signalling contributes to house dust mite-induced epithelial barrier dysfunction. ***Eur Respir J*** 2010;36(5):1016-26.
14. Martínez-Girón R, Ribas A. Asthma, cockroaches, and protozoal forms: chance or not chance? ***Ann Allergy Asthma Immunol*** 2006;97(6):818-9.
15. Berenji F, Parian M, Fata A, Bakhshaee M, Fattahi F. First Case Report of Sinusitis with Lophomonas blattarum from Iran. ***Case Rep Infect Dis*** 2016;2016:2614187.
16. Mokhtarian K, Taghipour S, Nakhaei M, Taheri A, Sharifpour A, Fakhar M, et al. Molecular Evidence of Emerged Pulmonary Lophomoniasis due to *Lophomonas blattarum* among Hospitalized Patients in Southwestern Iran: A National Registry-Based Study. ***Interdiscip Perspect Infect Dis*** 2022;2022:6292823.
17. Alam-Eldin YH, Abdulaziz AM. Identification criteria of the rare multi-flagellate Lophomonas blattarum: comparison of different staining techniques. ***Parasitol Res*** 2015;114(9):3309-14.
18. Zhou YP, Zhu XJ, Li M, Liu H, Chen YS. Clinical analysis of bronchopulmonary infection with hypermastigotes: a report of two cases and review of the Chinese literature. ***Zhonghua Jie He He Hu Xi Za Zhi*** 2006;29(1):23-5.