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Short Communication

Detection of *Lophomonas blattarum (Order: Hypermastigida)* from Iranian Patients with Allergic Rhinitis

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Abstract

Background: Allergic rhinitis (AR) is one of the most common allergic diseases triggered by indoor and outdoor allergens. Certain arthropods, such as mites and cockroaches, contain protozoa like *Lophomonas blattarum* in their intestines to help with digestion that may have some role in AR. We aimed to determine the frequency of *L. blattarum* in nasal smears of patients with AR in comparison with healthy controls.

Methods: In this prospective cross-sectional study (March 2015-March 2016), 36 patients with a clinical presentation of AR (with a positive prick test including mites) and 34 normal controls were included at ear, nose, and throat (ENT) clinic at Imam Reza Hospital of Mashhad, Iran. Nasal secretions were evaluated to examine presence of *L. blattarum* in the patients and control group by direct method. Diagnosis of *L. blattarum* was based on microscopic observation both on direct smear and Giemsa stained specimens.

Results: Patients with AR had a higher frequency of *L. blattarum* in their nasal smears than the control group (25% vs. 2.9%) (P=0.001).

Conclusion: We found *L. blattarum* more frequently in the nasal secretion of AR patients compared with healthy subjects; this protozoon may have some role in this condition. However, the relationship between *L. blattarum* and AR requires further studies to allow a greater understanding.

Introduction

Allergic rhinitis (AR) is one of the main allergic diseases triggered by house dust mite allergens as well as outdoor allergens. Certain arthropods, such as mites and cockroaches, carry protozoa in their intestines, which help them in their digestive process. With present medical means, allergic rhinitis remains inadequately controlled. Researchers isolated flagellated protozoa from the gut of the mite under a light microscope (1). Xia et al. examined the structural features, transmission, clinical features, laboratory diagnosis, and treatment of *L. blattarum* (2).

Pathogens including bacteria and protozoa are absorbed into the respiratory pathways through the inhalation of mite feces (3). One of the components of the vast range of the inflammatory diseases, which involve the unified airway, is allergic rhinitis (AR). Therefore, the protozoa are expected to occur at any point in the airways, including the nasal cavities.

These inhaled house dust mite protozoa might have possible pathogenic effects on the nasal epithelium which can be explained in the following ways: First, many inflammatory processes may depend on toll-like receptors (TLRs), and TLR signaling may be involved in the pathogenesis of airway inflammatory diseases (4). The crucial role of TLRs in the recognition of microbial pathogens including protozoa have been heavily emphasized in in vitro studies. The function through inducing signals is responsible for an effective host defense, especially pro-inflammatory cytokines (5). Second, similarly to alterations produced by disruption of the epithelial barrier, as is the case of flagellated protozoa such as *Trichomonas vaginalis* and *Giardia duodenalis (6, 7)*. A disruption of epithelial tight junctions might occur due to the proteases secreted by multiflagellated protozoa, which in turn increase airway permeability, increasing a loss of epithelial barrier function. Asthma is a defect in epithelium barrier function that causes an infiltration of the airway tissue through allergens and pathogens; *L. blattarum* was found in the sputum of asthmatic patients, but this required further evaluation (4, 8). Allergic rhinitis can be developed by this protozoan like asthma; however, despite its significance, scarce organized study has been conducted in this filed, but one study (4).

We aimed to examine this protozoan in the nasal smears of patients with persistent AR and compare the findings with a healthy control group.

Methods

This was a pilot cross-sectional study (March 2015-March 2016). Because no previous studies had been reported on this subject, we determined our sample size based on the requirements of a pilot study. We selected all patients who came to the Ear, Nose, And Throat (ENT) Clinic, Imam Reza Hospital, Mashhad, Iran as a tertiary center with clinical symptoms and signs of rhinitis, including nasal obstruction, watery rhinorrhea, and nasal itching and sneezing.

As the diagnostic gold standard, a prick test (mite included) was performed for all patients to confirm the allergic basis for their rhinitis. Thirty-six patients with a positive prick test including dust mite were evaluated by direct nasal smears to examine presence of *L. blattarum* directly. Secretions of patients' and control's nose blowing was examined directly and immediately and for those with difficulty to blow the nasal secretion was gathered by nasal swab.

Diagnosis of *L. blattarum* was based on microscopic observation both on direct smear and stained specimen by two parasitologists of Parasitology Laboratory of Imam Reza hospital of Mashhad, Iran separately (Fig.1A, 1B).

All samples were stained by Giemsa (Fig.1C) for more clarification. Important morphological characteristic features of *L blattarum* are listed as follow: 1. Round to ovoid flagellates measures 20-60 micron 2. *L.blattarum* has numerous irregular flagellates 3. The nucleus is under the flagellates' origin 4. It has no terminal bar while the bronchial cells have a terminal bar and otherwise nucleus in bronchial cell is visible at the posterior end of the cell (9).

Thirty-four volunteers 'healthy subjects who accompanied the patients of Otolaryngology Clinic with no history of recurrent rhinitis or AR were involved as control group in this project. This entire control group was evaluated by ENT physical exam and they did not have any problem. We did not evaluate control patients with Prick test because they did not have any indication for this test. Nasal secretion of control group examined for *L. blattarum,* using the same method. The parasitologists responsible for evaluating the protozoa were blinded to the study group. They did not know anything about the patients and control group. They only examined and reported the nasal smear results.

This study was approved by the ethics committees of Mashhad University of Medical Sciences (MUMS). All patients and controls provided written consent prior to participation in the study, according to the requirements of the ethics committee (Ethical Code: IR.MUMS.fm.REC.1395.203).

Statistical analyses were performed using statistical software (SPSS version 16; SPSS Inc., Chicago, IL). Absolute numbers and percentages were computed to describe data. Data were expressed as mean ± standard deviation (SD) for continuous variables. Categorical variables were compared between the two groups using the chi-square test, and the *t*-test was utilized to analyze continuous variables between the two groups. Differences were considered to be statistically significant for $P<0.05$.

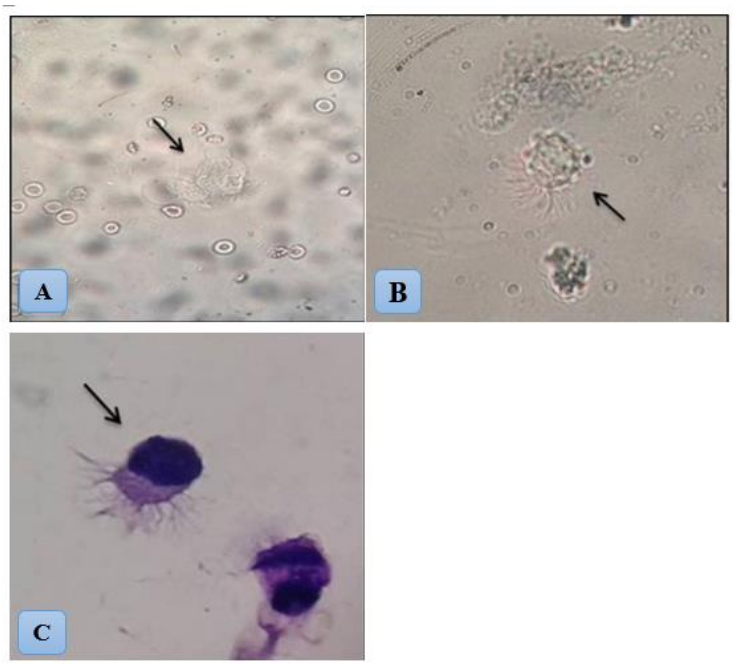

Fig. 1: Trophozoites of *Lophomonas blattarum* in nasal secretion (Direct smear) (×400) (A, B), Giemsa stain (×1000) (C)

Results

There were 21 male (58.3%) and 15(41.75%) female subjects in the case group and17 (50%) male and 17(50%) female subjects in the control group (*P*=0.48). The mean age in the case group was 27.8±11.2 years compared with 32.35±7.1 years in the control group (*P*=0.0669> 0.05).Watery rhinorrhea, nasal itching, nasal congestion and sneezing were seen in 100%, 61%, 81.6%, and 47.2% of patients, respectively in case group. Five cases (13.9%) had underlying diseases including asthma 4(11.1%) and chronic rhinosinusitis 1 (2.78%). None of the patients had gastro-esophageal reflux disease (GERD), addiction, or a history of cigarette smoking.

Among AR patients,9 cases (25%) had a positive nasal smear for *L. blattarum* while there was only one (2.9%)positive nasal smear for *L blattarum* in the control group.This difference was statistically significant (*P*=0.008). All patients had persistent AR checked for 15 common regional allergens with prick test. The results of prick tests and common allergens are mentioned in (Table1).

Table 1: Common allergens among allergic rhinitis patients

Allergen	*Frequency*	*Percent*
Russian thistle	19	51.35
Ash	21	56.75
Grass mix	12	32.43
Tree mix	20	54.05
Lamb's quarter	11	29.72
Cockroach	12	32.43
Dermatophagoides farine	10	27.02
Dermatophagoides pteronyssinus	13	35.13
Pigweed mix	23	62.16
Alternaria	5	13.51
Aspergillus	4	10.81
Cat fure	10	27.02
Feather	17	45.94
Penicillum	4	10.81
Cladosporium	25	67.56

Discussion

AR as a common human disorder needs greater investigation with respect to pathophysiology, etiology, and management. Although recent data and our previous knowledge clarify many aspects of this condition, there is still much to understand. The basis of management of the disease is the detection of causative agents, allowing individuals to avoid these factors.

While there are some studies on the relationship between *L. blattarum* and bronchopulmonary diseases, including asthma (8, 10, 11), there is no study in the field of AR and lophomoniasis. Through a search of the literature, we identified some case reports describing this protozoan in the sinonasal area. Wang Hue et al. reported one case of *L. blattarum* in maxillary sinusitis in 1998 (12), while Gunzhong et al. reported two cases of *L. blattarum* in 2008 and 42 cases in 1993–2007 (13) and Berenji et al. reported the first case of rhinosinusitis with *L. blattarum* in in Iran (14). Finally, Rafael Martinez et al. reported a 23-year-old man with a 15-year history of AR

with protozoa in his nasal secretion in 2009, and suggested a probable relationship between AR and a flagellated protozoan (4). In addition, a case of this protozoon has been reported by Jorjani et al. in a 37-year-old male with chronic respiratory allergy (15).

Ribas et al. found a significant similarity between extracted protozoa from mites and protozoa in the sputum of asthmatic patients and the nasal secretions of AR patients and suggested a probable relationship between these organisms and AR (1). Martinez et al. investigated the relationship between *L. blattarum* and respiratory problems in 2014 and concluded that the diagnosis and treatment of this organism is very important (11). Berenji et al. examined the alveolar contents of patients with respiratory disorders who were candidates for broncho-alveolar lavage (BAL). One-hundred-and-seventy BAL samples were evaluated in this study, of which 45 were positive for *L. blattarum* (10). In a progressive study, the effects of battlefield exposure on respiratory and sinonasal diseases were examined. Exposure to chemical agents may have lasting respiratory effects. The reported upper respiratory manifestations include rhinorrhea, congestion, and acute sinusitis (16). In accordance with the unified airway theory, we found a high frequency of 25% for this protozoan in patients with AR, which could indicate a significant association between the presence of *L. blattarum* and the symptoms. Based on the unified airway theory, we can deduce that such associations between AR and *L. blattarum* is conceivable.

One problem in the examination of a nasal smear for this protozoan is the similarity with ciliated respiratory epithelial cells, which could cause a misdiagnosis of the pathological condition in the normal environment of the nose. We tried to control for this bias by adopting a blinded study design and using two separate parasitologists.

It is a difficult task for those who are not familiar with the microscopic evaluation technique of *L. blattarum* and differentiate it from respiratory ciliated cells. It has not been possible to make an appropriate culture medium or specific gen analysis for this organism yet so experts' observation under microscope is currently the only option for detection of this multi-flagellated protozoan.

A limitation of our study was not to confirm the results by the molecular method for this protozoan. In spite of diagnostic limitation, we believe in our preliminary study on investigating the association between persistent AR and this protozoan may lead to future studies to explore further aspects of this association. Exploration of any aspects of AR pathophysiology has an important role in the management of this condition by controlling this protozoan environmentally as a probable causative agent that could trigger the allergic cascade.

In most of the above-mentioned studies, most patients with respiratory symptoms had a complete immune system. In addition, in this research all the patients were immune competent. As a result, considering that the clinical respiratory symptoms in different respiratory disorders associated with this protozoan are significant, it can be even considered as a probable pathogenic protozoan. However, there is still a need for more research around the world on patients who have a history of respiratory signs with presence of *L. blattarum* and poor response to the antibiotics.

Furthermore, future studies on molecular diagnosis of *L. blattarum* and the treatment of *L. blattarum* and the effect of treatment on clinical and laboratory findings of patients with AR is recommended.

Conclusion

We found *L. blattarum* more frequently in the nasal secretion of AR patients compared with healthy subjects. However, the relationship between this protozoa and AR requires

further studies to allow a greater understanding.

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Conflict of interest

The authors declare that there is no conflict of interests.

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