Acanthamoeba Keratitis Among Sudanese Patients With Corneal Ulcer In Khartoum State-Sudan

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Abstract: *This study aimed to determine Acanthamoeba keratitis among Sudanese patients with corneal ulcer in Khartoum State-Sudan. A cross sectional study was conducted in the period between April-June 2011. The study was conducted on 20 patients and 5 healthy persons regarded as control. The age ranged between 15-100 years old. Eye swab and corneal scraping samples were taken from all subjects included in the study. Direct light microscopy of eye swab and corneal scraping stained by Gram stain and Giemsa stain technique was used. The results showed that 5 (25%) of patients with corneal ulcer were positive for Acanthamoeba keratitis. The results demonstrated that the prevalence of infection was higher (50.0%) in the age group 46-55 years old. Also the prevalence rate of infection was high (26.7%) in the males than in the females (10.0%). The results were showed that prevalence of infection was high (22.2%) among patients who comes from rural areas than those from urban areas (18.8%).*

Keywords— Acanthamoeba keratitis; Sudanese Patients; Corneal Ulcer; Khartoum State

1. INTRODUCTION

Acanthamoeba keratitis (AK) is a very rare, sight-threatening corneal disease that results from the infection of a free-living amoeba [1]. The estimated rate of AK is 1.2 per million people in the United States and 0.2-2.0 per 10,000 soft contact lens wearers per year in the United Kingdom [1]. *Acanthamoeba* penetrates the eye through minimal corneal trauma, which usually occurs in individuals who routinely wear soft contact lenses [2]. Patients with AK will experience pain and photophobia, and the infection runs progressively leading to corneal perforation if left untreated [3]. Initial manifestation is characterized by a complete or partial ring infiltrate in the anterior stroma, which may respond well to steroid therapy [2]. Atypical manifestations, such as diffuse punctate epitheliopathy, dendritic epithelial lesion, or feathery-edge lesion, are also observed in other cases of *keratitis*. Therefore, AK is often mistreated as herpes simplex virus *keratitis* or even fungal *keratitis* in common practice [4]. The diagnosis of AK is often difficult as the manifestations could mimic other types of *keratitis*. Furthermore, its treatment is time-consuming; hence, a proper and accurate diagnosis is crucial. A delayed diagnosis could also lead to a delay in treatment, and the prognosis of the disease could worsen. Here, we present a challenging diagnostic and treatment process of a contact lens-associated infective *keratitis* case caused by *Acanthamoeba*, which was treated with antibiotics and amniotic membrane transplantation (AMT) to enhance the healing process. The

detection can be done in laboratory using non nutrient agar saline plate seeded with gram negative bacteria such as *Escherichia coli*. The PCR is used to confirm the diagnosis especially when the contact lenses are involved. Molecular methods also available for detection and identification of Acanthamoeba, these methods are also suitable for both clinical and epidemiological purposes [5,6]. The fluorescent *in situ* hybridization technique has also been successfully employed for the purpose detection [7].

2. Materials and methods

2.1 Study design:
It is a cross-sectional study.
2.2 Study area and study duration:
The study was conducted in Makka and Abd Elfadeel Almas eyes hospitals in Khartoum state, during the period between April-June 2011.
2.3 Study population:
The study was carried out on patients with corneal ulcer who attended in eyes hospitals for follow up.
2.4 Sample size:
The study was conducted in 20 patients with corneal ulcer and 5 healthy persons regarded as control.
2.5 Sample collection:
Eye swabs and corneal scraping were collected with a sterile instrument under topical anesthesia at the slit lamp.
2.6 Data collection:
25 designed questionnaires were filled by participants.

3. Methods

3.1 Direct microscopy:
Eye swab or corneal scraping were collected in clean slide and stained with Gram stain and Giemsa stain and examined under microscope.

3.2 Gram stained smear:
Dried smear was fixed by rapidly passed the slide three times through the flame. Smear was cooled then was covered with crystal violet for 1 minute then was washed with clean water. Smear was covered with Lugol's iodine for 1 minute then was washed with water. Smear was decolorized rapidly with 70% ethyl alcohol then washed with water. Smear was covered with sufranin for 2 minutes then washed with water. Back of slide was cleaned and placed in draining rack to air dry. Then slide was examined under microscope (X100).

3.3 Giemsa stained smear:
Smear was fixed by absolute methanol then was dried. Smear was covered with diluted Giemsa stain (10%) for 10 minutes then washed with water. Back of slide was cleaned and placed in draining rack to air dry. Then slide was examined under microscope (X100).

3.4 Data analysis:

Results were analyzed using the computerized program of statistical package of social science (SPSS). Then data were presented in tables.

3.4 Ethical consideration:
Approval was taken from the College of Medical Laboratory Science-Omdurman Islamic University and hospital management. The patients were informed for the purpose of the study before collection of the specimen and verbal concept was taken from them.

4. Results
The study was carried out on 20 patients and 5 healthy persons regarded as control, 15 (75%) of patients were males. Their age ranged between 15-100 years old. 5 (20%) were drivers, 3(12%) were farmers, 3(12%) were free job and 10 (40%) were students. 9 (45%) patients reside in rural areas, 11(55%) in urban areas, 9 (45%) of patients have past history of trauma, 7 (35%) have been exposed to foreign body and only 4 (20%) had past history of contact lens. 12 (60%) were under treatment. *Acanthamoeba* parasite was detected in 5 (25%) patients, 4 (80%) of them were males. 2 (40%) were drivers, 1 (10%) was farmer and 1 (10%) was a student.4 (80%) were found to have past history of trauma and 1 (20%) foreign body.

Table 1: Prevalence of *Acanthamoeba keratitis* in the study sample

Acanthamoeba	Frequency	Percentage (%)
Positive	5	25
Negative	15	75
Total	20	100

Table 2: Prevalence of *Acanthamoeba* according to gender

Gender	No. examined	No. positive	Percentage (%)
Male	15	4	26.7
Females	10	1	10.0
Total	25	5	20.0

Table 3: Prevalence of *Acanthamoeba* according to age groups

Age Groups (Years)	No. examined	No. positive	Percentage (%)
15-25	12	2	16.7
26-35	4	1	25.0
46-55	2	1	50.0
56-65	4	0	0.0
66-75	2	0	0.0
More than 75	1	1	100.0
Total	25	5	20.0

Table 4: Prevalence of *Acanthamoeba* according to occupation

Occupation	No. examined	No. positive	Percentage (%)
Driver	5	2	40.0
Farmer	3	1	33.3
Free job	3	0	0.0
Student	10	1	10.0
Teacher	2	0	0.0
Merchant	1	0	0.0
Jobless	1	0	0.0
Total	25	5	16.7

Table 5: Prevalence of *Acanthamoeba* according to residence

Residence	No. examined	No. positive	Percentage (%)
Rural	9	2	22.2
Urban	16	3	18.8
Total	25	5	20.0

Table 6: Prevalence of *Acanthamoeba* according to past history

Past history	No. examined	No. positive	Percentage (%)
Trauma	9	4	44.4
Contact lens	4	0	0.0
Foreign body	7	1	14.3
Control	5	0	0.0
Total	25	5	20.0

Table 7: Prevalence of *Acanthamoeba* according to exposure to treatment

Exposure to treatment	No. examined	No. positive	Percentage (%)
Yes	12	4	33.3
No	8	1	12.5
Control	5	0	0.0
Total	25	5	20.0

Table 8: Prevalence of bacterial infection in the study sample

Bacteria with gram reaction	No. examined	No. positive	Percentage (%)
G+ve cocci	10	1	10.0
G+ve bacilli	1	1	100.0
No bacteria	14	3	21.0
Total	25	5	20.0

Table 9: The majority of cells

The majority of cells	No. examined	No. positive	Percentage (%)
Neutrophilia	10	5	50
Lymphocytosis	2	0	0.0
No cell	13	0	0.0
Total	25	5	20.0

Table 10: Prevalence of *Acanthamoeba* according to systemic disease

Systemic disease	No. examined	No. positive	Percentage (%)
Yes	3	0	0.0
No	22	5	22.7
Total	25	5	20.0

5. Discussion

The present study was carried out on 20 patients with corneal ulcer who attended eyes hospitals and 5 healthy persons as control. The results showed that the overall prevalence rate of *Acanthamoeba* was 25%, these findings were disagreed with the study carried by Bairagi (2017) [8] who found the prevalence was 1.04%. Although (AK) is a rare disease, we found a relatively large number of infected patients. This may indicate that the disease is not as rare as we thought and it may be misdiagnosed due to lack of knowledge or poor competence of laboratory technologists. This will lead to wrong treatment and eventually increase severity of disease as we have seen the most ulcers of acanthamoebic patient were large in size (>5mm in diameter). Several studies reported the use of a soft contact lens as one of the greatest risk factors for corneal infection [9,10]. However, a study by Jiang *et al*. [11] reviewed clinical characteristics of AK between 1991 and 2013. Out of the 260 cases included, it was revealed that the most common risk factor was ocular trauma (53.1%), these findings were agree with the present study, and then followed by contact lens wear (29.8%), these findings were disagreed with the present study. In this case, the patient regularly rinsed her contact lenses and the case with commercial disinfectant solutions. However, she also frequently used tap water, which is known as one of the sources of *Acanthamoeba*. *Acanthamoeba* is found worldwide in air, dust, soil, and fresh waters, and it is relatively resistant to normal levels of chlorine in tap water [2]. In Cipto Mangunkusumo Hospital, the most common etiologies of the corneal ulcer-related soft contact lens are bacterial (68%) and fungal (9.55%) these findings were disagreed with the present study.

6. Conclusion

The study concluded that the prevalence rate of *Acanthamoeba keratitis* was 25%. The prevalence was high among males than females and most of them comes from rural areas and work in hard jobs which require more contact with dust.

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