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Severe, sight threatening microbial keratitis: Co-infection of Acanthamoeba and Pseudomonas in Contact Lens Associated Keratitis

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ABSTRACT

Contact lens-associated microbial keratitis (CLAMK) is a severe condition with sight-threatening potential and increasing incidence. Information regarding the etiological agents is essential in guiding management and may vary geographically. The aim of this study was to analyze the possibility of a concurrent infection of *P. aeruginosa* and *Acanthamoeba* in cases with contact lens-related keratitis. A prospective review of all cases presenting with CLAMK at Cornea Outpatient department of Research Institute of Ophthalmology (RIO) from September 2013 to September 2015, were included in this study. Full ophthalmic examination with slit lamp bio-microscopy was performed and corneal scrapings were sent for microbiological and parasitological diagnosis methods. Results revealed that of the 80 patients with CLAMK who attended the outpatient department 56 (70%) patients were confirmed to be positive for microbial keratitis 24(30%) showed no growth, 25 (44.6%) of positive cases were *P. aeruginosa*, 20 cases (25%) revealed *Acanthamoeba*. The coinfection of both *Acanthamoeba* and *P. aeruginosa* was detected in three cases. Culturing the suspected bacterial samples is the most accurate tool for diagnosis of these agents. However; still it is a more time consuming method. A sensitive method, such as PCR was done to detect the specific genotypes in very short time and was considered useful for confirming the diagnosis.

KEYWORDS: Microbial keratitis- CLAMK -Etiological agent -Contact lens- Acanthamoeba- *P. aeruginosa*

INTRODUCTION

Keratitis (corneal ulcer) is an umbrella term for an inflammatory or infectious event that is characterized by redness, pain and sometimes decreased vision.

A corneal ulcer is caused by a break in the corneal epithelium with or without stromal involvement and can lead to the entrance of a micro-organism through the break. Although more common unilaterally, it can present bilaterally and can vary in size and severity. Bacterial keratitis is a sight-threatening. Either untreated or severe it may result in perforation and endophthalmitis [1].

The use of contact lenses (CLs) has been widely associated with corneal alterations ranging in symptomatology and severity. Lesions may vary from small peripheral sterile infiltrates to infectious central ulcers with sight-threatening potential. CL wear has been repeatedly described as the most important predisposing factor to microbial keratitis worldwide. The incidence of microbial keratitis has increased over the past decades following the introduction of soft lenses in the 1970s [2].

Free-living amebae belonging to the genus *Acanthamoeba* are found worldwide in air, dust, and water and are relatively resistant to normal levels of chlorine in tap water. These amebae occur in two forms in humans: as an active, invasive trophozoite stage and as a dormant, cystic stage. Several species of *Acanthamoeba* can cause a chronic, progressive ulcerative keratitis of the eye, which is a painful and potentially sight-threatening condition. Many such ocular infections are associated with minor corneal trauma, and it is thought that contact lenses (usually soft lenses) may predispose the wearer to corneal infection. *Acanthameba* keratitis is being recognized with increasing frequency in both the developed and the developing world. This is probably due to a greater understanding of the disease process and the development of sophisticated, non-invasive diagnostic techniques, such as use of the confocal microscope. This microscope enables direct visualization of the *Acanthamebae* upon slit lamp examination. It has been postulated that Acanthamoeba species may have been the cause of many cases of clinically presumed herpes simplex virus keratitis, bacterial keratitis, and other corneal diseases. In one study, 84% of the *Acanthameba*-positive patients were found to have had a clinical diagnosis other than *Acanthameba* keratitis made upon initial examination [3].

Acanthamoeba keratitis manifests as an extremely painful ring-shaped infiltrate possibly associated with either swimming while wearing contact lenses or generally poor contact lens disinfection (the use of either tap water or saline instead of multipurpose solution). The patient usually has severe pain disproportionate to clinical findings. The condition develops over a period of several weeks [4].

Acanthamoeba keratitis (AK) is a destructive disease characterized by significant visual morbidity, and prompt diagnosis is important for a good visual outcome. Like AK, *Pseudomonas aeruginosa* keratitis usually progresses rapidly and presents with suppurative stromal infiltrate and marked mucopurulent exudate [5].

While other devastating microbes have come and gone in “outbreaks” *P. aeruginosa* has been a staple consistent problem throughout the history of the soft lens. Indeed, prior to the introduction of soft CLs to the market, *P. aeruginosa* keratitis was a rare occurrence. *P. aeruginosa* is ubiquitous in nature, and is likely to access ocular tissues often in the course of our daily lives. As a “water bug”, all lens wearers can be exposed to it whether or not they use solutions. While non-lens wearers have the benefits of blinking to regularly sweep the ocular surface.

P. aeruginosa is highly destructive and difficult to neutralize because of its virulent structure, adaptability and high rate of survival under different conditions. The strong association between *P. aeruginosa* and CL-related infection is intriguing. Although *P. aeruginosa* can elaborate a wide range of cell associated and extracellular virulence factors, which can initiate and potentiate the infection process and activate the host defense mechanisms, the link with CL wear has not been fully elucidated. The lens, storage case, and ocular environment may offer a suitable survival niche for this environmental organism. *P. aeruginosa* can adhere to and colonize lens materials during wear and survive in CL storage cases, partly through its ability to grow as a resistant biofilm on lenses and cases, and partly due to innate or acquired resistance to CL disinfectants.[6].

Acanthamoeba spp. and *P. aeruginosa* share many characteristics as eye pathogens. Both can adhere to, colonize, and invade injured corneal tissue; both produce tissue-destructive enzymes; and both have been recovered individually as contaminants of contact lens care systems. *P. aeruginosa*, is the most commonly recovered causative organism in CL-related disease, followed by Gram-positive bacteria *Staphylococci* [7].

MATERIALS AND METHODS

Patients: A total of 80 patients with CLAMK of different age and sex who attended the outpatient department and also admitted in the Ophthalmology ward of Research Institute of Ophthalmology during a two years study from 2013 till 2015 were included in the study.

This study was assessed and approved by the institutional review board (National Research Centre Ethical Research Committee) of the Research Institute of Ophthalmology ,Giza, Egypt, in compliance with the Declaration of Helsinki.

Patients were divided into four groups:

- Group (1): 30 CLAMK cases examined by corneal scrapes for corneal infections.
- Group (2): 20 CLAMK cases examined by corneal scrapes for corneal infections together with their contact lenses.
- Group (3): 6 CLAMK cases examined by corneal scrapes for corneal infections together with their contact lenses and their cases which were examined also.
- Group (4): 20 healthy controls.

Collection of Samples:

Demographic details like name, age, sex, clinical history and associated findings were recorded onto standard clinical history form. It was ensured that clinical microbiology material was collected before giving antibiotic therapy or 48 hours after discontinuing local antibiotics.

All patients underwent thorough slit-lamp bio microscopic examination by an ophthalmologist. After a detailed ocular examination, corneal scrapings were taken by an ophthalmologist with all aseptic precautions .Five minutes after instillation of local anesthetic to the affected eye, corneal scrapings were taken. The material obtained was spread onto labeled slides for Gram stain and was also inoculated onto the surfaces of agar plates for bacterial growth.

Bacterial Culture [8,9]:

Gram's staining was performed. The swab was inoculated onto Blood agar, MacConkey's agar, Chocolate agar and Wilkins media. The inoculation technique consisted of multiple "C" shape streaks on the culture plate, with the idea to localize the site of implantation of the corneal scraping on the agar media, then incubated at 37°C for 24 hours. The culture media were inspected for growth. If organism had not grown, plates were further incubated and finally declared as culture negative after 5 days. To insure 5-10% CO_2, Chocolate agar plates were incubated in- CO_2 incubator and Wilkins media were incubated anaerobically. Culture positive growth was identified by their colony, morphology, Gram staining and motility testing by hanging drop preparation, pigment production and relevant biochemical tests.

Acanthamoeba Culture [10]:

Corneal scrapings were performed also for detection of *Acanthamoeba*. Corneal scrapings were cultivated on non-nutrient agar plate layer co-seeded with live *Escherichia coli*. Plates were incubated at 30°C, observed and examined daily microscopically for 7 days. Acanthamoeba were characterized according to cyst (wrinkle double walled) and trophozoites (Acanthopodia and Pseudopodia) morphology.

Molecular Examination:

Sample Collection, DNA Extraction and Amplification:

Corneal scrapings for PCR were obtained by sterile swabs, which were then placed into a sterile micro centrifuge tube, capped and immediately transferred to –20°C for storage until processing. DNA extraction was performed using Gene Jet. Genomic DNA purify kit (Cat.No.00005743, Sigma Product) according to manufacturer's instructions. In this study DNA was amplified by PCR in a DNA thermal cycler(Perkin Elmer Cestus, Norwalk, CT) using oligonucleotide primers specific to amplify specific sequence for the detected organism synthesized by (Invitrogen life technologies, Carlsbad, Ca. USA). Amplicons were visualized on 2.5% Agarose gel Microkit from Qiagen, stained with Ethidium bromide and observed using a UV trans illuminator & samples run with DNA ladder from Solis Biodyne. Control samples for the strain used (*Pseudomonas aeruginosa)* was laboratory isolates from Microbiology laboratory at RIO.

PCR program for Pseudomonas aeruginosa:

DNA extraction protocol for bacteria:

For each sample a 2ml tube properly labeled was used, 3 ml of bacterial suspension was centrifuged and deposit was used. Enzymatic lysis buffer was added, 360µ, to each tube. Tubes were incubated at 37C for 30 minutes. Proteinase K 40µ was added to each tube. Buffer AL 400µ was added to each tube. Samples were vortexed briefly. Incubated at 56C for 30 min. 400µ of 100% ethanol was added to each tube and vortexed briefly. Using a micropipette, the entire contents 1200µ of the tube was added to labeled spin column in two steps each step uses 600µ. Columns were centrifuged at 10,000 x g for 1 min. Columns were removed from collection tube. Placed in new collection tubes. 500µ of buffer AW1 were added to the column and centrifuged at 10,000 x g for 1 minute. Columns were removed from collection tube. Placed in new collection tubes. 500µ of buffer AW2 were added to the column and centrifuged at 20,000 x g for 3 minute. Tubes were removed carefully from centrifuge, spin column were transferred to a 1.5 ml tube and 200µ of buffer AE were added to the column. Incubated at room temperature for 1 minute. Centrifuged at 7,000 x g for 1 minute. Columns were discarded and eluted DNA was used or stored at – 20 for PCR testing. [11]

PCR Amplification:

Master mix preparation:

We used Hot start master mix ready to use in a volume 12.5µ with 0.5µ from each primer (las f-las r) (tox f-tox r) (nan f-nan r) (PS1-PS2) (uni f-uni r) & 1.5µ PCR water & 10µ from extracted sample. Using the following cycle, hold at 95^{o}C for 15min following by 40 cycles of 95^{o}C for 45sec _ 55^{o}C for 45sec _72^{o}C for 1 minute & final extension at 72^{o}C for 5min. Detection of products was done by agarose gel electrophoresis [12].

Results:

Demographic data of 80 patients with CLAMK attending the corneal unit at Research Institute of Ophthalmology with different age groups together with 20 healthy controls included in the study are represented in table (1).

Sequences of used Primer Sets:

Organism	primers sequence
Pseudomonas aeruginosa	Psudomonas specific genes 1-Las gene Las F 5' GGAATGAACGAAGCGTTCTC3' Las R 5' GGTCCAGTAGTAGCGGTTGG3'2- 2-Tox gene Tox F 5' GGTAACCAGCTCAGCCACAT3' Tox R 5'TGATGTCCAGGTCATGCTTC' 3-Nan gene Nan F 5'AGGATGAATACTTATTTTGAT3' Nan R 5' TCACTAAATCCATCTCTGACCCGATA3' 4-PS gene Ps1 5'ATGAACAACGTTCTGAAATTCTCTGCT3' PS2 5'CTTGCGGCTGGCTTTTTCCAG3'
Universal primer for bacterial DNA detection 16S RNA	Uni F 5'GATTAGATACCCTGGTAGTCCAC3' Uni R 5'CCCGGGAACGTATTCACCG 3' 601bp

Patients presented with progressively increasing pain, redness, photophobia, mucopurulent discharge, and diminution of vision in the affected eye. Slit lamp examination showed a central stromal infiltrate with radial spokes .There was severe anterior chamber reaction, and intraocular pressure was elevated on digital examination.

It is important to be mentioned that patients wearing contact lenses were in age group ranged from 11-40 years old with most infections in 18-28, females were more susceptible than males Table (1).

Out of 80 cases, 56 cases (70%) were culture positive while the remaining 24 cases (30%) were negative. *Acanthamoeba* was detected in 20 cases (25%) in Table (2).

The bacterial growth pattern in the culture positive cases is shown in Table (3). It revealed that Out of 56 positive cases for bacterial culture, 25 (44.6%) cases were positive for *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* being the commonest Gram positive cocci 10 (18%) cases.

Table (4), shows that 88% of the population in this study used daily wear contact lens while only 12% used extended wear contact lens.

Regarding Acanthamoeba culture (Table 5):

- 20 cases (25%) had positive culture from the 80 examined cases with CLAMK while the remaining 60 cases of CLAMK were negative for Acanthamoeba.

Estimating the association between *Pseudomonas* and Acanthamoeba in the examined groups of patients revealed that:

- The positive 20 cases for Acanthamoeba culture were examined for bacterial association which was detected in 19 cases (95%) of them.
- Only one case (5%) had no bacterial association.
- Among those 19 cases only 3 cases (15%) presented with *Pseudomonas aeruginosa* while the rest of them had been associated with other bacterial species (80%).

Table (6) depicts microbial distribution among examined groups. It is seen that in the first group corneal scrapes were only performed, the second group corneal scrapes and culture of CL were performed in 20 cases of laboratory-proven bacterial keratitis and the CL culture led to the isolation of the *Acanthamoeba* in 6 cases. In the third group, corneal scrapes and cultures of CL and storage case were performed in 6 cases but only two cases showed positive results.

Table 1: Demographic Data of Examined Cases

Demographic Data		Number	+ve Bacterial culture	+ve Acanthamoeba culture
Sex	Males	40		
	Females	60		
Age in years	11-20	30	20	6
	21-30	50	30	12
	31-40	20	6	2

Table 2: Percentage of Positive Cultures among Examined Cases

Patients	Culture Type	No Examined	No of +ve	%	No of -ve	%
CLAMK	Acanthamoeba Culture	80	20	25%	60	75%
	Bacterial Culture	80	56	70%	24	30%

Table 3: Bacterial growth patterns in culture of CLAMK patients

Name of Bacteria Isolates	cases	%
Pseudomonas aeruginosa	25	44.6
Propionibacterium	1	1.8
staphylococcus aureus	10	18
staph.aureus& strept.pneumoniae	4	7.1
8staph.aureus& Micrococci	4	7.1
staphylococcus epidermidis	8	14.2
Moraxella species	2	3.6
streptococcus pneumoniae	2	3.6
Total	56	100

Table 4: Duration of contact lens wearing among population of the study

Duration						
	Cases	%	Control	%	Total	%
Daily	68	80	20	100	88	88
Extended	12	20	0	0	12	12
	80	100	20	100		

Table 5: Associations between Bacterial Isolates and Acanthamoeba Culture

Bacteria Type	No. of positive *Acanthamoba*	%
Pure Pseudomonas	3	15%
Other Bacteria	16	80%
Pure Acanthamoeba	1	5%
Total	20	100%

Table 6: Microbial distribution among examined Groups

Groups	Examined Sample					
	Corneal Scrapes		Lenses		storage cases	
	Bacterial Culture	Acanth. Culture	Bacterial Culture	Acanth. Culture	Bacterial Culture	Acanth. Culture
Gp I (30)	30	12	-	-	-	-
Gp II (20)	20	6	20	6	-	-
Gp III (6)	6	2	6	2	6	2
Gp IV (Control Group)	-	-	-	-	-	-

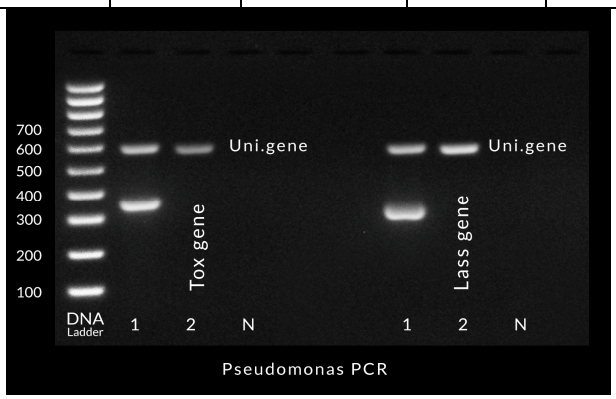

Fig. 1: Agarose gel showing PCR product of *pseudomonas aeruginosa* ,the Uni genes were detected at 601 bp while the Lass and Tox genes were detected at 350 bp.

Discussion:

Microbial keratitis (MK) is a potentially blinding corneal infection, which occurs rarely in the normal eyes. Predisposing factors have included ocular surface disease, ocular trauma, contact lens wear, systemic diseases, and ocular surgery. In a working age population, the two major preventable predisposing factors are ocular trauma and contact lens wear [13].

Contact lens wear is highly influential on the incidence of ulcerative keratitis worldwide, particularly in developed countries. The association between Acanthamoeba keratitis and contact lens wear is firmly established; it may account for up to 95% of the reported cases. Before the popularization of soft contact lens wear, Acanthamoeba keratitis was extremely rare .Contact lens-related problems depend on many factors, such as lens material, wearing modality, lens hygiene, type of lens-caring solution, the degree of compliance of the lens user with lens wear and care procedures, lens over wear, sleeping in lenses, rate of changing lenses, and lens case hygiene, wearing no disposable contact lenses, using homemade sodium chloride solution to clean the lenses, and wearing lenses while swimming. [14]. Isolation of *Acanthamoeba* from swimming pool water is not unusual. Acanthamoeba cysts are very resistant to chlorine. A higher percentage of isolates from swimming pools have been shown to be pathogenic than those isolated from natural fresh water [15].

An outbreak of Acanthamoeba keratitis, a potentially blinding corneal infection, was detected in the United States in 2007, showed an increase in the number of hospitalized patients due to contact lens-related corneal ulcers, which was correlated with the increase in the number of lens wearers. They added that most common predisposing factors included, history of ocular trauma, contact lens use, frequency of replacing old lenses with new ones, swimming in a lake or river while wearing lenses, washing the face while wearing lenses, lack of hand washing before inserting lenses, cleaning lenses at the bathroom sink, failure to always cap the solution bottle after use, and ever topping off solution (adding new solution to old solution in the lens case) [16].

In our study the results revealed that Acanthamoeba keratitis associated contact lenses wear was (95%) and with trauma was (5%) which was in accordance with a study done by Zimmerman *et al.* [17] stated that the primary risk factor for acquiring Acanthamoeba keratitis was contact lenses (85%-93%) and trauma was (7%-15%). In case of bacterial keratitis the primary risk factor was contact lenses (33%-50%) followed by trauma (15%-36%), then ocular surface diseases (7%-21%) [16], and also with a study done in Italy, medical history evaluation for acquiring Acanthamoeba keratitis revealed that (90.9%) of the patients wore contact lenses (CL) while (9.1%) of patients reported ocular trauma [18].

As regards microbial isolates in our study tables (2, 3) revealed that, out of 80 examined cases by microbial culture: 56 cases (70%) were positive while the remaining 24 cases (30%) were negative. From the 56 positive bacterial cultures, 25 (44.6%) cases were positive for *Pseudomonas aeruginosa*. followed by *S.aureus* (18%) then *S.epidermidis* (14.7%) while *S.aureus* with *S.pneumoniae* and *S.aureus* with micrococci were detected each in (7.1%), *Acanthamoeba* was detected in (25%) of the infected cases.

In a study done by Fong *et al.* in Taiwan showed that *Pseudomonas species* were the most commonly isolated organisms (37.7%) in cases of contact lens microbial keratitis, followed by *fungi* (13.5%), *staphylococci* (8.4%), nontuberculous mycobacteria (7.9%), *streptococci* (7.6%), and Acanthamoeba (4.4%)[19]

In a study done by Zimmerman *et al.* reported that the most common microorganisms responsible for contact lens related microbial keratitis were bacterial (71%) followed by fungal(5%) then Acanthamoeba (4%). The study showed that the most common gram positive bacteria detected were *Staphylococus species* (20%) while the most common gram negative bacilli were mainly *Pseudomonas* detected in (19%-73%) of the cases [17].

A retrospective analysis was done in Brazil during a 5-year period from January 2002 to December 2007, on 239 patients who were clinically diagnosed with contact lens-associated microbial keratitis and were culture proven .This study showed that the bacterial agent accounted for 166 cases (69.46%), while Acanthamoeba was verified in 95 patients (39.75%) and fungus was isolated in 4 cultures (1.67%). In the gram-positive group, the most frequent agent was coagulase-negative *Staphylococcus (*CoNS), which was found in 74 patients (27.71%). *Corynebacterium spp.* was present in 20 (7.49%) of the examined cultures. *Pseudomonas spp*. was the most common gram-negative etiological agent and was isolated in 32 (11.98%) cases. *Serratia spp*. was isolated in 15 cases and *Enterobacteraerogenes* in 5 cases [20].

Our results in tables (1, 4) showed, the cases were predominately females (60%) and the median age was (21-30 years). Of these contact lens microbial keratitis cases (80%) wore daily soft contact lenses while (20%) wore the extended contact lenses, while (100%) of the controls wore daily soft contact lenses.

An outbreak was described in 2007 involving a multipurpose contact solution and a study was done on 105 cases, they were predominantly female (64%) with a median age of 29 years. Of these case-patients, (89%) wore contact lenses, (88%) used soft contact lenses, and (94%) reported using some type of cleaning or disinfecting solution [16].

In a study done in Italy, on 11 cases suffering from Acanthamoeba keratitis, revealed that (36.3%) were females while the males were (63.6%) the median age was (34 years). Of these 11 cases, ten patients (90.90%) were soft contact lens (CL) wearers: 6 admitted not following appropriate standard hygiene procedures, using

unsuitable solutions to clean contact lenses (CL) (saliva or tap water), 3 were swimmers and 1 had associated the onset of symptomatology with ocular trauma while wearing contact lenses (CL).The patient who did not wear contact lenses(CL) reported an accidental trauma caused by an organic/plant source, that had occurred a few days before being admitted. In a study done in Brazil showed the mean age was 29.75 years (ranging from 9 to 84 years) and the male to female ratio was 1:1.26. The type of lens used was available from 109 of the 166 cases of bacterial keratitis as (88.9%) patients were soft-lens wearers, (10.9%) were rigid gas-permeable lens [18].

In our study, table (6) revealed, out of the 80 examined cases by bacterial culture: 56 cases (70%) were positive while the remaining 24 cases (30%) were negative. From the 56 positive cases for bacterial culture, the first group included 30 positive bacterial corneal swab cases, from which 12 cases were associated with Acanthamoeba. The second group included 20 positive bacterial corneal swabs cases, from which Acanthamoeba was also present in both the corneal swabs and lenses in 6 cases. In the same time the third group 6 cases had contaminated lens storage in addition to the corneal swabs and lenses, also Acanthamoeba was detected in only 2 cases.

A study done by Zimmerman *et al*. 2015 stated that in case of bacterial keratitis the positive cultures from the cornea were (37%-63%), from the case lens were (80%-83%) and from the lens were (67%_92%). In case of *Acanthamoeba* keratitis the positive cultures from the cornea were (23%-86%), from the case lens were (23%) and from the lens were (23%) [17].

A study done in Brazil, culture of contact lenses was performed in 30 cases of laboratory-proven bacterial keratitis and in 13 cases of amebic keratitis. In the 30 cases of bacterial keratitis, the CL culture was positive in 22 examinations, among which 10 (45.45%) demonstrated growth of the same bacterium as in corneal scraping cultures and 12 (54.55%) did not. In 7 cases, the lens culture was positive for Acanthamoeba, while corneal material showed bacterial growth and provided no evidence of protozoal eye infection. Among the 13 cases of corneal scraping culture-proven Acanthamoeba keratitis, contact lens culture led to the isolation of the protozoa in 6 cases (46.15%) and showed no growth in 7 cases (53.85%). Nine cases (20.93%) of the total 43 CL cultures displayed polymicrobial growth [20].

A study was done for examination of the contact lens care systems of 100 asymptomatic patients who used hard or soft contact lenses for correction of refractive errors for the presence of bacteria, fungi, Acanthamoeba. Of 100 patients, 52 %had contaminated contact lens care systems, and (13%) of commercial contact lens care solutions were contaminated. Contaminated commercial solutions were opened and used for a longer period of time than uncontaminated solutions. Contamination was not found in bottles of preserved commercial solutions that were opened and used for less than 21 days. All the bottles of homemade saline were contaminated with bacteria, and Acanthamoeba was isolated from two of these bottles. Pseudomonas was found in the care systems of 12 patients [21].

A survey done by Gray *et al.* established the incidence of protozoal, bacterial, and fungal contact lens case contamination in 101 asymptomatic daily wear cosmetic contact lens wearers. Eighty two (81%) contact lens cases were found to be contaminated, while 19 (19%) were sterile. Of all contact lens cases, 78 (77%) grew bacteria, 24 (24%) fungi, and 20 (20%) protozoa. Fifty six (55%) contact lens cases yielded mixed bacterial contamination. The most common bacterial contaminants isolated were non-fermentative Gram negative bacilli pseudomonas (60.2%) This study confirms that Acanthamoeba contamination of contact lens cases is far more common than Acanthamoeba keratitis. This is the first contact lens case survey in which hydrogen peroxide disinfection was the major method of contact lens disinfection and no homemade saline was used. All the contaminating organisms were shown to possess the enzyme catalase that breaks down hydrogen peroxide to oxygen and water. The polymicrobial nature of the biofilms found in many contact lens cases are recognized as potential source of pathogens associated with corneal ulcers [22].

The contamination of lens care systems with bacteria is an essential association in the development of Acanthamoeba keratitis. The bacterial microorganisms that adhere to the surfaces of contact lenses provide a good medium that facilitates attachment, feeding, survival, and growth of Acanthamoeba [14].

In our study, Table (5)the positive 20 cases for Acanthamoeba culture were examined for bacterial association which was detected in 19 cases (95%) of them. Only one case (5%) had no bacterial association.

Acanthamoeba can easily attach and grow on a lens surface previously loaded with bacterial microorganisms. Gorlin *et al.* [23] found that about 50% of the eyes infected with Acanthamoeba had positive cultures for bacteria. Other study done Kelly *et al.* [24] showed that 85% of contact lens systems infected with Acanthamoeba were contaminated with bacterial strains, mainly with the aerobic gram-negative bacilli *P. aeruginosa* and *Xanthomonasmaltophilia*.

Pseudomonas aeruginosa remains the commonest cause of contact lens-related corneal infection probably because of its unique virulence characteristics and ability to survive in the contact lens/storage case/ocular environment which offers a suitable survival niche for this environmental organism. *P. aeruginosa* can adhere to and colonies lens materials during wear and survive in contact lens storage cases, partly through its ability to grow as a resistant biofilm on lenses and cases, or acquired resistance to contact lens disinfectants. The initiation

of microbial keratitis probably requires a combination of unique bacterial virulence characteristics plus the physiological impact of contact lens wear on the cornea [13].

Among those 19 cases, 3 cases (15%) presented with *Pseudomonas aeruginosa* while the rest of them had been associated with other bacterial species (80%) table (6).

Both *P. aeruginosa* and Acanthamoeba are potentially devastating causes of microbial keratitis. It is important to consider the possibility of a concurrent infection in cases with contact lens-related keratitis (25).In previous work by the same authors Taher *et al.* [26] discussed the relation between Acanthamoeba infection and *S.epidermidis*&*S.aureus.*

The potential utility of PCR based techniques for improving the diagnosis of ocular infection is well recognized and expanding. This was in agreement with Khattab *et al.* [27] as they mentioned that PCR detects microbial DNA in the majority of bacterial and fungal corneal ulcers and identifies microorganisms in a high proportion of culture-negative cases. Although being expensive; PCR remains a promising tool for faster and highly sensitive diagnosis of microbial keratitis (Fig. 1).

Conclusion:

Contact lens wear is the main cause of ulcerative keratitis, which could get seriously complicated with corneal scarring and lead to permanent vision loss. The association between Acanthamoeba keratitis and contact lens wear is firmly established. Contact lenses have a great impact on corneal epithelium integrity. This, added to the greater affinity of Acanthamoeba to adhere to either corneal or lens surfaces, increase the risk in contact lens wearers. Lens hygiene, lens care solutions, wearing modalities and the compliance of lens users are important factors in the lens-keratitis relationship. Every lens wearer should be aware of what the main risk factors are and, when given the routine instructions regarding lens fitting and care.

Acanthamoeba and *P. aeruginosa* share many characteristics as eye pathogens, they can adhere to, colonize, and invade injured corneal tissue; both produce tissue-destructive enzymes; and both have been recovered individually as contaminants of contact lens care systems. In spite of that concurrent infection between the two organisms was only found in three cases in this study.

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