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COULD ACANTHAMOEBA KERATITIS BE MORE COMMON IN NON-CONTACT LENS-WEARING NIGERIANS THAN REPORTED? EVIDENCE FROM ABAKALIKI
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COULD ACANTHAMOEBA KERATITIS BE MORE COMMON IN NON-CONTACT LENS-WEARING NIGERIANS THAN REPORTED? EVIDENCE FROM ABAKALIKI

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ABSTRACT

Background: **Although acanthamoeba keratitis (AK) is more common in contact lens wearers, there have been many reports in those who do not wear contact lenses, especially in developing countries and particularly among agricultural workers and manual labourers. Available literature suggests that acanthamoeba keratitis is not a common cause of corneal ulcer in Nigeria. This might be due to a low index of suspicion as a result of low use of contact lens by the general population in Nigeria. Perhaps a routine search for the amoebic organism in corneal scrapings may actually reveal more cases of AK than reported among non-contact lens wearers. Despite the lack of sophisticated laboratory facilities, in resource-constrained settings, for definitive diagnosis of this amoebic organism in corneal scrapings, several stains are available for the detection of amoebic cysts in samples. However, the modified Field's stain, which is readily available, gives a very good colour contrast as compared with other stains, and has been found to be very useful for the early detection. This study is therefore aimed at presenting the utility of modified Fields stain for the rapid diagnosis of acanthamoeba keratitis with a view to highlighting the need to routinely search for amoebic organism in patients with corneal ulcers, particularly in agrarian communities with muddy farmlands; using a readily available simple stain such as the modified Field's stain.**

Objectives: **To demonstrate the utility of modified Field-stain in the microscopic diagnosis of acanthamoeba keratitis in a resource –constrained setting.**

Materials and Methods: **This is a cross sectional descriptive study of all consenting corneal ulcer patients managed at the FETHA eye clinic over a 4-month period (May to August 2015). Acanthamoeba diagnosis was based on Giemsa and modified Field's staining techniques. Potassium hydroxide (KOH)**

wet mount and Gram's stain were used for diagnosis of fungi and bacteria respectively, before culture results were available.
Results: **The microbial diagnoses were staphylococcus aureus (37.5%), Fungal keratitis (fusarium spp. and aspergilus, 25%) and acanthamoeba (25%). None of the patients ever used contact lenses.**
Conclusion: **The use of modified Field-stain in the microscopic examination of corneal ulcer scrapings yielded a high microscopic diagnosis of acanthamoeba among the corneal ulcer patients seen at Federal Teaching Hospital Abakaliki. It is recommended that a larger study be done to confirm the usefulness of the modified Field's stain in the diagnosis of acanthamoeba keratitis**

INTRODUCTION

Acanthamoeba keratitis (AK) is a sight – threatening infection of the cornea. It is caused by a free-living amoebic parasite of the genus *Acanthamoeba* that is ubiquitous in nature, found commonly in water; soil; air; cooling towers; heating, ventilating, and air-conditioning (HVAC) systems; and sewage systems. [1,2] The organisms are typically harmless to humans, but in rare instances can cause severe infection,[1]. Although more common amongst contact lens wearers, cases of AK have also been reported in non-contact lens wearers.[1] Acanthamoeba keratitis (AK) was actually first documented in the United States in 1973, in a farmer who accidentally scratched his cornea with wire and hay, and then washed out the wound with irrigation water.[3] The number of reported cases is increasing worldwide with increased rates of contact lens wear.[4] AK is reported to affect over 1.2 million people each year.[4]

Patients with AK may experience pain with photophobia. Characteristic ocular findings include corneal epithelial defect and ring-like stromal infiltrate. Initially a diffuse superficial keratopathy is found; later multifocal infiltrates are almost always observed in the stroma. If AK is not treated adequately and aggressively, it can lead to loss of vision. [2,5]

Clinical suspicion of AK is aided by suggestive history and ocular findings. Besides the decreased vision, photophobia and redness which occur in all forms of keratitis, the classical symptoms attributed to AK include ocular pain that is out of proportion to findings on examination. Unlike bacteria keratitis there may be no discharge from the eye.[5,6] Ocular signs depend on the stage of the disease. Early manifestations in the cornea can be seen as punctate keratopathy, pseudodendrites, and epithelial or subepithelial corneal deposits which can be confused with a viral keratitis such as Herpes zoster virus or Herpes Simplex Virus. As disease progresses the classic "ring infiltrate" may develop (in about 50% of cases); later followed by corneal ulceration, hypopyon and even perforation, which can also be confused with bacterial and fungal keratitis.[1,5]

Diagnosis of AK is challenging, and the available treatments are not fully effective against all strains[1]. Generally, AK should be considered in all contact lens wearers and in any case of corneal trauma with exposure to soil or contaminated water. [1,] Clinical diagnosis of AK is easier when the characteristic ring infiltrate is present but this is only seen in approximately 50% of patients. Sometimes the clinical presentation can be confused with *Herpes simplex* keratitis (in early stages) or bacterial keratitis in advanced stages. [1]

Where adequate facilities exist, a tentative diagnosis of AK can often be made by *in vivo* confocal microscopy (IVCM). The *Acanthamoeba* cysts appear as well-defined, hyper-reflective, spherical

structures with double wall. The trophozoites are difficult to distinguish from leukocytes and keratocyte nuclei.[1] Culture and microscopic examination of corneal scraping tissue material is the conventional method for identifying *Acanthamoeba* but several PCR-based techniques are also well established and usually increase sensitivity significantly. The corneal scraping/biopsy is received in transport medium (ideally 200 μL of sterile saline). While the sample itself is used for DNA isolation, the transport medium is used for culture[5,7]

In severe infection, the amoeba density is very high and can be detected by direct microscopy (200×–400× magnification) of the clinical sample without enrichment. The trophozoites or cysts exhibit auto-fluorecence and are also readily recognizable in phase contrast microscopy, as well as by use of several stains.[27,] The KOH preparation has also been reported to be a sensitive diagnostic tool for the detection of Acanthamoeba.[8]

It must be noted that nucleated corneal cells of deeper cornea layers may resemble amoebae, but acanthamoebae can be discriminated from other mononuclear cells by their large central nucleolus, their contractile vacuole and their hyaline pseudopodia with characteristic hyaline protrusions (acanthopodia) in the direction of movement. The trophozoites are 15–45 μm in size and have an oval to elongated outline.[1]

Several stains are available for the detection of cysts in samples,[9,10] Fast and easy stains include lactophenol-cotton blue or Giemsa. Calcofluor white and acridine orange usually give very good results as well. Also use of a silver stain is particularly well-suited for the investigation of the cysts. The draw-back of these stains is that other cells, particularly fungi also stain well in them. Immunostain is a specific staining technique using anti-*Acanthamoeba* antibodies and is the recommended stain of choice for tissue sections. Alternatively, tissue section can be stained with haematoxylin & eosin (HE).[1]

Field's stain, which contain methylene blue and eosin, has been found to be a very useful staining technique for the early detection of protozoans in general. It is readily available in resource-constrained settings and already in common use as a rapid dagnostic test for identification of malaria parasite in blood smears. In 1999, Pirehma et al compared different types of stains such as Field's stain, modified Field's stain, Wright's stain, Giemsa stain, Ziehl-Neelsen stain, and trichrome stain to determine the best stain for the identification of amoeba and concluded that the modified Field's stain gives a very good color contrast as compared with other stains. Furthermore, it takes only 20 seconds to carry out using the least number of reagents, making it suitable for both laboratory and field use.[10]

Available literature suggests that acanthamoeba keratitis is not common in Nigeria, probably due to relative low use of contact lenses.[11,12,13] It is, however, possible to misdiagnose cases of AK based on low index of suspicion. Moreover, the laboratory diagnosis of acanthamoeba is challenging in a resource-constrained environment. In this study, we present the use of the Modified Field's stain as a rapid test for acanthamoeba in corneal scrapings.

MATERIALS AND METHODS

Study area: This study was carried out at the eye clinic of the department of Ophthalmology of the Federal Teaching Hospital Abakaliki (FETHA), which is the only tertiary hospital in Abakaliki, the state capital of Ebonyi State, South eastern Nigeria. The population of Ebonyi State is approximately 2.9 million, with a land mass of 5,530 square kilometers.[14] The vegetation is a mixture of savanna and semitropical

forest. Agriculture is the mainstay of the economy.

Study Design: This is a cross sectional descriptive study of all consecutive corneal ulcer patients managed at the FETHA eye clinic, which consented to be recruited into the study from 1st May to 31st August 2015. Following adequate information concerning the study, a written informed consent was obtained from each patient who had clinical features of microbial keratitis. Patients were recruited consecutively at diagnosis and refusal by any patient to participate in the study was respected.

Ethical Clearance: Ethical approval consistent with the tenets of 1964 Helsinki Declaration on research involving human subjects was obtained from Health Research Ethics Committee of FETHA. Confidentiality was maintained throughout the study.

Corneal Sample Collection and Staining Procedure: Corneal scraping was performed by an ophthalmologist using a sterile Bard-Parker blade size 15. The details of the KOH mount, gram stain and details of culture have been reported in a previous publication on the predisposing factors for corneal ulcers in this population.[15]

Acanthamoeba diagnosis was based on 10% Giemsa and modified Field's staining techniques as follows: The corneal. scrapping transferred onto a clean grease free glass slide was allowed to air dry at room temperature. The dried slide was stained with 10% Giemsa stain and allowed for 20 minutes. Then stained smear was washed with distilled water and allowed to air dry at room temperature. The dried stained smear was examined microscopically using x40 and x100 objective lenses for 10 minutes spending about 1 minute per field. To confirm the detection of Acanthamoeba spp, another dried smear of corneal scrapping was stained with four drops of Field's stain B and 8 drops of Field's stain A. The slides were agitated until a golden scum appeared on the surface of the smear within 15 seconds. The slides were rinsed for 2 seconds under a stream of water and allowed to dry at room temperature. The smear was examined microscopically using x40 and x100 objective lenses for 10 minutes spending about 1 minute per field.

Data Collection and Management: Data was collected using an interviewer-administered questionnaire. Information obtained included socio-demographic data and relevant patient history including presenting symptoms, duration of symptoms before presentation, history of eye trauma and use of traditional eye medication. Ocular examination was done and relevant data included visual acuity at presentation and at discharge. Data collected were transferred into a personal laptop computer and analyzed using the Statistical Package for Social Sciences (SPSS), version 23 (SPSS Inc, Chicago, Illinois, USA), and reported as frequency distributions, simple percentages and charts.

RESULTS

There were 8 patients in this study, 5 males and 3 females. The ages ranged from 24 to 74years with a mean age of 47.1 ± 20.3. Majority (62.5%) of the patients were farmers. All patients presented late after unsuccessful attempts at treatment. The mean duration before presentation was 2 weeks. A history of recent corneal injury or trauma was present in 4 (50%) of the patients. All cases which started spontaneously (non-traumatic), as red eye was mismanaged, either by the use of TEM or intervention by quacks/self. The details of the sociodemographic variables and visual outcome are reported a previous publication.[15]

Microbiological Diagnosis: Corneal scrapings in this series yielded microbial diagnosis in 7 (87.5%) of the patients, out of which 3 (42.9%) were positive for bacteria, 2 (28.6%)

had fungal isolates and 2 (28.6%) had Acanthamoeba. The bacterial isolates were identified to be staphylococcus aureus while the fungal isolates were fusarium and aspergilus species respectively. See Figure 1

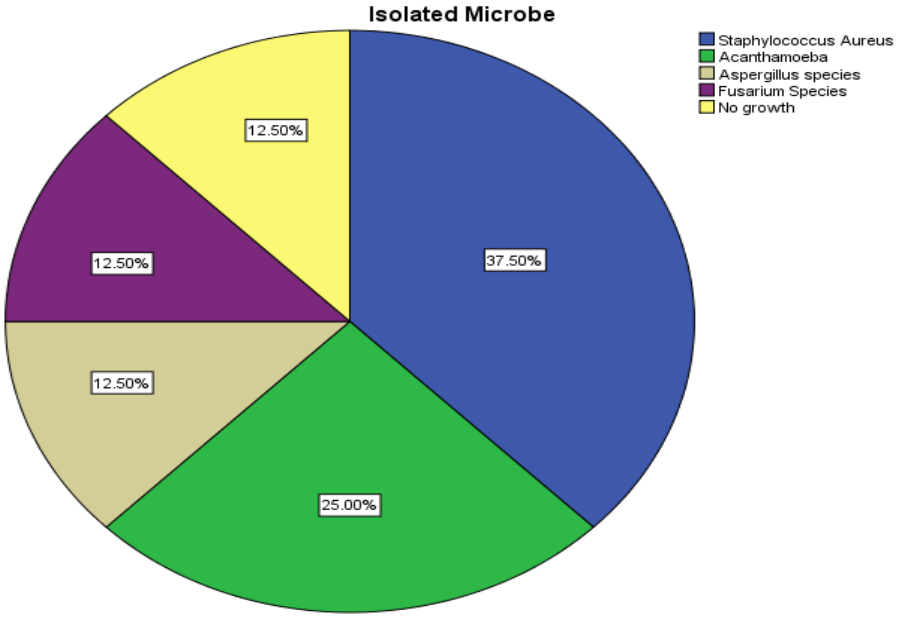

Figure 1: Microbiological diagnosis of isolated microbes

Outcome of Treatment for the Microbial Isolates: There was no visual improvement with the acanthamoeba keratitis after treatment. On the other hand, there was visual improvement following treatment in 2 of the three patients with staphylococcus keratitis and one patient with aspergilus keratitis. See table 1.

Table 1

Outcome of Treatment for the Microbial Isolates

Microbial Isolate	No of patients	Visual improvement After treatment (%)
Staphylococcus Aureus	3	2 (66.7%)
Aspergillus spp	1	1 (100%)
Fusarium spp	1	0 (0)
Acanthamoeba	2	0 (0)
No Growth	1	0 (0)

DISCUSSION

There have been many reports of acanthamoeba keratitis (AK) in those who do not wear contact lenses, especially in developing countries.[6,8,] Although more common in contact lens wearers, AK can occur both in contact lens wearers as well as in any case of corneal trauma with exposure to soil or contaminated water.[1,4,6,16] The finding of acanthamoeba organism in 2 of the corneal scrapping specimens in this study is in keeping with these reports. In non-contact lens users, the greatest risks for

developing acanthamoeba infection are trauma and exposure to contaminated water.[1, 6, 16]

Sharma et al suggested the existence of *acanthamoeba* pathotypes specifically associated with non-contact lens keratitis and unique to certain geographical area.[6,] Furthermore, Garg et al highlighted risk factors such as warmer weather, poor socioeconomic conditions, agricultural workers and manual labourers.[16] Ebonyi state of Nigeria is known for rice farming, and therefore has a large population at risk. Moreover, the use of traditional eye medications (TEM) and local remedies from quacks are often the first line of treatment following eye trauma, red eye and other eye symptoms amongst rural dwellers.[17] These local remedies are often prepared using water from sources contaminated by micro-organisms and therefore a potential source of free-living amoeba. This may explain the high prevalence of acanthamoeba diagnosis in this population.

AK is often clinically misdiagnosed in the early stages as atypical herpes simplex keratitis or fungal keratitis. Diagnosis is also complicated by the frequent occurrence of secondary bacterial infections. Furthermore antibacterial, antiviral, antifungal, or corticosteroid treatment may complicate the diagnosis because an initial improvement may occur followed by worsening of the disease. Proper diagnosis is important for early treatment, since the amoebic organisms are known to form amoebic cysts in tissues and become resistant to many drugs. In most settings, the laboratory diagnosis of Acanthamoeba is based on a combination of microscopic examination of smears and inoculation of corneal scrapings on various culture media that allow growth of bacteria, fungi, and Acanthamoeba.[20] Non-nutrient agar plate coated with *Escherichia coli* and incubated at approximately 37 C, is particularly useful as culture medium for acanthamoeba organisms as they feed on the bacterial overlay and multiply rapidly.[13] However this takes at least 72 hours and may sometimes need to be cultured for 2 weeks before concluding on a negative culture. [3]KOH preparation has been reported to be a sensitive diagnostic tool for the detection of Acanthamoeba.[8] Bharathi et al reported a remarkable visibility of acanthamoeba cysts under 10% KOH wet mount with a high sensitivity.[8] However a high magnification microscope (200×–400× magnification) is required to enhance visibility. In resource-constrained settings stains provide immediate diagnosis to guide treatment. Available stains include calcofluor-white, gram stain. lactophenol-cotton blue, acridine orange, Field's stain, modified Field's stain, Wright's stain, Giemsa stain, Ziehl-Neelsen stain, and trichrome stain among others. Some of the stains can identify the trophozoites and cysts while some can identify only the cysts (e.g Z-N AFB stain). [1, 10,18] While the trophozoites may be recognized by their acanthaphobia, it is sometimes difficult to distinguish from leukocytes and keratocytes.[1] The cysts, on the other hand, are seen as well-defined spherical hyper reflective structures with double wall. In our study the Modified Field test was used to confirm the presence of acanthamoeba.

The utility of Modified Field's stain in the diagnosis of the amoebic organism has been corroborated by other studies[10,19] The modified Field's stain has the advantage of good colour contrast and short testing time (less than 5 minutes). Moreover, it is readily available in resource-constrained settings and already in common use as a rapid diagnostic test for identification of malaria parasite in blood smears.

Poor visual outcome is common with acanthamoeba keratitis despite appropriate treatment[3,4,6,16] This was the case in this study where none of the patients with AK had visual improvement after treatment, unlike

those with bacterial keratitis, among which 66.7% had visual improvement with treatment. The tendency for the organism to form amoebic cysts in tissues makes it often resistant to many drugs. [1, 6,20] This may explain the poor treatment outcome in patients with acanthamoeba keratitis. Early diagnosis and treatment may help to battle the organisms in other forms.

CONCLUSION AND RECOMMENDATION

The use of modified Field-stain in the microscopic examination of corneal ulcer scrapings yielded a high microscopic diagnosis of acanthamoeba among the corneal ulcer patients seen at Federal Teaching Hospital Abakaliki. It is recommended that a larger study be done to confirm the usefulness of the modified Field's stain in the diagnosis of acanthamoeba keratitis and the sensitivity in comparison with other stains.

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