Original articles

Causative organisms in microbial keratitis, their sensitivity pattern and treatment outcome in western Nepal

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Abstract

Introduction: Corneal infection is one of the major causes of monocular blindness in developing countries.

Objective: To determine the epidemiological characteristics, predisposing factors, microbiological pattern, sensitivity pattern and treatment outcome of microbial keratitis.

Materials and methods: A retrospective analysis of hospital records of 414 patients with diagnosed infective keratitis was done. The outcome measures were microbial isolates, their sensitivity to therapeutic agents and treatment outcome.

Results: Of the total, 312 (75.4 %) patients were farmers by profession, 138 (33.3 %) had a history of ocular trauma and 17(4.1 %) were using topical corticosteroids. Among the 138 cases of the corneal ulcer with trauma, 52 (37.68 %) had fungi isolated in culture (RR=0.54, 95% CI = 0.44 – 0.68) and 32 (23.19 %) had a bacterial growth. Cultures were positive in 300 (72.5 %) cases, having 138 (33.3 %) patients with pure fungal infection, 121 (29.2 %) with pure bacterial and 41 (9.9 %) with mixed infection. Fusarium spp was the most common fungal pathogen while Staphylococcus epidermidis was the commonest bacterial isolate. The most sensitive antibiotics for the Gram positive bacteria was cephazolin (84.92 %), while for Gram negative, ciprofloxacin and ofloxacin were the most effective (79.31 %). Of 414 cases of corneal ulcers, 363 (87.7 %) cases healed completely.

Conclusion: Microbial keratitis is mostly seen in farmers in this part of the world. Fusarium and Staphylococcus epidermidis were the most common isolates. Cephazoline and ofloxacin were the most effective antibiotics for Gram positive and Gram negative organisms respectively.

Key-words: corneal ulcer, microbial keratitis, western Nepal

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Introduction

Microbial keratitis is one of the leading causes of avoidable blindness in developing nations. Surveys in many parts of the world (African, Asian, Middle East etc) have revealed that corneal scarring is an important cause of blindness and visual impairment

(Upadhyaya et al 1991; Srinivasan et al 1997; Norina et al 2008; Whitcher et al 1997; Chirambo et al 1986). Blindness survey in Nepal (Brillian et al, 1985) too showed that corneal trauma and ulceration are second leading cause of unilateral blindness after cataract and are responsible for 7.9 % of all blind eyes.

The incidence of microbial keratitis varies from places to places from 11/100,000 persons /year in the United States (Erie et al 1993) to 799/100000 person/year in Nepal (Upadhyaya et al 2001). On the other hand, the epidemiological features and causative organisms for keratitis vary from country to country or even region to region within the same country.

The clinical diagnosis of microbial keratitis often relies on a thorough specific history of infectious exposure, epidemiological trends and the morphological features of corneal inflammation (Upadhyaya et al 2001). Furthermore, the course of disease and patient management is directly affected by the lack of proper diagnosis and initiation of appropriate antimicrobial therapy. To begin the proper management required quick and accurate identification of causative microorganisms. So, the evaluation of the etiological diagnosis region wise and sensitivity pattern of isolated microorganisms provide the valuable information for initiation of their management. For this, some studies in Nepal and several studies in Indian subcontinent have provided the important data but a comprehensive study in Western Region of Nepal is not available. So this study was conducted in Lumbini Eye Institute Nepal, which provides eye care not only to the people of Western Region of Nepal but also covers the large geographical area of Gangetic planes of north India.

Most of the studies about microbial keratitis have primarily evaluated epidemiological features, predisposing factors and clinical features of corneal ulceration (Whitcher et al, 1997; Srinivasan et al, 1997; Norina et al, 2008; Upadhyaya et al, 1991; Williams et al, 1987), but this study, along with the epidemiological pattern and identification of causative microorganisms, tried to include the sensitivity pattern of bacterial isolates, treatment modalities and their outcome.

Materials and methods

The retrospective study was conducted in Lumbini Eye Institute, Siddharthanagar, Nepal between the periods of 1st January to 31st December 2007.

All patients with infectious corneal ulcers presenting to cornea clinic at Lumbini Eye Institute, Siddharthanagar, Nepal from 1st January to 31st December 2007 were included in the study. Patients were seen consecutively after the initial clinical diagnosis of corneal ulceration was made. Ulceration was defined as a loss of the corneal epithelium with underlying stromal infiltration and suppuration associated with signs of inflammation with or without hypopyon.

Typical viral ulcers and healing ulcers were excluded as were Mooren's ulcers, marginal keratitis, interstitial keratitis, sterile neuro-trophic ulcers and any ulcers associated with autoimmune conditions. ***Statistics:*** All patients' data were entered in pre-designed format documenting socio-demographic and clinical parameters including history of trauma, duration of symptoms, previous treatment, predisposing ocular conditions and associated risk factors and analyzed on SPSS version 11.0.

Clinical procedure

Every patient was examined on the slit-lamp biomicroscope by an ophthalmologist. The size of the epithelial defect after staining with fluorescein was measured with the variable slit on the slit-lamp and recorded in millimetres on a standardised form. In similar fashion, the size and depth of the stromal infiltrate was recorded. A sketch of each ulcer was also drawn on the form using standardised frontal and cross sectional diagrams and the presence or absence of a hypopyon was recorded; and the height of the hypopyon was measured in millimetres. Associated ocular conditions such as blepharitis, dacryocystitis, dry eyes, corneal anaesthesia, lagophthalmos, any surgery on the cornea, use of

contact lens, or ocular leprosy were noted. The use of topical medication including topical corticosteroids was also noted.

After a detailed ocular examination, corneal scrapings were performed under aseptic conditions on each ulcer by ophthalmologist using a flame sterilised Kimura spatula. Scrapings were performed in the slit lamp after instillation of 4 % lignocaine (lidocaine). Material was obtained from scraping, the leading edge and the base of each ulcer, was inoculated directly onto Blood agar, Chocolate agar and Sabaraud-Dextrose agar (SDA). Material from the corneal scraping was also taken on two separate glass slides for smear: one for Gram stain and the other for microscopic examination in the clinic as a KOH wet mount. All KOH smears were then sent to the laboratory for confirmation.

Laboratory procedure

All bacterial cultures were incubated aerobically at 37°C. Cultures on blood agar, chocolate agar were evaluated at 24 hours and at 48 hours and then discarded if there was no growth. Fungal cultures inoculated onto SDA were incubated at 27°C, examined daily, and discarded after 2 weeks if no growth was present in culture.

Microbial cultures were considered positive only if growth of the same organism was demonstrated on two or more solid media; or there was semi confluent growth at the site of inoculation on one solid medium associated with the identification of the organism of appropriate morphology and staining characteristics on Gram stain or KOH mounted corneal smears. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, and biochemical properties using standard laboratory criteria. Fungi were identified by their colony characteristics on SDA and by their microscopic appearance in lacto phenol cotton blue.

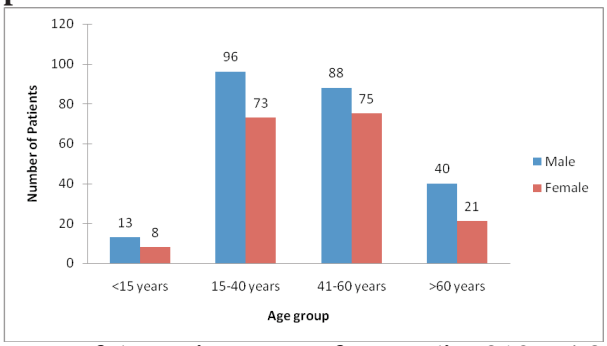
All culture positive samples were tested for their sensitivity pattern with commonly available/ used antimicrobials. It was reported on standardized format (Modified Kirby Bauer's disc diffusion method on Blood Agar Medium using antibiotics discs of Oxoid Diagnostics, UK) with a grade assigned to them. Treatment decision was based on clinical judgment and response to empirical treatment. Culture & sensitivity pattern was taken into account after 48 hours when culture sensitivity report was available of bacterial growth.

Results

Epidemiological characteristics

414 patients with the clinical diagnosis of corneal ulcers were enrolled in this study. Among them 310 cases were from North India (74.9 %) and 104 (25 %) were from Nepal. 237 (57.2 %) cases of total cases were males and 177 (42.8 %) were females. Male: female ratio was 1.3:1. Ulceration occurred most frequently in the age group of 21 – 40 years in 169 (40.8 %) cases, followed by 163 (39.4 %) cases in the age group of 41 – 60 years. Ulceration was distributed more or less evenly in all age group except in patients of less than 15 years and more than 60 years of age, in which age group, males were affected twice frequently than females (Fig. 1).

Fig 1: Age and sex distribution of 414 patients with corneal ulceration



Most of the patients were from India (310; 74.9 %) and rest from Nepal (104; 25.1 %). The majority of them (311; 75.1 %) were farmers or related to agricultural works. The next frequently seen group was housewives (54.13 %) followed by students 42 (10.1 %), businessman 3 (0.7 %) and children (< 6 years of age) 2 and 2 others were in service.

Seasonal variation of out flair of corneal ulcer noticed in May - August, the period when agricultural activity increases, mainly during crop planting period and then in November – December, crop harvesting period. This seasonal variation was similar as noticed in the study done by Basak et al (2005).

Duration of illness was also recorded. 179 patients (43.2 %) were examined within 15 – 30 days after the onset of illness which showed that most of the patient seeking medical help came from far place, while 116 patients (28 %) were examined within 7 – 14 days. Only 66 patients (15.9 %) presented in less than 1 week of onset of disease and 53 (12 %) seeked the medical help after 30 days or more from the onset of their first ocular signs and symptoms (Table 1) .

Table 1
Duration of illness

Duration of illness	Frequency	Percentage
<1 week	66	15.9
7-14 days	116	28.0
15-30 days	179	43.2
>30 days	53	12.8
Total	414	100.0

As most of the patients seeking medical help were far away from LEI, 215 (51.9 %) patients were hospitalized and only 199 were treated as outpatients. Among total 414 patients, only 5 (1.2 %) had second episode of disease, all the others 409 (98.8 %) patients had no previous history of eye disease. Factors predisposing to corneal ulceration were reported in 172 cases out of total 414 patients (41.55 %). The common predisposing factor was trauma in 138 (33.3 %) patients. The agent of trauma were mainly the vegetative matters mostly paddy or paddy stock. 17 of 414 (4.1 %) patients had a history of using topical corticosteroid and 3 (0.7 %) patients used traditional medicine like goat's milk and rati gedi (seed of the plant) etc. Other leading predisposing conditions included corneal foreign body 20 (4.8 %), lagophthalmos (1.9 %), chronic dacryocystitis (1.9 %), exposure keratitis (1.2 %), conjunctivitis (1.4 %). And systemic condition mostly detected was diabetes mellitus (1.7 %).

Microbial diagnosis

Cultures were positive and fulfilled the criteria established for the presence of infection in 300 (72.5 %) of 414 corneal ulcer (Table 2). Of 414 cultures, 121 (29.2 %) exhibited pure bacterial growth, although 20 (4.8 %) of cultures grew multiple spp. of bacteria. Pure fungal growth was present in 138 (33.3 %) of all cases of corneal ulcers and 41 (9.9 %) cultures showed a mixed growth of bacteria and fungi. Among those 41 cases, 36 cases (8.69 %) had single species of bacteria with fungi while 5 cases showed multiple species of bacteria with fungi. None of the cultures showed multiple fungal growth as in the study done by Srinivasan et al (1997).

Table 2
Microbial growth pattern in 414 corneal ulcers

Growth pattern	No. of cases	Percentage %
Pure bacterial growth	121	29.2
Single spp. of bacteria	101	24.4
Multiple spp. of bacteria	20	4.8
Pure fungal growth	138	33.3
Mixed microbial growth	41	9.9
Single spp. of bacteria with fungi	36	8.69
Multiple spp. of bacteria with fungi	5	1.20
Cases with positive cultures	300	72.5
Cases with negative cultures	114	27.5
Total	**414**	**100**

Out of 186 isolates, Gram positive bacteria were predominant accounting 157 cases (84.41 %) of total isolates. Among Gram positive isolates, Staphylococcal epidermidis was the most frequently isolated species with 55 cases (29.57 %), followed by Streptococcus viridians 28 cases (15.05 %). Though diphtheroids are nonpathogenic species of corynebacteriae spp. they may be opportunistic pathogens mainly in immunosuppressed individuals which in this study were isolated in 31 cultures. Staphylococcus aureus was the next species mostly grown after diphtheroids.

Gram negative organisms constituted 29 cases (15.59 %) of all the total isolates. Among the Gram negative isolates, Pseudomonas aeruginosa (26 i.e. 13.98 %) was the most frequent isolate. The data was followed by Moraxella, Neiseria and Enterobacter species.

Table 3
Bacterial isolates from corneal ulcers in LEI

Bacteria	Single sp. Isolated	Mixed with other bacteria	Mixed with fungi	Mixed with other bacteria and fungi	Total (%)
Gram Positive organisms isolated					
Staphylococcus epidermidis	30	10	12	3	55 (29.57)
Staphylococcus aureus	12	4	6	1	23 (12.37)
Streptococcus pneumoniae	14	2			16 (8.6)
Streptococcus viridans	13	7	5	3	28 (15.05)
Staphylococcus pyogenes	1				1 (0.54)
Bacillus spp.			1		1 (0.54)
Nocardia asteroids	1				1 (0.54)
Corynebacterium spp.	1				1 (0.54)
Diphtheroids	8	13	8	2	31 (16.67)
Gram Negative organisms isolated					
Pseudomonas aeruginosa	17	5	3	1	26 (13.98)
Moraxella	1				1 (0.54)
Neiserria spp.		1			1 (0.54)
Enterobacter spp.			1		1 (0.54)
Total (percentage)	98(52.69)	42 (22.58)	36 (19.35)	10(5.37)	186 (100)

Fungi were grown from 179 corneal ulcers. Of 179 fungal isolates, 55 were Fusarium spp (30.73 %), 18 (10.06 %) were Aspergillus spp. (Aspergillus fumigatus 11 , Aspergillus flavus 3, Aspergillus niger 4) and 6 were Candida species. Dematiceous fungi (pigmented filamentous organisms) cultured were Curvularia spp 16 (8.94 %), Bipolaris spp 3(1.68 %) and Alternaria spp 2 (1.12 %). Rest of the fungi cultured were unidentified dematiceous fungi (57; 31.84 %) and unidentified Hyaline fungi (22 i.e. 12.29 %). Of all the fungal organisms cultured 138 (77.09 %) were pure fungal isolates while 41(22.91 %) were mixed with single or multiple baterial spp. but none of the culture grew multiple fungi.

Table 4
Fungal isolates from corneal ulcers in Nepal

Fungi	Pure isolates	Mixed with bacteria	Percentage (%)
Fusarium spp	44	11	55 (30.73)
Aspergillus fumigatus	7	4	11 (6.15)
Aspergillus flavus	2	1	3(1.68)
Aspergillus niger	3	1	4(2.23)
Candida spp	6		6 (3.35)
Curvularia spp	10	6	16(8.94)
Bipolaris spp	3		3(1.68)
Alternaria spp	2		2(1.12)
Unidentified dematiceous fungus	46	11	57(31.84)
Unidentified hyaline fungus	15	7	22(12.29)
Total	**138 (77.09)**	**41 (22.91)**	**179 (100)**

The most sensitive antibiotics againt the Gram positive bacterial isolates were cephazolin (84.92 %) and gentamycin (76.19 %) followed by chloramphenicol (73.81 %) and ofloxacin (71.43 %). Ciprofloxacin and tobramycin were the next sensitive for Gram positive bacterial isolates. The least effective antibiotic was norfloxacin (40.48 %). The most effective antibiotics againt the Gram negative isolates were ciprofloxacin and ofloxacin with the same percentage of effectiveness (79.31 %) followed by tobramycin (68.97 %), norfloxacin (65.52 %) and gentamycin (48.28 %). The least effective antibiotic was cephazolin (10.34 %).

Table 5

Antibiotic sensitivity test of Gram positive isolates

Antibiotics	S. epidermidis n=55		S. aureus n=23		S. pneumoniae n=16		S. viridans n=28		S. pyogenes n=1		Bacillus spp. n=1		Nocardia n= 1		Corynebacterium spp. n=1	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Cephazolin	43	12	22	1	15	1	24	4	1		1			1	1	
Chloramphenicol	36	19	15	8	15	1	25	3		1	1			1	1	
Ciprofloxacin	36	19	10	13	14	2	19	9		1	1			1	1	
Ofloxacin	35	20	13	10	15	1	25	3		1	1			1	1	
Tobramycin	39	16	17	6	7	9	17	11	1		1			1	1	
Norfloxacin	19	36	6	17	11	5	13	15		1	1			1	1	
Gentamycin	42	13	19	4	10	6	22	6	1		1			1	1	
S= Sensitive R= resistant																

PS. Here Diphtheroids (n= 31) were not included for sensitivity test.

Table 6

Antibiotic sensitivity test of gram negative isolates

Antibiotics	Pseudomonas aeruginosa n= 26		Moraxella n=1		Neiserria spp. n=1		Enterobacter spp. n=1	
	S	R	S	R	S	R	S	R
Cephazolin	10	25		1	1		1	
Chloramphenicol	25	16	1		1		1	
Ciprofloxacin	21	5	1		1			1
Ofloxacin	22	4	1			1		1
Tobramycin	17	9	1		1		1	
Norfloxacin	18	8	1			1		1
Gentamycin	12	14	1		1			1
S= sensitive **R**= resistant								

In vitro sensitivity pattern test showed that, cephazolin (78.18 %) was the most effective antibiotic against Staph epidermidis followed by gentamycin (76.36 %) and tobramycin (70.91 %). The least effective was norfloxacin (34.55 %). The most sensitive antibiotic was cephazolin followed by gentamycin and tobramycin for the Staphylococcus aureus and least sensitive was norfloxacin. While for Streptococcus pneumoniae, cefazolin, chloramphenicol and ofloxacin were more effective followed by ciprofloxacin and gentamycin. And for Streptococcus viridans, chloramphenicol and ofloxacin were most effective followed by cefazolin and gentamycin, the least effective being norfloxacin. for gram negative isolates, like pseudomonas aeruginosa, ofloxacin (84.62 %) was the most effective antibiotic followed by ciprofloxacin (80.77 %), norfloxacin (69.23 %), tobramycin (65.38 %) and gentamycin (46.15 %), while the least effective was cefazolin (3.85 %).

In conclusion, for Gram positive bacteria isolated in this study, the most effective first line drugs are cephazolin and gentamycin followed by ofloxacin, ciprofloxacin and chloramphenicol. While for Gram negative bacteria, the most effective antibiotics are ofloxacin and ciprofloxacin followed by norfloxacin and tobramycin.

Treatment of the corneal ulcers was started with broad spectrum antibiotics for bacterial corneal ulcers which were modified later according to the culture and sensitivity reports. For fungal corneal

ulcers treatment started with topical antifungals and systemic as and when necessary with the clinical judgment like peripheral involvement and involvement of anterior chamber etc.

Along with medical treatments, surgical interventions were required for 124 cases out of 414. Surgery included mostly conjunctival vasculoplasty for 104 (25.1 %), total Gunderson flap for 4 (1 %) and therapeutic penetrating keratoplasty for 1 case (0.2 %). Ten of the cases i.e. 2.4 % were subjected to evisceration in spite of all medical treatments. Other surgeries included dacryocystectomy, dacryocystorhinostomy, entropion and ectropion correction and lateral tarsorrhaphy for the ulcers with local predisposing factors.

Out of 414 cases with corneal ulcers of different etiologies, 363 cases i.e. 87.7 % resulted with healing corneal scar with or without recovering functional visual acuity. Out of 368 eyes healed with corneal scarring, 104 cases were post conjunctival vasculoplasty and 4 were post total Gunderson flap.

Discussion

In many parts of the world, mainly in the developing countries, corneal ulceration is the major cause of blindness. As a result, corneal scarring is the second only to cataract as the most important cause of visual disability in the world today (Whitcher et al 1997; Srinivasan et al 1997; Norina et al 2008; Upadhyaya et al 1991; Chirambo et al 1986; Brilliant LB et al, 1985).

Since LEI is located near North Indian border, more than half of the patients included in this study were from India, 310 cases (74.9 %) and only 104 cases (25 %) from Nepal. So, our hospital LEI provides eye care not only for Nepalese population in Southern part of Nepal but also large areas of Gangetic planes of Northern India especially form Uttar Pradesh and Bihar.

Since Nepal is an agriculture based country, the occupation of the patients included in this study shows the pattern of employment in terai belt of Nepal. The vast populations of North India are also farmers. Of 414 patients, 314 cases (75.4 %) were farmers followed by individuals involved in household activities (13 %). The occupation profile is similar to Gangetic West Bengal region of India and southern India (Srinivasan et al, 1997; Basak et al, 2005). But in contrast, in Ghana only 16.1 % of the patients were agriculture workers (Hagan et al 1995). Seasonal variation of out flair of corneal ulcer was noticed in May- August, the crop planting period and then in November – December, crop harvesting period which was similar to that seen in West Bengal (Basak et al 2005).

Unlike South India where 60 % of cases reported earlier than one week (Srinivasan et al 1997), only 66 (15.9 %) of the patients presented in LEI within a week or two after the onset of symptoms. 179 (43.2 %) patients were seen within 15-30 days which shows that most of the patients seeking medical help came either from far way away or could not use the transportation system or reported ultimately to the hospital because of the worsening condition of the disease.

Microorganisms were isolated from 72.5 % of the 414 corneas that were cultured. This rate of positive cultures is similar to the other study (Srinivasan et al 1997; Williams et al 1987; Basak et al 2005). But it is less than the 80 % isolation rate reported by Upadhaya et al (1991). This may be due to use of several enriched culture media for inoculation and repeated scrapping.

More than half (43.2 %) of all corneal ulcers with positive cultures were fungal in origin. This figure approaches the fungal isolation rate 46.8 % in South India (Srinivasan et al, 1997) and 51.9 % in Ghana (Hagan et al 1995) but it differs from the Study in Kathmandu Nepal, mid eastern region of Nepal where only in 68 (16.85 %) cases fungi were grown (Upadhyaya et al, 1991). This may be due to the climatic variations as Kathmandu is in hilly region with cold weather and the LEI in the sub-tropical low land. Similar findings were observed in the study done in eastern region of Nepal where only 20 % of cultures had fungal growth (Lavaju et al, 2009).

Among the fungal positive cultures, 30.73 % cases were Fusarium spp, 10.06 % Aspergillus spp and the remaining were the mix of large number of fungal pathogens including unidentified hyaline fungi, unidentified dematiceous fungi spp. This pattern of fungal organisms dominated by Fusarium spp is similar to spectrum of microbial keratitis reported from South India, Madurai by Srinivasan et al. Aspergillus spp were predominant in the study done by Upadhyaya et al (1991) in Nepal, in the study done by Basak et al (2005) in West Bengal region and also in the study by William et al (1987) in Bangladesh.

Among 186 bacterial isolates in this study, 29.57 % was Staphylococcus epidermidis while Streptococcus pneumoniae was the most common isolate in South India and even in mid eastern part of Nepal (Upadhyaya et al, 1991). Staphylococcus aureus was the most common isolate in West Bengal region (Basak et al, 2005) while Pseudomonas spp. in Ghana (Hagan et al, 1995) and Bangladesh (William et al, 1987).

The sensitivity pattern, of bacterial isolates, in this study showed cephazolin and gentamycin are the most sensitive antibiotics against Gram positive bacteria. ciprofloxacin and ofloxacin were most effective for Gram negative bacteria and cephazolin was least sensitive to them.

In summary, this study was performed primarily to determine the specific pathogens responsible for the infective keratitis in western part of Nepal. An attempt was made to identify the epidemiological characteristics of the population at risk for corneal ulceration as well as predisposing factors of corneal ulcers. Along with this, we also tried to include the sensitivity pattern of different bacterial isolates in this study to develop a guideline for the choice of antibiotics before culture report in this geographical area.

Conclusion

Microbial keratitis is mostly seen in farmers in this part of the world. Fusarium and Staphylococcus epidermidis were the most common isolates. Cephazoline and ofloxacin were the most effective antibiotics for Gram positive and Gram negative organisms respectively. This knowledge can be helpful to make an informed choice of antimicrobial agents for the treatment of corneal ulcer in the population not only in Western part of Nepal but also in large areas of Gangetic Planes of North India.

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