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Incidence of common aerobic microbiological agents and outcome of ulcerative keratitis in patients who refer to Ahvaz Imam Khomeini hospital

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Abstract

To evaluate the incidence of common aerobic microbiological agents and outcome of ulcerative keratitis in patients who refer to Ahwaz Imam Khomeini hospital during a year. Patients presenting with suppurative corneal ulcer to ophthalmology emergency center of Imam Khomeini Hospital of Ahwaz University of medical science from October 2015 to October 2016 were prospectively followed. Demographic data, risk factors and clinical sign were carried out. Visual acuity measured and converted to logarithm of the minimum angle of resolution(logMAR). Corneal scraping was performed and obtained material was smeared on glass slid for gram stain. material obtained from corneal scraping transferred to platinum loop and wet swab. platinum loop smeared on chocolate agar and wet swab transferred into liquid tryptic soy broth (TSB). Blade directly inoculated in sabour aud dextrose agar (SDA). empirical treatment started and patients were followed up after 1 week,2 weeks,1month and 2 months. Most of the patients were between 60 to 80 years old and only three patients were under 40 years old. The commonest predisposing factor was Ocular surface disease (OSDs) followed by prior ocular surgery and trauma. The coagulase negative staphylococcus was the commonest microorganism (38.9%). Average log MAR BCVA at the inrolment and 2 month of treatment was respectively1.99 and 1.84, this difference was statistically significant(p=.01). We revealed that patients who were between 60-80 years old were more susceptible to the ulcerative keratitis, moreover, the commonest predisposing factor for suppurative keratitis was ocular surface disease (OSDs) and prior ocular surgery. coagulase negative staphylococcus was the commonest agent and average logMAR BCVA was improved with treatment.

Keywords: Suppurative Keratitis, Ulcerative, Keratitis, Prospective, Iran

Full-length article **Corresponding Author*, e-mail:

1. Introduction

Microbial keratitis is a significant cause of corneal blindness and visual impairment throughout the world (1). It can be caused by a range of pathogens including bacteria, viruses, protozoa, and fungi (2,3). Corneal ulcer is a sight-threatening condition that requires prompt diagnosis and treatment to prevent unfavorable outcomes (4). The most prevalent symptoms and signs are pain, photophobia, redness, anterior chamber reaction, corneal infiltration, and corneal edema (5). In patients with untreated supportive keratitis, the symptoms can be more severe including endophthalmitis, corneal perforation, and blindness (5). Depend on the region, several groups of pathogens are involved in the suppurative bacterial keratitis such as gram-positive, gram negative, aerobic and anaerobic microorganisms and fungus (6). Bacterial keratitis is a rare condition in normal eyes, however, prior ocular surgery, trauma, contact lens wear, dry eye, ocular surface disorder (OSDs) and immunosuppression are the most important predisposing factors (7).

Geographically, the risk factors of bacterial keratitis vary, for example in the developed countries the most important risk factor is contact lenses and in developing countries, trauma is the common cause (8). The incidence of suppurative keratitis varies from 11 per 100.000 in developed countries such as Unites State of America to 299 per 100 000 in developing countries such as India (9). The rapid initiation of empirical and then targeted treatment is crucial.the choice of a targeted drug depends on the culture result and drug resistance of the cultured microorganism (10). Therefore, the laboratory evaluation of ulcerative keratitis is essential to perform the right diagnosis and treatment. We conducted this prospective study to define the clinical demographic state, risk factor, microbiological pattern and visual outcome of ulcerative keratitis in patients who refer to Ophthalmology

Emergency Center of Ahwaz Imam Khomeini hospital during a year.

2. Materials and Methods

This study was approved by the ethic committee of Ahwaz university of medical sciences, moreover, the study procedure was explained to all patients and written informed consent was obtained. All patients who were diagnosed with orneal ulcer presenting to ophthalmology emergency center of Imam Khomeini hospital of Ahwaz university of medical science from October 2015 to October 2016 were included in this prospective study.

Inclusion criteria were any patient with suppurative corneal stromal infiltration with overlying corneal epithelial defect in central 3 mm of cornea that greater than or equal to 1mm in size, peripheral ulcer more than 2 mm and peripheral ulcer less than 2 mm in the presence of anterior chamber reaction. Exclusion criteria were patient with viral keratitis, acanthamoeba keratitis, sterile keratitis like vernal keratopathy and marginal keratitis, age below 18 years,who were unable to provide consent, no light perception(NLP) visual acuity at the time of refer and history of taking more than one species of topical antibiotic.

The patients were interviewed, and demographic data include name, sex, age, and location of life were recorded. The history of predisposing factor including prior ocular surgery, history of ocular trauma and type of trauma, history of contact lens wearing, use of topical corticosteroid, keratorefractive surgery and systemic disease like diabetes were listed. Visual acuity was measured using the Snellen chart at a distance of 4 meters and then converted to the logarithm of the minimal angle of resolution (log MAR). For patients with severe vision loss including counting fingers (CF), hand motions (HM), light perception (LP) and no light perception (NLP) were recorded as LogMar values 1.875,2.700, 2.301and 3.000 respectively (11,12).

Full ophthalmologic examination was performed using slit lamp and eyelid examination was carried out and trichiasis, distichiasis, entropion, ectropion, dacryocystitis and meibomian gland dysfunction (MGD) was evaluated. The size (largest diameter) and depth of corneal stromal infiltration and corneal epithelial defect measured in millimeter. Location of ulcer was drawn based on the corneal zone (central 3mm, peripheral, total cornea). Thinning, descemetocele and vascularization of the cornea were evaluated and anterior chamber examination for cell and hypopIon was done.

After a full ocular examination, corneal scraping from edge of infiltration under sterile conditions with the help of slit lamp was performed. the cornea was anesthetized using tetracaine 0.5% and corneal scraping was performed with a sterile Bard-Parker surgical blade number 15. Some of the collected material was transferred to a platinum loop and some to a sterile wet cotton swab. A platinum loop smeared onto glass slide the surface of the chocolate agar. wet cotton swab transferred to tryptic soy broth (TSB). Remained materials on blade directly inoculated in sabouraud dextrose agar (SDA). The samples were sent to microbiology laboratory of Ahvaz Jundishapour University of medical science and incubated at 37°C for 24 to 48 hours. Then swab was removed from liquid TSB medium and cultured on the diagnostic media such as blood agar and chocolate agar, then incubated at 37°C for 24 hours again. To isolate gram-positive from gram-negative bacteria, the bacteria were stained (gram staining) and cultured on the McConkey agar, mannitol salt agar and bile esculin agar. if isolated bacteria were gram positive then identified with specific tests such as MSA, DNAse, coagulase and catalase and if it was negative, a differential tube such as TSI, citrate, malonate, SIM, and lysine decarboxylase was used. Finally, for the all isolated bacteria, antimicrobial susceptibility test was performed based on the Clinical and Laboratory Standards Institute (CLSI) guideline. Antibiotics, including ciprofloxacin, co-trimoxazole, vancomycin, amikacin, gentamicin, rifampin, cefazolin and bacitracin was used for gram-positive bacteria and ceftazidime, ciprofloxacin, imipenem and meropenem for gram negative.

For fungal cultures inoculated SDA incubated at room temperature and was inspected daily for up to14 days and declared as fungal culture negative after two weeks. Empirical medical therapy was started for all patients immediately after the corneal scraping. For all patient treatment with a fluoroquinolone (ciprofloxacin or levofloxacin) had been started and for moderate to severe case fortified eye drop added. For patients who were suspected to have fungal keratitis based on clinical features, natamycin eye drop had been started. All the patients were given homatropine 1% eye drop, 2gram oral vitamin C daily and doxycycline capsule 100 milligram Bd.

2.1. Statistical Analyses

The data were analyzed using Statistical Package for Social Studies version 20.0 (SPSS Inc, Chicago, Ill). Categorical data are presented as numbers (%), and continuous data as mean ± SD. We used the Chai_2 test to compare categorical variables and the student's t-test to compare continuous variables. $P<0.05$ was considered significant.

3. Results

3.1. Demographics

For one year, 54 patients were examined in the emergency department of ophthalmology, ahwaz imam khomeini hospital with a corneal infiltrate that was compatible with a diagnosis of supporative keratitis. Thirty (55.6%) patients were admitted to the hospital for therapy while the other 24(44.4%) were treated in the outpatient clinic. Of these 24 patients, 14 needed hospitalizations but did not consent to hospitalization. The time interval between first symptom and presentation to hospital range from 0 to11 days (average 3.2± 3.7). The period of admission ranged from 7 to 36 days (average 9±11). 26 rural and 28 urban patients (28 males,26 females) were studied. The average age was 64.63±13.77 years, ranging from 22 to 85 years. Most of the patients were between 60 to 80 years old (63%) and only three patients were under 40 years old. All 54 patients had unilateral corneal ulcer, 28 (51.9) in right eye and 26(48.1) in left eye. Interestingly, the number of cases during autumn time was significantly less than in other seasons.

3.2. Predisposing factor

The commonest predisposing factors were Meibomian gland dysfunction (79.6%), prior eye surgery (40.7%), and trauma (13%).

Table 1: The patients' characteristics and predisposing factors

Variables	**Subtype**	**N %**
Sex	Male	28(51.9%)
	Female	26(48.1%)
Age	20-39	3(5.6%)
	40-59	12(22.2%)
	60-79	34(63%)
	>80	5(9.3%)
Location	Rural	26(48.1%)
	Urban	28(51.9%)
Eye	Right	28(51.9%)
	Left	26(48.1%)
Hospitalization	Yes	30(55.6%)
	No	24(44.6%)
Trauma	No	47(87%)
	Mineral particle	4(7.4%)
	Finger nail	2(3.7%)
	vegetable	1(1.9%)
Surgery	No	32(59.2%)
	Cataract extraction	17(31.4%)
	Vitreoretinal surgery and cataract extraction	2(3.7%)
	Penetrating keratoplasty and cataract extraction	3(5.6%)
Diseases	No	47(87%)
	Diabetes	6(11.1%)

	malignancy	1(1.9%)
Contact lens wear		3(5.6%)
Meibomian gland dysfunction		43(79.6%)
Entropion		3(5.6%)
Ectropion		1(1.9%)

Table 2: Clinical sign of ulcerative keratitis

Sign		**N (%)**
Location	Central	48(88.9)
	Peripheral	6(11.1)
Depth	>30%	48(88.9)
	<30%	6(11.1)
Size	<3 mm	8(14.8)
	>3mm<6mm	23(42.6)
	>6mm	23(42.6)
A/C reaction		8(14.8)
Hypopion		16(30)

Table 3: Smear, culture and antibiogram

Smear	Gram-positive cocci	26(48.1%)
	Gram positive bacillus	2(3.7%)
	Gram negative bacillus	3(5.5%)
	Negative	23(42.6%)
Culture	No growth	13(24%)
	Coagulase-negative staphylococcus	21(38.8%)
	Staph aureus	4(7.4%)
	Pseudomonas	3(5.5%)

	Gram positive bacillus	3(5.5%)
	Enterococcus and coagulase-negative staphylococcus	3(%)
	Corynebacterium	1(1.8%)
	Fusarium	2(3.7%)
	Yeast	1(1.8%)
	Staph aureus and coagulase-negative staphylococcus	2(3.7%)
Cefoxitin	Sensitive	26(48.1%)
	Resistance	2(3.7%)
Rifampin	Sensitive	27(50.0%)
	Resistance	2(3.7%)
Cotrimoxazole	Sensitive	6(11.1%)
	Resistance	23(42.6%)
Gentamycin	Sensitive	33(61.1%)
	Resistance	0
Vancomycin	Sensitive	17(31.5%)
	Resistance	0
Ciprofloxacin	Sensitive	25(46.3%)
	Resistance	2(3.7%)
Azithromycin	Sensitive	14(25.9%)
	Resistance	20(37%)
Amikacin	Sensitive	26(48.1%)
	Resistance	3(5.6%)
Imipenem	Sensitive	4(7.4%)
	Resistance	0
Meropenem	Sensitive	4(7.4%)
	Resistance	0
Levofloxacin	Sensitive	4(7.4%)

	Resistance	
Norfloxacin	Sensitive	3(5.6%)
	Resistance	0
Cefotaxime	Sensitive	1(1.9%)
	Resistance	2(3.7%)
Cefepime	Sensitive	1(1.9%)
	Resistance	3(5.6%)
Ceftazidime	Sensitive	1(1.9%)
	Resistance	2(3.7%)

Table 4. Univariate analysis of factors effective on visual acuity during follow-up period using linear regression model

Variable	LogMAR At inrollment		logMAR after 1 week		logMAR after 2 weeks		LogMAR AFTER 1 Month		LogMar After 2 months	
	Regression Coefficient	P value	Regression Coefficient	P value	Regression Coefficient	P value	Regression Coefficient	P value	Regression Coefficient	P value
Age	0.02	<0.001**	0.03	<0.001**	0.03	<0.001**	0.03	<0.001**	0.03	<0.001**
Sex										
Male	1*	-	1	-	1	-	1	-	1	-
Female	-0.46	0.002**	-0.53	0.002**	-0.45	0.02**	-0.49	0.01**	-0.48	0.01**
Time hospital stay	-.01	0.59	-0.01	0.35	-0.01	0.52	-0.01	0.45	-0.01	0.39
Hospitalization										
No	1	-	1	-	1	-	1	-	1	-

Yes	0.01	0.95	-0.04	0.83	-0.01	0.94	-0.03	0.89	-0.05	0.81
Culture										
not growth	1	-	1	-	1	-	1	-	1	-
coagulase neg. staph	0.02	0.90	0.08	0.82	0.07	0.78	0.04	0.86	0.05	0.86
staph aureus	-0.29	0.34	-0.32	0.39	-0.39	0.30	-0.49	0.21	-0.50	0.20
Pseudomonas	-0.34	0.36	-0.40	0.37	-0.29	0.53	-0.27	0.58	-0.39	0.42
gram pos.bacillus	0.08	0.82	0.06	0.90	0.07	0.87	0.10	0.83	0.11	0.82
Enterococcus	-0.17	0.70	-0.12	0.82	-0.16	0.78	-0.13	0.82	-0.12	0.83
corinebacterium	-0.77	0.21	-0.57	0.43	-0.56	0.47	-0.53	0.50	-0.62	0.42
fusarium	-0.02	0.97	0.23	0.67	0.19	0.73	0.22	0.69	0.28	0.62
yeast	-0.19	0.75	-0.09	0.90	-0.46	0.55	-0.43	0.58	-0.42	0.59
staph aureus and coag.neg.staph	-0.30	0.42	-0.30	0.49	-0.39	0.41	-0.36	0.45	-0.35	0.46
Smear										
Negative	1	-	1	-	1	-	1	-	1	-
g p cocci	0.03	0.84	-0.03	0.86	-0.09	0.66	-0.12	0.54	-0.14	0.48
g p ba	-0.58	0.16**	-0.82	0.09**	-0.86	0.09**	-0.99	0.05**	-0.99	0.05**

g n b	-0.28	0.41	-0.61	0.13**	-0.64	0.13**	-0.62	0.15**	-0.61	0.16**

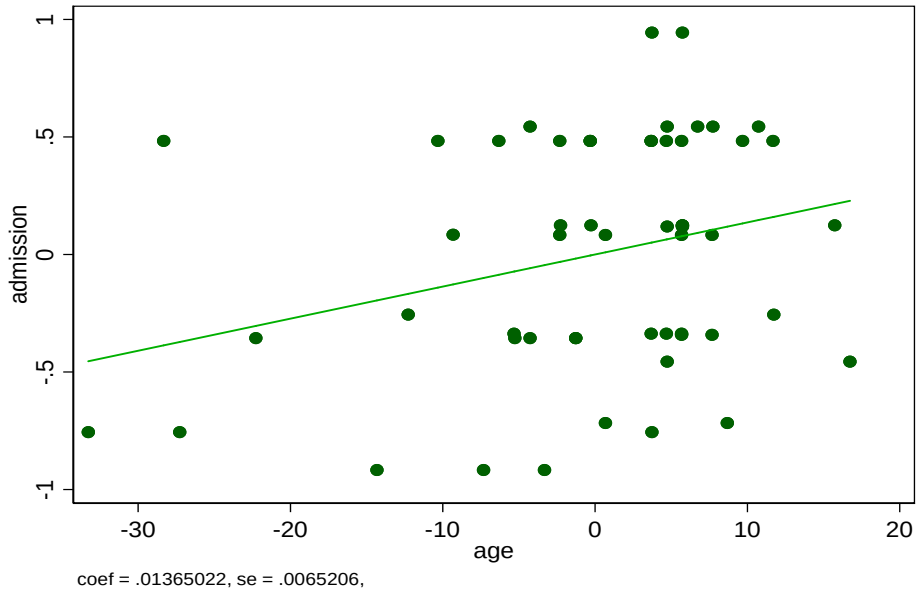

Figure 1. The relation between age and Log MAR BCVA at enrollment in multivariate model

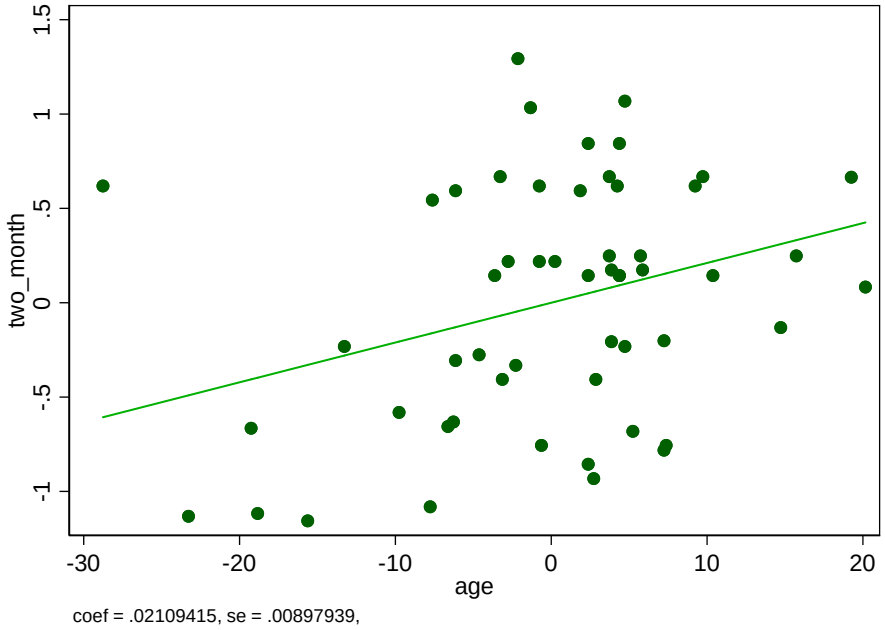

Figure 2: The relation between age and LogMAR BCVA after 2 months in multivariate model

Cataract extraction in 17 (31.4%), penetrating keratoplasty combined with cataract extraction in 3(5.6%), and vitreoretinal surgery combined with cataract extraction in 2(3.7%) patients. There was a history of trauma in 7 (13%) patients. Mineral particle in 4 patients, trauma with fingernail in 2 patients and organic material(vegetable) in 1 patient. Eye lid abnormality includes entropion in 3(5.6%) and ectropion in 1(1.9%) patient were observed.6 patients had diabetes mellitus and 1 patient had malignancy(lymphoma). moreover, ulcerative keratitis occurred in three patients with history of contact lens wearing.

2.1. Clinical sign

Most common location of ulcer was in central 3 mm (88.9%) and 85.1% had size large than 3mm (Table 2).

2.2. SMEAR

The smear was negative in 23 patients, however, in the smear positive patients (57.4%) the most prevalent microorganism was gram- positive cocci (26,48.1%). gram- negative bacilli (5.5%) and gram-positive bacilli (3.7%) also positive in 5 patients.

2.3. Culture

The culture did not grow in 13 (24%) patients. among 41 (76%) patients with positive culture the coagulase-negative staphylococcus was the commonest organism (21 patients). Staphylococcus aureus in 4 patients, pseudomonas in 3-patients, gram-positive bacilli in 3 patients, gram-negative bacilli (enterobacter) in 1 patient, and Corynebacterium in 1 patient. Also, in 2 patients grew both coagulase-negative staphylococcus and staphylococcus aureus and in 3 patients both coagulase-negative staphylococcus and enterococcus. fungi isolated in 3 patients,2 fusarium and 1 yeast.

2.4. Antibiogram

Among antibiotics, most of the patients were sensitive to ciprofloxacin, amikacin, gentamycin, rifampin and cefoxitin, on the other hand, resistance to the cotrimoxazole among the patients was more than other antibiotics (Table 2).

2.5. Treatment outcome

Treatment was considered successful where the stromal infiltration resolved, and the overlying corneal epithelium healed completely (with or without scar). Out of 54 patients,46(85.1%) patients achieved good outcomes in the form of complete healing of corneal infiltration among them only 3 patients healed without scar and 43 patients had dense stromal corneal scars. The enucleation was performed for 2 patients, conjunctival flap for 4 patients and penetrating keratoplasty for 2 patients.

2.6. Visual outcome

At the time of inrollment, 14 patients had LP vision, 10 HM in 30cm,13 CF in 30 cm and 17 patients had vision between 20/200 to 20/800. Average LogMar BCVA at the time of enrollment was 1.99 and after one week of treatment was 1.92. It means that 3.5 letters increased in LogMar chart and difference was statistically significant (p =.0001). average LogMar BCVA after 2 weeks,1 month and 2 months respectively was 1.89,1.85and 1.84 and LogMar BCVA after 2 month increased 2 line in LogMar chart and difference was statistically significant(P=.013).

3. Limitations

The study was carried out in a tertiary referral center and therefore was treating a high portion of more difficult and sever cases.

4. Conclusions

We revealed both genders tend to develop corneal ulcer between 60-80 years old. the present study demonstrated that the commonest predisposing factor for suppurative keratitis was ocular surface disease (OSDs) and prior ocular surgery followed by and the most prevalent pathogen was coagulase negative staphylococcus.

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