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Unexpected high rate of *Lophomonas blattarum* in resistant upper and lower respiratory infection

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ABSTRACT

Lophomonas blattarum inhabits the intestines of cockroaches and mites. This study aimed to evaluate the existence of this protozoan parasite in bronchoalveolar lavage (BAL) samples of patients with respiratory disorders and nasal secretion samples of patients with sinusitis resistant to common treatments in Mashhad, Iran (between September 2014-October 2015). 133 samples were obtained from the patients, including 127 BAL samples (95.4%) and six nasal secretion samples (4.6%). Unexpected high rate of rarely known parasite "L blattarum" and its connection to upper respiratory infections and pneumonia cases were discovered. In total, 50 samples were positive for L. blattarum infection, 45 of which (33.8%) were BAL samples and five cases (3.7%) were nasal secretion samples. Since delayed diagnosis and treatment of pulmonary and sinus infection in our region due to L.blattarum contributes to higher mortality and morbidity, therefore, L.blattarum infection requires special attention as an emerging pathogen globally.

Keywords: Lophomonas blattarum; pulmonary infection; sinusitis; emerging disease

INTRODUCTION

Lophomonas blattarum is a protozoan parasite, which belongs to the Phylum of flagellates and super group of excavata. Two of the main species in this subfamily are *L. blattarum* and *L. Striata*, which belong to the class of *Parabasalia* [1]. *L. blattarum* inhabits the intestines of different insects, namely cockroaches, termites and mites. Risk of *L. blattarum* infection increases in patients with pulmonary diseases if they live in infested environment by cockroaches, termites and dermatophagoides mite [2]. Cockroaches are considered as vectors for some diseases, and due to the large number of termites and cockroaches in South China, most cases of *L. blattarum* infection have been reported in this country.

Although *L. blattarum* is a rare parasite, it is considered as a potential pathogen for bronchial and respiratory tract infections and various respiratory symptoms. This parasite has been detected in children with pneumonia and asthma [1]. Mechanisms of the damage caused by *L. blattarum* involve the adhesion or reduced function of the epithelial

cells and cell apoptosis. Clinical symptoms of *L. blattarum* infection are similar to other respiratory diseases, including high fevers, mild coughs with discharge of white, bloody purulent sputum, and sneezing due to the incompetence of the respiratory tract for purulent discharge. Moreover, *L. blattarum* infection is associated with reduced lung capacity, and in general, all the symptoms of this disease are similar to asthma [3-5].

This protozoa was first identified by Lee and Brugerolle through observing the morphological features of human biological samples using optical microscopes. *L. blattarum* is 20-60 micrometers long and 12-20 micrometers wide with granular cytoplasm, which contains phagocytized material. In one of the poles of this protozoan, there are large numbers of flagellates with different rotation directions [3, 5]. The nucleus of this parasite is typically seen as dark and circular, and the cystic form has been observed in both types of *L. blattarum*. Surrounded by the membrane, this cyst has a spherical form and contributes to the survival of the protozoan in harsh conditions.

L. blattarum damages pulmonary epithelial cells, and the infection caused by this parasite could be diagnosed by bronchoscopy and computed tomography [3-5]. Thin bronchiols filled with mucus could be seen in bronchoscopy. Moreover, increased amount of eosinophils in the peripheral blood has been reported in some cases [3].

In sampling for the detection of *L. blattarum* infection, provided smears must be fresh, and samples should be collected from the airways (e.g., bronchoalveolar lavage (BAL))and tracheal fluid [3-5]. Under optical microscopes, evaluation of morphological features of *L. blattarum* could be performed on fresh smears or samples stained by methods such as Giemsa, Papanicolaou and Trichrome [5]. Distinguishing these poly-flagellated protozoa from epithelial cells is difficult, and there is the possibility of misdiagnosis in samples observed by optical microscopes. However, the risk of misdiagnosis is likely to diminish with the advancement in different molecular approaches [6]. Treatment of *L. blattarum* infection generally involves the use of metronidazole, which is commonly prescribed for protozoan diseases and has favorable effects on these infections. Alternative treatments for *L. blattarum* infection include the use of tinidazole and albendazole [3-5].

To date, several studies have focused on *L. blattarum* infection, 86.9% of which are from China, 9.8% are from Peru, and 3.3% are from Spain. It is noteworthy that most cases of this infection have been reported in men [3]. Recently, a few cases of sinusitis and pneumonia with various lung lesions have been reported in Mashhad, located in northeast of Iran. This study aimed to evaluate the prevalence of *L. blattarum* protozoan parasite in bronchoalveolar lavage (BAL) samples of patients with respiratory disorders and nasal secretion samples of patients with sinusitis resistant to common treatments.

MATERIALS AND METHODS

This study was conducted during September 2014-October 2015. Sampling was performed on patients with respiratory disorders who were candidates for BAL. Informed consent was obtained from all patients prior to the study, and required data were collected using prepared checklist.

After bronchoscopy and normal saline wash, alveolar contents of the patients were extracted and sent to parasitology laboratory of Imam Reza Hospital of Mashhad, Iran. Initially, direct smears were obtained to evaluate the presence of *L. blattarum*, and afterwards, Giemsa and Papanicolaou staining were performed on the smears.

In some of the patients with symptoms of sinusitis, samples were obtained from nasal secretions. Direct microscopic examination and staining were performed on these samples. SPSS 11.5 software (SPSS Inc., Chicago, Illinois, USA) was used for all statistical analyses. Standard descriptive statistics were applied to describe the pattern of the data. Prevalence of *L. blattarum* in the collected samples was determined. Chi-square test was used to examine the significance of the association between categorical data. Normality of the data was checked with Kolmogorov–Smirnov test. Mann-Whitney-U test were applied in case. All tests were 2-tailed, and probability values 0.05 were considered significant.

RESULTS

In total, 133 samples were evaluated in this study, including 127 BAL samples and six nasal secretion samples. According to our observations, 50 samples were positive for *L. blattarum* infection, 45 of which (33.8%) were BAL samples, and five cases (3.7%) were nasal secretion samples. Among positive BAL samples for *L. blattarum* infection, 26(57.7%) and 19(42.3%) cases belonged to male and female patients, respectively (P-valu=0.75). As for nasal secretion samples, two (40%) and three cases (60%) were positive for *L. blattarum* infection in male and female patients, respectively.

In this study, patients with positive nasal secretion samples were within the age range of 31-58 years, with mean age of 43.14 years. Patients with positive BAL samples for *L. blattarum* infection were within the age range of seven months to 84 years, with mean age of 37.85 years. Figure 1 depicts the distribution of *L.blattarum* infections according to age groups.



Figure 1. Diagram of distribution of *L. blattarum* infections according to age groups

Clinical symptoms in patients with positive BAL samples for *L. blattarum* were fever (22.22%) and chills (15.55%), perspiration (13.33%), haemoptysis (24.44%), sputum discharge (22.22%), shortness of breath (31.11%), and chest pain (20%). Moreover, radiography of some of these patients revealed other symptoms such as pulmonary infiltration, pulmonary cavity (4.44%), lung collapse (4.44%), atelectasis, and in peripheral blood eosinophilia (30%). The main clinical symptoms in patients with sinusitis included allergic rhinitis, persistent sneezing, headache, earache, and chronic sinusitis irresponsive to common medications.

Prevalence of underlying diseases among 35 studied patients is presented in Table 1.

Diseases	Patients (%)
Mucormycosis*	1 (2.85)
Brain Tumor	1 (2.85)
Kidney Transplant	4 (11.42)
Diabetes	2 (5.71)
Heart Failure	1 (2.85)
Sinus Adenocarcinoma	1 (2.85)
Chronic Leukemia	1 (2.85)
Dermatomyositis and Corticosteroid Therapy	1 (2.85)
Cystic fibrosis	1 (2.85)
Wegener's Granuloma and Corticosteroid Therapy	1 (2.85)
Hodgkin Lymphoma	1 (2.85)
Hydatid cyst of lung	1 (2.85)
Acute Leukemia	1 (2.85)
Heart Transplant	1 (2.85)
Metastatic cancer of lung	1 (5.71)
Renal failure	1 (2.85)
Addiction	3 (8.57)
Chronic bronchitis	1 (2.85)
Corticosteroid Therapy	5 (14.28)
COPD	4 (11.42)
Tuberculosis	1 (2.85)
Total	35

DISCUSSION

According to previous studies, rate of pulmonary infections caused by protozoa has increased significantly during recent decades. However, no cases of these infections have been reported in Iran. This emerging pulmonary infection normally occur with non-specific symptoms in the presence of immunosuppression.

L. blattarum infection should be considered in the differential diagnosis in immunocompromised patients presented with symptoms such as pulmonary infection, asthma, fever and coughs and dyspnea, who may also have eosinophilia and lack of response to common treatments [5, 7-10].

In Mashhad (Iran), *L. blattarum* was first observed by a laboratory employee in his own nasal secretion. The person had previously showed symptoms of sinusitis for a few months, and the disease could not be treated by common medications, such as antibiotics [11].

After the identification of this protozoan, we decided to evaluate the prevalence of this parasite in laboratory samples of BAL referred to our lab and also nasal secretion obtained from patients with sinusitis resistant to common treatments in Imam Reza Hospital in Mashhad, Iran. For this purpose, we collected and evaluated 133 samples, including 127 BAL samples and six nasal secretion samples.

According to our results, 33.8% of BAL samples were positive for *L. blattarum* infection, which was significantly higher than the value obtained by another study conducted in Turkey (2014) on immunocompromised patients (8.2%) [12]. However, it should be noted that our samples were collected from different patients and not immunocompromised patients only.

In another study performed in Spain in 2005, Ribas et al. evaluated HIV-positive and HIV-negative patients in terms of *L. blattarum* infection. According to the findings, the protozoa had a higher prevalence in HIV-positive patients (86.7%). In addition, 34.8% of HIV-negative patients were positive for *L. blattarum* infection, which is in agreement with the results of the present study.

Although in most recent studies reported L. *blattrum* as a rare infection, in this research unexpectedly we found many cases with positive sample for *L*. *blattarum* in Mashhad, Iran. It must be mentioned that Mashhad is the second largest city of Iran and also it is the major center of tourism in Iran. Authors believe that the rarity of this parasite may be due to many facts as below:

1. This parasite is unknown in many countries and to many pulmonologists, because many of our cases in this study refer to our lab for diagnosis of other infections like nocardiosis or pnuomocystosis, and because of awareness of this infection we diagnosed this protozoa as a causative agent of pulmonary and sinus infection in present study. After reporting *Lophomonas* as a causative agent and introducing it to clinicians during the period of this research they are more aware of this parasite and for treatment use metronidazole and with near 99% cure rate.

2. Diagnosis of this parasite is difficult and may be mistaken by bronchial epithelial cells and lab technicians must be aware of the features of this protozoa for differential diagnosis from bronchial epithelial cells. The authors believe that many cases of *Lophomonas* infection may be undiagnosed all over the world.

In the current study, patients with positive pulmonary secretions for *L. blattarum* infection were within the age range of 31-58 years, with mean age of 37.85 years. Mean age of these patients has been estimated at 45 years in China [2]. In only one study performed in Peru, Zerpa (2010) reported some cases of *Lophomonas* infection in children [12]. In the present study, 30% of positive samples for *Lophomonas* infection accounted for infants and children, the majority of whom were aged less than 12 years. These patients were mainly presented with symptoms such as chronic pulmonary infection resistant to treatment, chronic persistent coughs, and atelectasis.

According to statistics in different countries, 70% of *Lophomonas* infections have been reported in men [2]. Similarly, this infection was found to be more prevalent among male patients (57.7%) compared to female ones (42.3%) in our study.

Lophomonas infection is associated with various clinical symptoms, ranging from mild coughs and wheezing to respiratory insufficiency, purulent discharge, high fevers, and symptoms of pulmonary consolidation [4, 13]. Patients in the current study showed many of these symptoms, including coughs, sputum discharge, shortness of breath, hemoptysis, pulmonary cavity and atelectasis. Furthermore, almost all the patients effectively responded to metronidazole treatment, and only one patient with cystic fibrosis died during the course of study.

With respect to underlying diseases, several cases were observed among our patients, including different types of cancer, chronic obstructive pulmonary disease, kidney transplant, Wegener's granulomatosis, cystic fibrosis, and diseases associated with the long-term use of corticosteroids (Table 1). This finding is consistent with the results obtained by another research in China [14].

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In the present study, treatment with metronidazole resulted in the improvement of the patients with positive nasal secretions for *L. blattarum* infection who had acute and chronic sinusitis symptoms irresponsive to antibiotics. In the literature, only a few cases of sinus infection accompanied with *L. blattarum* infection have been reported [5, 11]. In our study, one of the patients with sinusitis used metronidazole and corticosteroid spray simultaneously, which exacerbated the symptoms. However, the patient had a significant improvement with after the elimination of corticosteroids.

Previous studies have confirmed the possibility of cyst formation in *L. blattarum*, which similarly occurs in other protozoa in harsh conditions [4]. This parasite could be transmitted through the inhalation and ingestion of parasite cysts excreted from the intestines of cockroaches.

Transmission of this infection has been deemed unclear in some studies, while a number of researchers have confirmed that the parasite is transmitted through the cysts excreted from cockroach faeces [1, 4]. In the present study, trophozoite of *L. blattarum* was frequently found in the sputum or nasal secretion samples of the patients (Figure 2 and 3).



Figure 2. Trophozoite of *Lophomonas blattarum* with irregular long flagella in BAL specimen at right (Papanicolaou stain), a bronchial epithelial cell at left is seen with regular short cilia .(×1000)



Figure 3. Trophozoite of *Lophomonas blattarum* in BAL specimen. Papanicolaou (PAP) stain.(×1000)

Some scholars believe that *L. blattarum* could be transmitted through the dust contaminated with cockroach faeces. Furthermore, considering the presence of *L. blattarum* cysts in the pulmonary secretions of some patients, during this study we had an assumption of *Lophomonas* transmission may be due through the respiratory particles coming from the coughs and sneezes of the infected patients. We believe that lack of hygiene and improper disposal of sputum may have role to the transmission of *L. blattarum* through dust and other floating particles.

According to the findings of the present study, direct observation of movement of flagellated parasites is more efficient compared to staining methods [4]. (Video 1, 2). It is noteworthy that many researchers believe that several cases of remain undiagnosed due to the similarity of the parasite to bronchial epithelial cells.

Identification of flagellated protozoa in symptomatic patients who are responsive to anti-protozoal treatments could be considered as *Lophomonas* infection. It is likely that this parasite commensally hosts the human body and it could become pathogenic under certain circumstances. Considering the variable morphological features in microscopic examinations, it is assumed that *Lophomonas* could have different species.

In general, the authors of this paper believe that *L. blattarum* infection must be considered as an emerging disease affecting human lungs and sinuses. In this regard, use of appropriate culturing methods and molecular evaluation of this parasite could pave the way for further research.

In conclusion unexpected high rate of rarely known parasite "*L blattarum*" and its connection to upper respiratory infections and pneumonia cases were discovered. *L. blattarum* infection should be considered in patients with pulmonary symptoms in immunodeficiency, and underlying medical conditions (even in those without immunodeficiency). Additionally, *L. blattarum* infection must be considered in chronic sinusitis irresponsive to common medications. Furthermore, pulmonary secretions should be evaluated with regards to *Lophomonas* infection in patients with fever and pulmonary symptoms (e.g., hemoptysis, lung collapse and pulmonary cavity). As for cases with sinusitis, nasal secretions should be assessed for *L. blattarum* infection. Metronidazole treatment is required if the flagellated protozoan parasite is observed in these populations. Considering the similarity of this parasite to bronchial epithelial cells, it seems that most of the cases with *L. blattarum* infection remain undiagnosed in different regions of the world. Therefore, it is necessary to regard *L. blattarum* as an emerging cause of pulmonary and sinus infections across the world.

Many of mortality and morbidity of pulmonary and sinus infection in our region may be due to *L. blattarum* and future research on more patients in wider range may reveal the greater prevalence of *L.blattarum* infection in our region and other countries as well.

Video 1 .Motile trophozoite of *Lophomonas blattarum* in nasal discharge.

Video 2. Irregular movement of trophozoite of *Lophomonas blattarum*(at right) in comparison with a bronchial epithelial cell(at left) without any movement in BAL secretion.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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