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Morphological Identification of *Acanthamoeba* spp. Isolated from Malignant Patients from Zanjan, Iran

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ABSTRACT

Background: Genus *Acanthamoeba* belongs to free-living amoebae, which could pose risk to the central nervous system and cornea and is considered to be a significant health concern. The present study aimed to evaluate the presence of *Acanthamoeba* spp. in malignant patients in Zanjan, located in the northwest of Iran, using morphological methods.
Methods: Nasal specimens were collected from 100 malignant patients using swabs in the hospitals in Zanjan, Iran during July 2017-August 2018. The samples were cultured on non-nutrient agar, and the *Acanthamoeba* genus was identified based on the morphological characteristics.
Results: Based on the morphological features, six samples (6%) were positive for *Acanthamoeba* spp.
Conclusion: This was the first report on the presence of *Acanthamoeba* spp. in malignant they patients in Zanjan city, which provides further evidence on the existence of *Acanthamoeba* spp. The findings emphasize that special attention should be paid to immunocompromised patients in order to prevent the infections associated with *Acanthamoeba* spp.

1. Introduction

Genus *Acanthamoeba* is one of the most important free-living amoebae spreading worldwide [1-3]. This opportunistic protist has been isolated from soil, clay, dust, sewage, sediment, fresh water, mineral springs, hot springs, sea water, contact lenses, and clinical specimens [4-7].

Based on morphological criteria, genus *Acanthamoeba* is classified into two categories of active trophozoites and non-active cysts [8].

Trophozoites are flat-shaped with the characteristics of acanthopodia, and the cysts are double-walled with a wrinkled or wavy ectocyst and various endocyst shapes, including star, triangular, square, and round shapes [6, 9].

Acanthamoeba spp. are cause of granulomatous amoebic encephalitis (GAE), subacute granulomatous dermatitis, sinusitis, pneumonitis, and disseminated infections in immunocompromised patients. Moreover, they could lead to amoebic keratitis in healthy individuals [10].

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Acanthamoeba spp. are able to carry and transfer numerous pathogenic microorganisms, including protozoa, yeasts, bacteria, and viruses [11, 12]. As such, they have adverse effects on human health directly or indirectly [13, 14].

Although the presence of unidentified encephalitis in immunocompromised patients has been reported in different regions in Iran, there are no reports on fatal GAE due to the lack of related studies [10, 15]. It is assumed that the delayed diagnosis and treatment of GAE leads to fatal complications [10, 16].

The present study aimed to identify *Acanthamoeba* spp. in malignant patients in Zanjan, Iran, based on morphological characterization. This was the first survey to determine the presence of *Acanthamoeba* spp. in immunocompromised patients in the hospitals in Zanjan, located in the northwest of Iran.

2. Materials and Methods

2.1. Sample Collection

This cross-sectional study was conducted in Zanjan city (36˚40′ N and 48˚31′ E), located in the northwest of Iran, during July 2017-August 2018. After obtaining written informed consent, nasal specimens were collected from 100 malignant patients using swabs. The patients were assured of confidentiality terms regarding their personal information and medical data. The collected samples were placed in a labeled tube containing sterile phosphate buffer saline and immediately transferred to the Department of Parasitology and Mycology in the School of Medicine at Zanjan University of Medical Sciences in Zanjan for further evaluation.

2.2. Isolation and Cultivation

Each sample was placed on 1.5% non-nutrient agar (NNA), overlaid with heat-killed Escherichia coli as a food source for amoebae outgrowth [11, 17]. The culture plates were incubated at room temperature and monitored daily for 60 days for the growth of *Acanthamoeba* spp. using an inverted microscope [18].

2.3. Morphological Identification

After using the Giemsa staining method, the prepared samples were examined for the presence of trophozoites and cysts [19].

The criteria for the recognition of genus *Acanthamoeba* were based on the morphology of trophozoites and cysts and taxonomy keys [18].

3. Results and Discussion

Genus *Acanthamoeba* was detected based on the presence of double-walled cysts (Figure 1) and flattened trophozoites with acanthopodium, spine-like pseudopodia, on the surface (Figure 2). Out of 100 collected samples from malignant patients, six cases (6%) were positive for *Acanthamoeba* spp. based on the morphological criteria of the NNA culture.

Genus *Acanthamoeba* belongs to free-living amoebae and is considered to be an important medical complication worldwide [20]. In addition, this amphizoic protozoan parasite is the etiological agent of several central nervous system infections, including *Acanthamoeba* meningitis/meningoencephalitis (AME) and fatal GAE. *Acanthamoeba* has been frequently detected in many environmental sources [3, 21]. Consequently, human exposure to *Acanthamoeba* spp. is highly common.

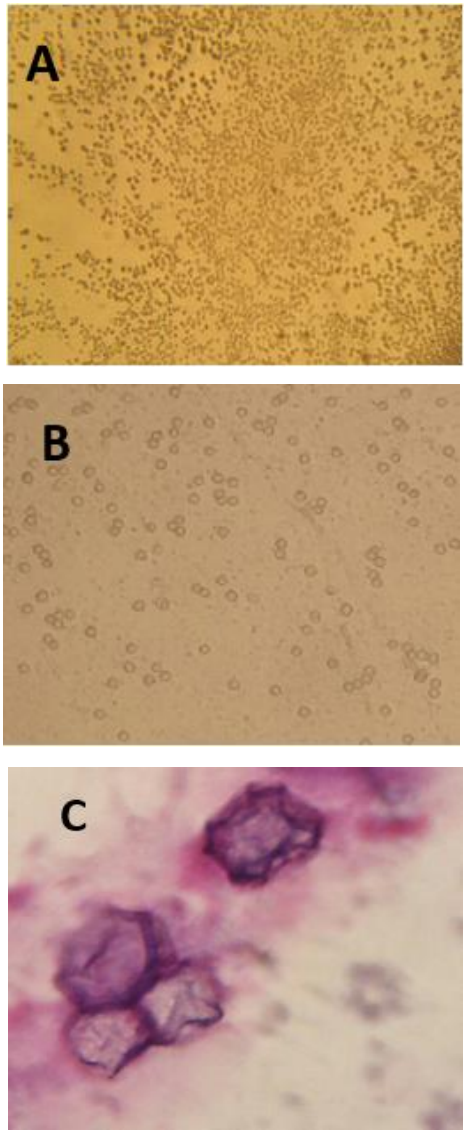

Figure 1: Light microscopy images of *Acanthamoeba* spp. cysts isolated from mucosal tissue of malignant patients in hospitals of Zanjan, Iran

A: Cysts on NNA (10X), B: Cysts on NNA (40X), C: Cysts in Giemsa stain (40X)

According to the literature, *Acanthamoeba* spp. has been isolated from water, soil, dust, and biofilm samples in various regions in Iran [6]. In a survey conducted by Pezeshki et al. (2017) *Acanthamoeba* spp. was identified in 50% of the water samples collected from Zanjan, Iran [8].

Therefore, the presence of *Acanthamoeba* spp. in various environmental resources should be considered a significant health concern, especially for high-risk populations such as immunocompromised patients and contact lens users [6, 20].

Figure 2: Light microscopy images of *Acanthamoeba* spp. trophozoite isolated from mucosal tissue of malignant patients in hospitals of Zanjan, Iran

Acanthamoeba trophozoite in saline (40X)

The previous investigations in this regard have also revealed that the rate of undistinguished encephalitis is on the rise in immunodeficient individuals in Iran [15].

To date, there have been no reports on encephalitis regarding *Acanthamoeba* and disseminated acanthamoebiasis [22]. This is mainly due to the lack of awareness on *Acanthamoeba* as an agent causing complications in the central nervous system [6].

It is notable that in the only study in this regard, which has been performed by Memari et al., in Iran, the presence of *Acanthamoeba* was documented in cancer patients [15].

According to the mentioned research, the early diagnosis and treatment of *Acanthamoeba* in the individuals with poor immune status could prevent GAE and other severe health problems [10, 16]. This was the first comprehensive study to identify *Acanthamoeba* spp. in malignant patients using morphological methods in Zanjan, Iran. To the best of our knowledge, the present study is the second research to detect *Acanthamoeba* spp. in immunocompromised patients in the hospitals in Iran. The obtained data in this survey provide further evidence on the presence of *Acanthamoeba* spp. in immunocompromised individuals in Iran, which is consistent with the other studies across the world [16, 21, 23, 24].

According to our findings, the infection rate in Zanjan city was lower compared to the results obtained in the previous studies conducted in Iran [15]. However, comparison of the differences in this regard would not be logical considering the lack of adequate data in Iran.

4. Conclusion

The current research provides baseline knowledge on *Acanthamoeba* spp. infection in cancer patients. It is recommended that further investigation be conducted in this regard in order to focus on the reconfirmation of the positive samples that were identified as *Acanthamoeba* spp. using molecular techniques and determining the *Acanthamoeba* genotypes in cancer patients so as to develop proper methods for the prevention and control of the subsequent life-threatening complications.

Authors' Contributions

P.A., designed the study and wrote the manuscript; H.A., and M.A., revised the manuscript; F.P., conducted the experimental work and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors report no conflict of interest.

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