Research in Molecular Medicine

Evaluating Pulmonary Samples of Immunodeficient Patients for a Free-Living Amoeba: Acanthamoeba in BAL Samples

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ABSTRACT

Background: Pulmonary disorders caused by parasites are common in many tropical regions. Acanthamoeba is an opportunistic parasite, and most of its dangerous complications are seen in patients with immune deficiencies. Considering the high dissemination of Acanthamoeba parasite in water, soil, and fine airborne dust in Iran, this research was performed to study the rate of pulmonary secretions infection to Acanthamoeba in patients with immune system disorder.

Materials and Methods: This cross-sectional study was done in one year (2017 to 2018) in Arak City. The study sample was selected from immunodeficient subjects who had chronic obstructive pulmonary disease, too. The demographic data were also collected from all study samples. The respiratory sample was obtained from each selected patient by Bronchoalveolar Lavage (BAL). Each sample was examined by smear staining, cultivation, and molecular methods.

Results: Out of 64 immunocompromised patients investigated in the current research, the Acanthamoeba infection was found in 100%, 98.4%, and 0% of their BAL with culture, molecular, and direct methods, respectively.

Conclusion: The outcomes of our research indicated that selecting the diagnostic method in agreement with the kind of sample has a remarkable role in recognizing the contamination. The results of this research showed that the direct microscopy test of Giemsa stained smear was not suitable for detecting this kind of parasitic infection in the BAL sample. Therefore, awareness of the occurrence of Acanthamoebiasis in immunocompromised subjects is essential for preventing the dangerous complications of this parasite.

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Introduction

Pulmonary infections are among the leading cause of diseases in the pulmonary system. The reasons for these infections can be viruses, bacteria, fungi, and parasites. Many organisms are introduced into the lower respiratory tract by breathing or by micro-espionage through the upper respiratory tract and digestive tract. Under standard conditions, the body's immune system keeps the lower respiratory tract sterile. But, this system now works with difficulty given the widespread outbreak of pneumonia and the increasing use of interventional technologies in medicine, particularly in people with immune deficiency and patients who use immunosuppressive drugs [1].

So far, parasitic infections have accounted for a small part of the pulmonary infectious diseases, which may be because of our failure to recognize the parasite in this organ. Recently, with the rising immunosuppressive illnesses, the case reports of pulmonary parasitic infection have increased. It seems that under suitable conditions, some parasites cause opportunistic infections in the lung. Even some parasites, reported in many cases in pulmonary secretions, also cause clinical symptoms when the immune system is suppressed. Some scientists have presented case reports of protozoa such as Plasmodium, Entamoeba histolytica, Acanthamoeba, Leishmania, Trypanosoma, Babesia, and Toxoplasma in lung secretions [2, 3].

Acanthamoeba is a free-living amoeba that is isolated from water, soil, air, dust, and the nasal mucosa and nasopharynx of healthy people and patients with an upper respiratory infection. Several diseases are associated with this parasite, but the most important ones are Acanthamoeba Keratitis (AK) and Granulomatous Amoebic Encephalitis (GAE). Apart from AK, other diseases caused by Acanthamoeba have been reported in immunocompromised patients and confirmed to be opportunistic in these individuals [4].

Because of the rising trend of immune deficiency disorders and extensive use of immunosuppressive drugs, reported cases of respiratory protozoa such as Microsporidium, Toxoplasma, and Cryptosporidium have increased [5]. Considering the vast distribution of Acanthamoeba parasite in water, soil, and fine airborne dust in Iran, this study was performed to measure the rate of pulmonary secretions infection to Acanthamoeba in a subject suffering from immune system disorder.

Participants and Methods

Study population

This cross-sectional study was carried out during one year (January of 2017 to 2018) in Arak City, Markazi Province of Iran. Figure 1 displays the inclusion and exclusion criteria of this research.

Study sampling

The respiratory sample was obtained from each selected patient with Bronchoalveolar Lavage (BAL sample). Demographic data, including gender, occupation, marital status, and history of immune disorder, were recorded, too.

Study experiments

Each sample was examined as follows. At first, a smear was provided from each sample and stained with Giemsa. Second, a portion of each sample was transferred to a Non-Nutrient Agar (NNA) plate, coated with E. coli (killed) at a temperature of 28° C, and kept under monitoring for 4 weeks [6]. Third, the rest of the samples were used for molecular analysis as follows. The phenol-chloroform technique was applied for DNA extraction [7]. For PCR amplification, genus-specific primers were used. To amplify a nearly 500-bp fragment of Acanthamoeba-specific 18S ribosomal DNA, reverse primer JDP2 (5′- TCTCACAAGCTGCTAGGGGAG TCA-3′), and forward primer JDP1 (5′-GGCCCAGATCGTTTACC-GTGAA-3′) were used [6].

Statistical analysis

Statistical analysis was performed in SPSS V. 16. The results were presented as descriptive data. Also, the Chi-square test was used to investigate the differences between the type of immunodeficiency and the presence of this parasite in subjects at a significant level of $P<0.05$.

Results

A total of 64 immunocompromised patients were eligible for participating in the research. The most common kinds of immunodeficiency were immunosuppression and immunotherapy (Table 1).

No positive sample was observed in the direct microscopy test of Giemsa-stained smear prepared from BAL samples. However, all samples were positive by culture and 63 samples (98.4%) with the molecular method (Figure 2).

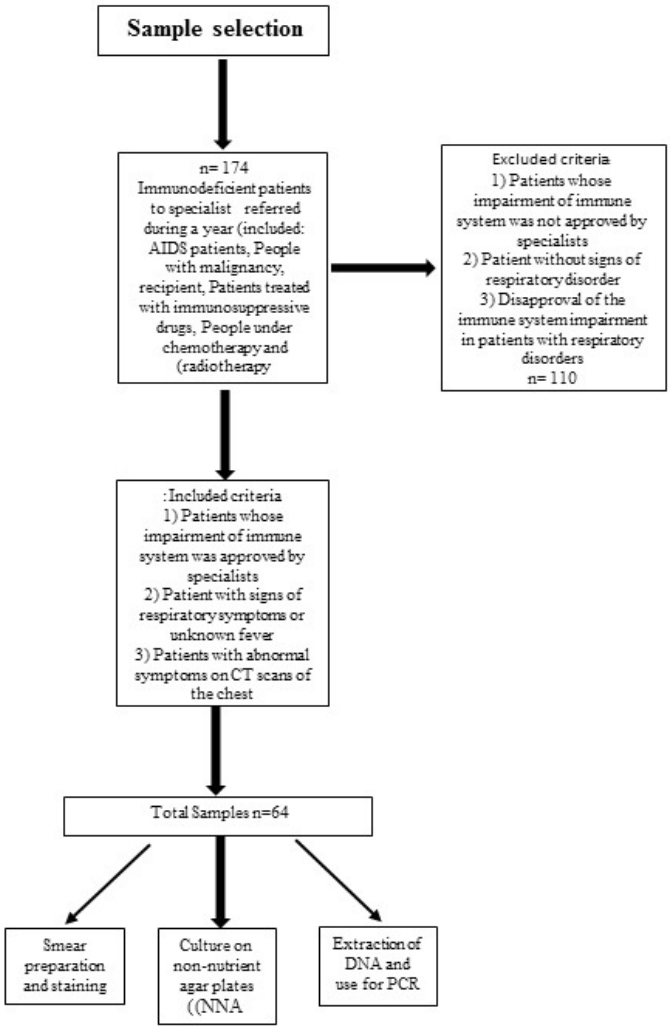

Figure 1. Flowchart of the disposition of samples and experiments

The statistical analysis indicated no considerable differences between Acanthamoeba infection and the kind of immunodeficiency (P>0.05).

Discussion

Pulmonary disorders caused by parasites are common in many tropical regions. Some factors, such as travel to endemic areas, contact with dogs and cats, and immune system disorders, are effective in the rate of parasitic in-

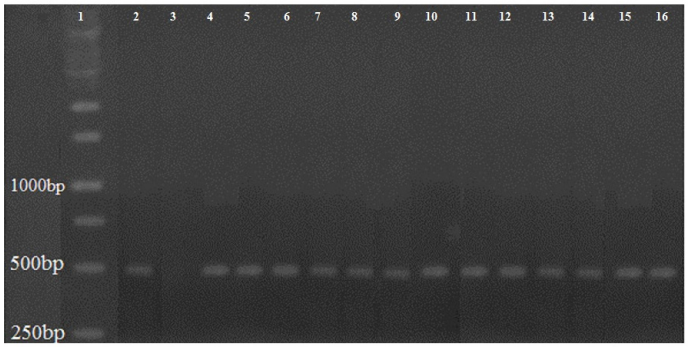

Figure 2. Lane 1: marker thermo scientific SM0313; lane 2: positive control; lane 3: negative control; lane 4-16: positive samples

Table 1. Overview of the demographic features of the immunodeficient patient in the present study

Variable		No. (%)
Gender	Female	35(54.7)
	Male	29(45.3)
Age groups, y	10-29	12(18.8)
	30-49	24(37.5)
	>50	28(43.7)
Marriage	Yes	58(90.6)
	No	6(9.4)
Type of immunodeficiency	Lymphoreticular	10(15.6)
	Immunosuppress	24(37.5)
	Immunotherapy	22(34.4)
	Malignancy	7(10.9)
	HIV	1(1.6)

fection and pulmonary complications [3]. The type of pulmonary secretion is also effective in identifying contaminations.

The diversity of microorganisms in the upper bronchial secretion is low, and they usually belong to the microbial flora of oropharynx. Considering that the BAL sample is taken from lower bronchial secretion, it can be used to found the pathogenic microorganisms in the respiratory tract and determine the difference between the microorganisms placed in the upper and lower parts of the bronchi [8].

In the current study, we investigated the Acanthamoeba contamination of BAL samples in the co-infection of immunodeficient subjects with COPD. Smear staining, culture, and molecular methods were used for diagnosis. The most useful diagnostic approaches in this study were culture and molecular techniques, which identified 100% and 98.4% of Acanthamoeba infection, respectively.

Our results indicate that choosing the right diagnostic method according to the sample, has a significant role in recognizing the contamination. The results of a study that performed on different samples of patients with Acanthamoeba keratitis confirm this point.

The results of the study showed that the sensitivity of the PCR method for corneal scrapes was much higher than the culture method. In contrast, the sensitivity of the culture method for the contact lens was higher than the PCR method [9].

Also, in this study, the microscopic examination of the smear staining prepared from the BAL specimens was not appropriate for detecting this kind of parasitic infection. Moreover, our findings confirmed the high cell density in BAL samples, which made it impossible to distinguish the parasite from other cells. It should be noted that even in healthy people, BAL samples contain different cells and parameters that could alter the cellular pattern and density of BAL specimens [10].

Owing to the abundant dissemination of Acanthamoeba in water, air, and soil around humans (especially in Arak, where the rates of water, soil and air contamination to this parasite have been reported to be 61%, 33%, and 27.3%, respectively), their entry into the human body is not unexpected [6, 11, 12]. It should be noted that under normal conditions and in healthy cases, bronchial tubes and lung parenchyma are sterile. Still, in the subjects with stable COPD, microorganisms that are potentially pathogenic could be isolated from these parts of the respiratory tract [8]. On the other hand, the co-infection of immune deficiency and COPD can be the cause of not removing the microorganism in the upper respiratory tract. Accordingly, in the current re-

search, Acanthamoeba infection was confirmed in all BAL samples (64 samples).

In another study, the Acanthamoeba contamination of 2 out of 64 BAL samples was reported from immunodeficient subjects with pneumonia syndrome [13]. This difference can be because of the high incidence of this parasite in environmental resources (i.e. water and soil) in Arak as the Acanthamoeba prevalence in water and soil in Arak was 61% and 31%, respectively [6].

Acanthamoeba is an opportunistic parasite, and most of its dangerous complications (i.e. granulomatous amoebic encephalitis) are seen in patients with immune deficiencies, organ recipients, and AIDS [14]. For this reason, awareness of the prevalence of Acanthamoebiasis in immunocompromised subjects is necessary for preventing its dangerous complications. Although recent investigations have indicated that Acanthamoeba can transmit symbiotic bacteria, the co-infection of Acanthamoebiasis with respiratory tract disease (such as COPD) does not have definitive parasitic etiology so bacterial etiology must be taken into account as well [14]. However, further investigations are required to confirm it.

Conclusion

The outcomes of this research indicate that Acanthamoebiasis is a common infection among the immunodeficient patients, but the direct microscopy test of Giemsa stained smear was not suitable to test for detecting a positive sample of Acanthamoeba in BAL samples.

Ethical Considerations

Compliance with ethical guidelines

This study received ethical code from the Ethics Committee of Arak University of Medical Sciences (IR.ARAKMU.REC.1395.347). The study sample was chosen from immunodeficient subjects who had Chronic Obstructive Pulmonary Disease (COPD) and was willing to contribute to the research (written consent was attained from selected patients).

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Authors contribution's

Conceptualization, investigation, writing-review & editing: All Authors; Methodolog: Zahra Eslamirad and Mojtaba Didehdar; Project Administration: Ali Arash Anoushirvani; Original Draft: Abdolatif Moini and Zahra Eslamirad.

Conflict of interest

The authors declare no conflict of interest related to this work.

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