Original article

Direct microscopy in suppurative keratitis: a report from tertiary level hospital in Nepal

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Abstract

Introduction: Infective keratitis is an ocular emergency that requires prompt diagnosis for appropriate management. This study was done todetermine the sensitivity, specificity and predictive values of Gram stain and potassium hydroxide (KOH) wet mount in the diagnosis of suppurative keratitis. **Materials and methods:** A prospective hospital based study of all patients with clinically diagnosed suppurative keratitis presenting between January 2011 and June 2012 was carried out. Corneal scrapes were taken and subjected to direct microscopy and culture.**Results:** Corneal scrapings were obtained from 108 eyes with suppurative keratitis. Direct microscopy was positive in 39.2% of cases and organisms were grown in 50.9 % of the cases. Bacteria were responsible in 76.4% and fungi in 23.6%. Of the bacterial isolates, 66.7% was Staphylococcus aureus and of the fungal isolates, 30.7% was Aspergillus species. Sensitivity in vitro showed that Cefazolin, Chloramphenicol and Ofloxacin were most effective against bacteria. Sensitivity of Gram stain in detecting bacteria was 50% (95% CI, 34.43 to 65.56) and specificity was 77.3% (95% CI, 65.0 to86.3) and sensitivity of KOH wet mount in detecting fungi was 53.8% (95% CI, 26.12 to79.6) and specificity was 98.9% (95% CI, 93.44 to 99.9). Positivity of direct smear (65.1%) was found to be higher among eyes with larger ulcers (>2mm) than eyes with smaller ulcers (<2mm). **Conclusion:** Direct microscopy is of great diagnostic value in the management of suppurative keratitis and it is recommended in all ophthalmic clinics without exception for establishing timely, appropriate and effective treatment.

Keywords: corneal ulcer, suppurative keratitis, microbiological diagnosis, direct microscopy.

Introduction

Corneal ulceration is defined as a loss of the corneal epithelium, with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon (Whitcher JP et al, 2001). Infective keratitis is an ocular emergency that requires prompt diagnosis for appropriate management to ensure the best visual outcome for the patient. Without adequate treatment, corneal infection leads to blindness through corneal scarring and endophthalmitis (Resnikoff S et al, 2004).

A clinical diagnosis of infective keratitis does not give an unequivocal indication of the causative organisms because a wide range of organisms can produce a similar clinical picture

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(Ostler HB, 1993; Florakis GJ, 1997; Liesegang TJ, 1980). Direct microscopic examination and culture for the detection of causative organisms are the two important microbiological investigations that are widely used. Although culturing of microbial pathogens is considered to be the gold standard, direct microscopic evaluation of smears provides immediate information about the causative organisms.

Several techniques have been used for the direct microscopic identification of microbes from corneal scrapes using stains like Gomori's methenamine silver (Xie L et al, 2001),periodic acid- Schiff (Sharma S et al, 1998),calcofluor white and fluorescein conjugated lectins (Robin JB et al, 1986) yield accurate results, but are time consuming and require special infrastructures. In addition, the cost of each test makes them inapplicable in primary, secondary and even in most tertiary centers. The conventional techniques, potassium hydroxide (KOH) wet mount, Gram stain and Giemsa stain, are widely used for the rapid detection of microbes (Xie L, 2001; Sharma S, 2002); however, owing to misinterpretation, presence of artefacts, and lack of detection of Candida and other yeasts, the sensitivity of these methods is highly variable (Xie L, 2001; Rosa RH, 1994; Rao NA, 1989; O'Day DM, 1996; Vajpayee RB, 1993). In addition, molecular diagnosis of pathogenic agents is a newer technology for accurate identification of the causative agents (Gaudio PA et al, 2002) but is inapplicable to all patients with corneal ulcer, as it is more expensive. Also, cultures require a longer time depending on the organisms (24 hours to 3 weeks).

Hence, examination of a smear can provide results in a short span of time, enabling clinician to start empirical treatment (Hagan M et al, 1995).In a study commissioned by the WHO Southeast Asia Regional Office in New Delhi (WHO/SEARO), it was estimated that 6 million corneal ulcers occur annually in the 10 countries of South-east Asia Region encompassing a total population of 1.6 billion (Gonzales CA et al, 1996). These estimates are based on the data from 4 countries where the incidence of corneal ulceration ranged from as low of 113 per 100,000 in India (Upadhyay MP et al, 2001)to as high as 799 per 100,000 in Nepal (Erie JC et al, 1993).

Suppurative keratitis is one of the leading causes of corneal blindness in Nepal. Prompt diagnosis and initiation of appropriate management could reduce severe visual loss and restrict corneal damage. Lack of microbiological laboratory facilities and initiation of primary treatment without etiological diagnosis are the main challenges in the management of corneal ulcers in the developing countries like Nepal.

Hence, there is a great need to perform simple methods of diagnosis like direct microscopy as it is easy, economic, less time consuming and can be carried out without sophisticated technicalities. To the best of our knowledge only a few reports are available in Nepal with regard to the sensitivity and specificity of the direct microscopy till date. This study has been undertaken to emphasize the role of direct microscopy and its importance as a simple method in the management of suppurative keratitis.

Materials and methods

This prospective hospital based study included all the diagnosed cases of suppurative keratitis attending the BPKLCOS, Kathmandu over a period of 18 months (1st January 2011 to 30th June 2012). Informed consent was taken from all the patients. Typical or suspected viral ulcer, interstitial keratitis, neurotrophic ulcer, Mooren's ulcer, any ulcer associated with systemic or metabolic disease, patients not willing to participate, small children and small ulcers from which enough sample could not be scraped for examination and patient who were not evaluated as per the protocol ,were excluded from this study. Patients already on

antimicrobials at the time of presentation were advised to stop medication for 48 hours before inclusion in the study.

Detailed clinical history, general physical examination and detailed ophthalmological examination were carried out in all the subjects as per ourstudy protocol.

The location of the ulcer was recorded as central, paracentral or peripheral or total. The shape, margins and the size of the ulcer were recorded. The size of the epithelial defect after staining with 2% fluorescein was measured with variable slit on the slit-lamp bio-microscope and recorded in millimeters on a standardized form. Firstly, the longest dimension of the defect was determined and then the dimension perpendicular to the first was measured. In a similar fashion the size and depth of the stromal infiltrate was recorded. Stromal infiltrate was looked for their colour, depth and margin along with surrounding corneal haze. The corneal ulcer depth was evaluated as <20%, 20-50% or >50% of the total corneal thickness. Corneal vascularisation was looked for and labeled as superficial or deep. A sketch of each ulcer was also drawn on the form using standardized frontal and cross sectional diagrams. Corneal sensation was assessed in all the cases. Ultrasound A and B scan were done in cases where the posterior segment could not be viewed to rule out the presence of vitritis and/or endophthalmitis. Photographic documentation was done whenever it was felt necessary.

Microbiological evaluation

Corneal scraping was performed under magnification of a Haag Streit slit lamp

bio-microscope or Konan operating microscope 700. The affected eye was anesthetized with a topical 0.5% Proparacaine hydrochloride. The Kimura's spatula or no.15 Bard Parker blade was scraped over the surface of the suppurative area first to remove the superficial necrotic material then in a series of short, moderately firm strokes to sample both the leading edges and the base of each infiltrated area in one direction from the peripheral margins towards the center of the suppurative area. The material obtained was initially smeared onto clean sterile labeled glass slides for 10% KOH wet mount and Gram stain. Giemsa stain was not included in the study. The material obtained by the next scrape was inoculated directly onto the surface of solid media such as blood agar, chocolate agar and Sabouraud's dextrose agar in rows of C-shaped streaks and also inoculated into depth of liquid medium such as brain heart infusion broth. The order in which the specimen was prepared was: Slide for Gram stain and KOH wet mount was prepared first, followed by inoculating the sample onto the solid media and lastly onto the liquid media.

Laboratory procedures

Smears were prepared by scraping the ulcer and gently transferring the material on the spatula on to the sterile glass slide over an area of approximately 1 cm in diameter. The glass slide was marked on the opposite side with a permanent marker to obviate the need to search for area smeared under the microscope. Two slides were prepared, one for Gram staining and the second for KOH wet mount. The specimen was then viewed with 10x objective and 100x oil-immersion objective of a microscope (Nikon, Japan, 183382). All inoculated media were incubated aerobically. The inoculated Sabouraud's dextrose agar was incubated at 27^{o}C, examined daily and discarded at 3 weeks if no growth was seen. The inoculated blood agar, chocolate agar and brain heart infusion broth were incubated at 37^{o}C, examined daily and discarded at 7 days if no growth was seen. For the entire bacterial isolates antimicrobial sensitivity test was performed by Kirby Bauer Disc diffusion method. Processing, reading of the results and zone size interpretation have been performed as per NCCLS (CLSI) guidelines (Wayne, Pa., 2006).

Treatment protocol

Initial therapy was decided on the basis of smear report. If it was negative, then on the basis of patient's history and clinical examination, therapy in the form of broad spectrum antibacterial or antifungal was started. After availability of culture sensitivity report, if needed treatment was changed to a suitable antibiotic or antifungal.

Bacterial Corneal Ulcer

For mild corneal ulcer- Fluoroquinolones (Ofloxacin) eye drops was started every hour for 2-3 days. Then it was tapered according to clinical response of the corneal ulcer. For moderate to severe corneal ulcer- Duo-therapy consisting of reconstituted Cefazolin 50mg/ml and Fortified Gentamycin 14mg/ml eye drops were used.However, sensitivity of the organism to an antibiotic was of prime importance in selecting a suitable antibiotic.

Fungal corneal ulcers

For mild fungal corneal ulcers- 5% Natamycin eye drops were started every hour and tapered according to the clinical response. For moderate to severe fungal corneal ulcers- 5% Natamycin eye drops and systemic Itraconazole (100mg BD) for 21 days or Fluconazole (150mg BD) for 2 weeks were given.

Adjuvant therapy

All patients with suppurative keratitis were given Atropine 1% eye drop, anti-glaucoma therapy if needed, Vitamin C along with antibacterial or antifungal therapy. In severe corneal ulcers not responding to the above treatment or in impending corneal perforation, treatment was modified in the form of systemic antibacterial or antifungal therapy. For perforated corneal ulcer, bandage contact lens with tissue adhesive for smaller perforations (<2 mm)and therapeutic penetrating keratoplasty for larger perforations were done.

Data Processing and Analysis

Detail findings were recorded in the Performa developed for this study. Pearson Chi-square test was used for the analyses of categorical data. The 95% confidence interval limits were provided and p value ≤0.05 was considered statistically significant. SPSS software version 18 for Windows was used for statistical analysis.

Results

One hundred and eight patients with the clinical diagnosis of suppurative keratitis meeting the inclusion criteria were included in the study.

The mean age of the patients was 46.76 ± 18.27 years, ranging from 14 – 89 years. The median age of the patients was 50 years. In this study 52% (n=56) cases were female and 48% (n=52) cases were male. Majority of the patients 77 (71%) were from the Hilly region of Nepal followed by those from the Terai region (26%). Corneal ulcers occurred throughout the year in Nepal, in this study it was seen that a slightly more number of patients presented to the Centre during the Spring season. Corneal ulcers were more prevalent in the agricultural workers (n=51, 47.2%).

The median duration of presentation was 7 days (Inter-quartile range was 11.75 days). About 32% patients presented between 4 to 7 days of onset of symptoms. History of corneal injury was the most common risk factor, encountered in 43 eyes (39.8%). Trauma with vegetative matter was found to be the most common predisposing risk factor in 23 patients (21.3%) and 20 patients (18.5%) had a history of trauma with non vegetative matter. Most of the patients were using topical eye drops at the time of presentation (n=74, 68.5%) among them 9 (8.3%) subjects were documented to be using steroid eye drops.

Out of 108 specimens examined, direct microscopy was positive in 43 (39.8%)

specimens and culture was positive in 55 (50.9%). Table 1 shows the yield percentage of direct smear and culture method.

Table 1: Yield percentage

Methods	Positive yield	Negative yield	Bacteria	Fungus
Direct Smear	39.8%	60.2%	83.7%	16.3%
Culture method	50.9%	49.1%	76.4%	23.6%

Fifty-five (51%) ulcers measured more than 2mm in diameter. In this study it was seen that smear positivity was seen in 50.9% ulcers those were larger than 2mm in size (p value =<0.02) as shown in table 2.

Table 2: Ulcer size vs. smear positivity

Ulcer size	Number of cases	Smear positive	Percentage
Less than 2 mm	53	15	28.3%
More than 2 mm	55	28	50.9%

Culture was positive in 55 samples. Out of the 55 samples, in 42 eyes (76.4%) bacteria were identified and 13 eyes (23.6%) showed fungal growth. No mixed organism was isolated from any eye. Out of the 42 bacterial isolates, 67% (n=28) grew Staphylococcus aureus , 28% (n=12) grew Streptococcus pneumonia and 5% (n=2) grew Streptococcus viridians. Pseudomonas species, E.coli, filamentous bacteria or Acinetobacter were not isolated in this study. Out of the 13 fungal isolates 4 (30.75%) grew Aspergillus, 3 (23.1%) each grew Fusarium and Penicillium. Candida species or any dematiaceous fungiwere not isolated in this study. Table 3 shows the different organisms isolated.

Table 3: Organisms isolated

Bacteria	42	Fungus	13
Staphylococcus aureus	28	Aspergillus spp.	4
Streptococcus pneumonia	12	Fusarium spp.	3
Streptococcus viridans	2	Penicillium spp.	3
		Acremonium spp.	1
		Rhodotorula spp.	1
		Others	1

The sensitivity of Gram stain in detecting bacteria was 50% (95% CI 34.43 to 65.56) and the specificity was 77.3% (95% CI 65.0 to 86.3). Hence, the positive predictive value of Gram stain was 58.3% and the negative predictive value was 70.8% as shown in table 4.

The sensitivity of KOH wet mount in detecting fungi was 53.8% (95% CI 26.12 to 79.6) and specificity was 98.9% (95% CI 93.44 to 99.9). Hence, the positive predictive value of KOH wet mount was 87.5% and negative predictive value was 94%, also shown in table 4.

Table 4:Sensitivity and Specificity of direct smears

Methods	Grams stain	KOH wet mount
Sensitivity	50%	53.8%
Specificity	77.3%	98.9%
Positive predictive value	58.3%	87.5%
Negative predictive value	70.8%	94%

Most of the patients were managed well with medical therapy but few required surgical intervention. Table 5 shows the overall treatment outcome.

Table 5:Treatment outcome

Outcome	Culture result			Total
	Bacteria (n=42)	Fungus (n=13)	Negative (n=53)	
Healed scar	22	5	15	42
Adherent leucoma	9	1	12	22
Worsening/ no response	5	2	10	17
Evisceration	0	1	3	4
Keratoplasty	2	3	7	12
No follow-ups	4	1	6	11

Results of different previous studies have been compared with the results of this study in table 6.

Table 6: Comparison with previous studies

Studies		Bharathi MJ et al, 2006	Gopinathan U et al, 2009	Dunlop AAS et al, 1994	Feilmeier MR et al, 2010	Current study
Culture yield %	Total	69.4%	60.4%	81.7%	40%	50.9%
	Bacteria	46.7%	51.9%	58.6%	39%	76.4%
	Fungus	49.7%	38.2%	41.4%	61%	23.6%
	Mixed	3.6%	9.9%	(5%)	0%	0%
Smear yield %	Total	74%	70.5%	70.4%	34.6%	39.8%
	Bacteria	48.1%	60.4%	50%	41%	83.7%
	Fungus	48.5%	39.6%	50%	59%	16.3%
	Mixed	3.4%	0%	(6%)	0%	0%
KOH wet mount	Sensitivity	99.3%	89.8%	97.9%	80.5%	53.8%
	Specificity	99.1%	93.7%	96.8%	98.9%	98.9%
	Positive predictive value	98.5%	-	94%	95.8%	87.5%
Grams stain	Sensitivity	100%	56.6%	61.7%	75%	50%
	Specificity	97.6%	97.8%	89%	97.3%	77.3%
	Positive predictive value	95.7%	-	84%	80.6%	58.3%
Most common micro-organism	Bacteria	Streptococcus pneumonia	Staphylococcus epidermidis	Streptococcus pneumonia	Streptococcus pneumonia	Staphylococcus aureus
	Fungus	Fusariumspp	Fusariumspp	Aspergillus fumigatus	Aspergillusspp	Aspergillusspp

Discussion

In our study of 108 patients with clinically diagnosed suppurative keratitis, the mean age of the patients was 46.76 ± 18.27 years ranging from 14 to 89 years. While evaluating the age distribution of the patients it was seen that 79 (73.1%) of the patients were between 21 to 60 years of age, which is the most productive socioeconomic population of Nepal. This was similar to a study conducted by M.P. Upadhyay et al in Nepal, where corneal ulcers were found to be least common in patients below 10 years and over 71 years of age (Upadhyay MP et al, 1982). Similarly, in a study conducted by U. Gopinathan et al it was seen that the mean age of patients with bacterial keratitis was 41.20 ± 20.36 years and that of patients with fungal keratitis was 30.90 ± 15.28 years, indicating a relatively increased occurrence of corneal infections in the middle age group (Gopinathan U et al, 2009) . In another study conducted by Lavaju P et al, it was seen that ulcers were more prevalent in the middle age groups, between 21-40 years of age (50%) (Lavaju P et al, 2009). Presentation in this age was more common due to the fact that persons belonging to this age group are more active and involve themselves in outdoor activities. Another factor could be that they are also the earning member of the family so are brought more frequently to the hospital (Saeed A et al, 2009).

In this study females were affected more (52%) than males (48%) similar to that seen in a study conducted by M.P. Upadhyay et al where 63.9% of patients with corneal ulcer were females and 36.1% were males (Upadhyay MP et al, 1982). While a study conducted by Samar K Basak et al in West Bengal, India showed that majority of the patients were males (70.6%) and 29.4%were females (Basak SK et al, 2005). In another study done by M.P. Upadhyay et al between September 1985 and August 1987 showed that the males and females were equally affected

(Upadhyay MP et al, 1991). In our study females seemed to be affected more than males because there is a significant contribution of the female population to the agricultural labour force in the country and also that a significant number of the male population venture to foreign countries in search of employment.

Although corneal ulcers occur in all seasons of the year, a slightly more number of patients (30.6%) presented to the hospital in the spring season between the months of March and May. A study done by M.P. Upadhyay (Upadhyay MP et al, 1982) et.al saw that corneal ulcers seemed to be more frequent during monsoon (June to August) and autumn (September to November) seasons, when 84 cases (63.2%) were seen.

Majority of keratitis cases were seen in the agricultural group (47.2%) similar to other studies done by Upadhyay MP et al (Upadhyay MP et al, 1991), Lavaju P et al (Lavaju P et al, 2009) 75% in Nepal and Samar K Basak et al (Basak SK et al, 2005) 57.6%; but a marked contrast was found in a study done in Ghana (Hagan M et al, 1995)where only 16.1% were involved in agricultural activity.

The median day of lag before presentation to our Eye Centre was 7 days, with the interquartile range of 11.75 days. More than half of the patients (57.4%) presented to the hospital before 7 days of onset of symptoms contrary to the study conducted by Samar K Basak (Basak SK et al, 2005) where the majority of patients (51.8%) were seen between 2-3 weeks of their illness. The findings in our study can be correlated with the regional distribution, where it was seen that the majority of patients came to hospital from the Hilly region of Nepal which falls in and around the Kathmandu Valley, where the tertiary Eye Centre is located.

Corneal injury has always been identified as a cause of microbial keratitis. In our study, a history of corneal injury was present in 43 eyes (39.8%). Similarly, a history of trauma could be elicited in 82.7% of cases of corneal ulcer in the study conducted by Upadhyay M.P et al (Upadhyay MP et al, 1982). Whereas Y W Ibrahim et al (Ibrahim YW et al, 2009) and Matthew Green et al (Green M et al, 2008) found that a significant number of patients had contact-lens use as the main risk factor (31% and 22% respectively).

Majority of the patients were using topical drops at the time of presentation to the hospital (n=74, 68.5%), of them 13% had received a combination therapy (antibiotics and antifungals). This reflects the tendency of general practitioners and ophthalmologists at the primary and secondary eye centres to prescribe a cocktail of drugs and refer patients to tertiary eye centres only when empirical treatment failed. Almost 16% had received antibiotics alone; many of them had received insufficient dosage, demonstrating the tendency of medical practitioners to treat the condition inadequately. UshaGopinathan et al (Gopinathan U et al, 2009) found 10% of patients using steroids similar to our study where 8.3% had received topical steroids, and many of them had acquired them as an over the counter drug from a local chemist, showing a lack of awareness of the disease and its complications.

In the total of 108 cases, 43 patients (39.8%) showed positive findings in direct microscopy and 55 patients (50.9%) were culture positive. These findings were comparable to other studies done in Nepal (Feilmier MR et al, 2010) where smear microscopy was positive in 34.6% of cases and 40% of the cases showed growth in the culture, Ghana (Hagan M et al, 1995) where 57.3% were culture positive. Similarly Matthew Green et al (Green M et al, 2008) found Gram stain was positive in 83 cases (33%) and cultures were positive in 164 (65%) cases and negative in 89 (35%) cases. The lower rate of isolation in our study could be attributed

to empirical use of topical antibiotics before presentation to our hospital and our inability to employ enriched and selective media. Use of additional media would help determine the pattern of microbes more fully, which in turn would permit the selection of antimicrobials that will have the best chance of curing corneal ulcers (Upadhyay MP et al, 1982).

Bacteria wereisolated in 42 (76.4%) samples and fungus in 13 (23.6%) of culture-positive samples. None of the isolates showed mixed organisms or Acanthameoba. Out of these, Staphylococcus aureus was isolated in the majority i.e.,28 patients (67%), followed by Streptococcus pneumoniae in12 cases (28.6%). Aspergillus species was seen as themost common isolates accounting for 4 (30.7%) of fungal isolates. Staphylococcus aureus was the most commonly isolated bacterial organism representing 70% of all positive bacterial growth and Aspergillus was seen as the most common of the fungal isolates (66.6%) in a study done by Samar K Basak et al(Basak SK et al, 2005) and Staphylococcus aureus was also the most common bacterial organism isolated and Aspergillus the most common fungi in a study conducted by AartiTewari et al (Tiwari A et al, 2012). Ashok Narsani Kumar (Narsani AK et al, 2009) and colleagues also found that the most frequent organism isolated among the 240 cases of corneal ulcer was Staphylococcus aureus (n=75, 60%). A study done by Lavaju P et al (Lavaju P et al, 2009) showed that among the 44 patients of infective keratitis culture positivity was observed in 20 (45.5%) samples. Staphylococcus aureus was the most commonly isolated bacteria (70%) in this study also. Penny Asbell et al (Asbell P et al,1982) also found Staphylococcus to be the most common isolate (239 cases, 48%), coagulase positive Staphylococcus was twice as common as coagulase negative Staphylococcus (33% and 16% respectively). Whereas M. P. Upadhyay et al (Upadhyay MP et al, 1982) found Pneumococcus to be the single most important isolate, contributing to 60.4% of corneal ulcers; Staphylococcus was responsible for 24.5%. Among the fungi, Aspergillus species was the most common (40.0%). Feilmeier MR et al (Feilmeier MR et al, 2010) reported fungi in 61% of the cases, of which Aspergillus species was most commonly isolated (35%) and among the bacteria Streptococcus pneumoniae was most commonly isolated (69%).

Antibiotic sensitivity in vitro showed that Cefazolin, Chloramphenicol and Ofloxacin were most effective (100%) effective against the bacteria. Gentamycin which is commonly used along with Cefazolin for the treatment of suppurative keratitis was seen to be effective (100%) against Staphylococcus aureus and Streptococcus viridians and 83.5% of Streptococcus pneumonia was sensitive to it. It was also seen that Staphylococcus aureus was 96.4% sensitive to Ciprofloxacin as seen in a report (Goldstein MH et al, 1999) which shows a rapid increase in Staphylococcus aureus resistance to ciprofloxacin. M.P. Upadhyay et al (Upadhyay MP et al, 1982) found that Carbenicillin, Chloramphenicol, Cephaloridine, Methicillin and Lincomycin were most effective against bacteria. In our study it was seen that Tobramycin was not as effective against the bacterial isolates, contrary to this LailaAkter et al (Akter L et al, 2009) found that Lomefloxacin, Tobramycin and Gentamycin were more effective drugs against most of the gram-positive and gram-negative bacteria. The disc susceptibility method provides quantitative measurements that are critical for epidemiology and drug resistance surveillance. Although this approach is both fast and cost effective (Hindler J et al, 1995), resistance criteria apply only to systemically achieved drug levels, which are different from those achieved with topical treatment.

Direct microscopy (Gram stain + KOH wet mount) was positive in 65.1% of the ulcers

(n=28) which were greater than 2mm in size (p value <0.016). Similar findings were seen in a study done by Bharathi MJ et al (Bharathi MJ et al, 2006) which showed that the positivity of KOH was 44.46% and Gram stain was 77.37% among eyes with larger ulcers (>2mm). In another study by Savitri Sharma et al (Sharma S et al, 2007) it was also seen that in ulcers with the infiltrate size of <2.5mm square, the smear negativity was 79.3% while the smear positivity rate was just 49.0%.

Although culture was used as a gold standard for detecting pathogens in this study, smear microscopy was also effective for rapid identification of micro-organisms. It was seen that the sensitivity of Gram stain in detecting bacteria was 50% (95% CI, 34.43 - 65.56) and specificity was 77.3% (95% CI, 65.0 - 86.3), thus the positive predictive value of Gram stain was 58.3% and negative predictive value was 70.8%. The sensitivity of KOH wet mount in the detection of fungi was found to be 53.8% (95% CI, 26.12 - 79.6), specificity was 98.9% (95% CI, 93.4 - 99.9). Its positive predictive value was 87.5% and negative predictive value was 94%. In a study done by M J Bharathi et al, the sensitivity of Gram stain in detecting bacteria was 100% and specificity was 97.6% and sensitivity of KOH in detecting fungi was 99.3% and specificity was 99.1% (Bharathi MJ et al, 2006). Prashant Garg et al found the sensitivity and specificity of Gram stain in detecting bacteria to be 56.6% and 97.8% respectively and sensitivity and specificity of KOH in detecting fungi was 90.6% and 94.3% respectively (Gopinathan U et al, 2009). A study done by Michael R et al in Nepal, Gram stain was 75% [95%, confidence interval (CI), 0.632–0.841] sensitive and 97.3% specific (95% CI, 0.951– 0.986) in identifying bacterial organisms. KOH wet mount was 80.5% sensitive (95% CI, 0.718–0.871) and 98.9% specific (95% CI, 0.971–0.997) in identifying the presence of fungal elements (Feilmeier MR et al, 2010). Similarly, another study done by AartiTewari et al shows the smear sensitivity corresponding to 64.4% and specificity to 93.8% for bacteria and for fungi, smear sensitivity was 75.6% while specificity was 100% (Tiwari A et al, 2012). Matthew Green et al found in their study, the sensitivity of Gram stain in identifying causative organisms as 53% and specificity as 89% (Green M et al, 2008). AAS Dunlop et al did a study on 142 cases of infective corneal ulcer where Gram stain had a sensitivity of 62%, specificity of 65%, positive predictive value of 84% and negative predictive value of 37% in detecting bacteria (Dunlop AAS et al, 1994). The factors which may have contributed to our findings are the size of the corneal ulcers, scraping technique, amount of scraped material and the ability to detect microorganisms in the field of microscopic examination of the scraped material. However, our findings do indicate that smear microscopy is a very important diagnostic test that provides rapid etiologic information and allows for initiation of the most appropriate antimicrobial therapy at the time of presentation. This is particularly important in developing countries such as Nepal, where follow-up is not always feasible and culture techniques are not always available.

Conclusions

Corneal ulcer is an ocular emergency that requires prompt and appropriate management for restoration and better visual outcome. This study was designed to find out the efficacy of available direct microscopic techniques in the detection of microbes from corneal scrapes which could lead to timely and appropriate treatment of the suppurative corneal ulcers.

Among the microbiological aetiology established, bacterial isolates were three times more frequent than the fungal isolates. This study found that there is a change in the trend of bacteria isolated from the cases of suppurative keratitis over the past decade.

About 10% difference was found between the direct microscopy of corneal smear and culture results. The sensitivity and specificity of Gram stain and KOH wet mount in our study were comparable to what was found in most other studies.

There was a statistically significant association between the smear positivity and the size of the corneal ulcers. It was observed that direct microscopy of corneal smear was an important diagnostic tool in the management of suppurative keratitis. Hence, the practice of performing direct microscopy in all the cases of infective keratitis should be emphasized and made mandatory in all the ophthalmic clinics.

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