Original Article

Giemsa Staining Findings and Clinical Characteristics of Viral Keratitis in a Tertiary Care Centre

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Abstract

Background: Herpes simplex virus is the most common cause of viral keratitis which can recur and cause progressive corneal damage and visual loss. Diagnosis is mainly clinical but Giemsa staining is a simple and cheap supportive test in resource-limited areas. Hence, the present study aimed to assess the positivity of Giemsa stain, pre-triggering factors, frequency of recurrence and epithelial or stromal pattern of recurrent viral keratitis in a tertiary care centre. **Material and Methods:** This was a prospective observational study of 72 cases of clinically diagnosed viral keratitis during one year at a tertiary care centre. Patients were evaluated in detail after informed consent, slit-lamp examination, fluorescein staining, visual acuity, and appropriate laboratory investigations. Corneal scrapings from the cases of epithelial keratitis were stained with Giemsa stain to look for the presence of multinucleated giant cells. Triggering factors and recurrence were recorded and data were analysed with descriptive statistics in SPSS. **Results:** Of 72 patients with viral keratitis, 34.7% were 10-20 years old, 54.2% were male and 87.5% were from urban areas. Unilateral involvement was seen in 62.5% with redness (68.1%) and decrease in vision (50.0%) being the most frequent symptoms. Epithelial keratitis predominated (72.2%). Epithelial cases showed positivity in 28.8% of the cases using Giemsa stain. Few triggers were identified (16.7%) and these were primarily fever (13.9%). Recurrence was seen in 5.6% and was mostly stromal keratitis (75.0%). **Conclusion:** Most cases of viral keratitis were unilateral, involving the epithelium, and occurred in young urban patients. Limited supportive diagnostic utility was seen with Giemsa staining, and triggers and recurrence were uncommon, but stromal involvement predominated for the recurrent cases, thus fulfilling the study objectives.

Keywords: Herpes Simplex Keratitis, Cornea, Giemsa staining, Recurrence.

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Introduction

Viral keratitis is an important cause of infectious corneal disease and preventable visual impairment worldwide. The most common etiological agent is herpes simplex virus (HSV), particularly HSV-1, and although diagnosis is largely based on slit-lamp findings, atypical presentations and recurrent disease may pose diagnostic problems, especially in resource-limited settings where advanced laboratory techniques such as polymerase chain reaction (PCR), viral culture, and immunofluorescence assays are not readily available.[1-7]

Corneal damage and visual morbidity are high due to recurrent HSV infection, which has been implicated in such events as febrile illness, psychological stress, ocular trauma, immunosuppression and ultraviolet light exposure.[3,9] Multinucleated giant cells may be seen on Giemsa staining of corneal scrapings, providing supportive evidence for HSV infection, particularly in clinically atypical cases.[6,8]

Given the limited evidence of practical utility of Giemsa staining and region specific data on triggering factors and recurrence patterns is limited, the present study was aimed at assessing the diagnostic utility of Giemsa staining and the clinical characteristics of viral keratitis among patients attending a tertiary care centre. The aims were to assess the positivity of Giemsa stain in epithelial viral keratitis, to describe triggering factors, to assess recurrence patterns and to determine the distribution of recurrent disease based on the type of keratitis (epithelial or stromal).

Materials and Methods

This is a prospective observational study for one year from October 2015 to September 2016 in the Department of Ophthalmology, A.J. Institute of Medical Sciences, Mangalore with approval from the Institutional Ethics Committee. The aim of the study was to assess the diagnostic usefulness of Giemsa staining and the clinical parameters of viral keratitis in patients

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who visited a tertiary care centre. Convenience sampling method was used and all the patients who were eligible were included during the study period. The study population consisted of 72 patients with viral keratitis.

Patients with clinical signs of viral keratitis including some with superadded bacterial/fungal infection were enrolled after written informed consent was obtained. Patients who refused to participate or who did not have clinical signs indicative of viral keratitis were excluded from the study.

A detailed clinical history was taken on a predesigned proforma from each participant and then a thorough ophthalmic examination was performed. Clinical examination comprised slit-lamp examination of the anterior segment, evaluation of the pattern of corneal involvement, and record of any history suggestive of possible triggering factors prior to onset of symptoms. Visual acuity was documented with Snellen's chart and fluorescein staining was done to detect epithelial corneal lesions. Lacrimal syringing was carried out whenever indicated. As part of the clinical evaluation, routine laboratory investigations were conducted, such as complete blood count, random blood sugar, HIV and hepatitis B surface antigen testing.

Microbiological assessment involved conjunctival and corneal scrapings stained with Giemsa stain to look for the presence of multinucleated giant cells suggestive of herpes simplex virus infection. In the case of epithelial keratitis, Giemsa staining was done. At the initial presentation, information on triggering factors such as fever and history of foreign body was recorded when available. Patients were also evaluated for recurrence of viral keratitis during the study period, and recurrent cases were categorised according to the type of keratitis as epithelial or stromal.

Data collected were entered in Microsoft Excel and analysed using SPSS software. All the study variables were categorical and data pertaining to age group, gender, place of residence, laterality, presenting symptoms, corneal layer involved, Giemsa staining findings, preceding triggering factors, recurrence status and type of recurrence were presented as frequencies and percentages. The study was descriptive in nature therefore appropriate descriptive statistical methods were used to present the results.

Results

Among the 72 patients with viral keratitis, the largest proportion belonged to the 10–20-year age group (34.7%), followed by those aged 21–30 years (26.4%). Males constituted 54.2% of the study participants, while females accounted for 45.8%. Most patients were residents of urban areas (87.5%), whereas 12.5% belonged to rural areas. [Table 1]

Table 1: Baseline demographic characteristics of patients with viral keratitis (N = 72)

Variable	Categories	n	%
Age group	10 to 20 years	25	34.7
	21 to 30 years	19	26.4
	31 to 40 years	13	18.1
	41 to 50 years	8	11.1
	51 to 60 years	5	6.9
	>60 years	2	2.8
Gender	Male	39	54.2
	Female	33	45.8
Place of residence	Urban	63	87.5
	Rural	9	12.5

Table 2: Clinical presentation of patients with viral keratitis (N = 72)

Variable	Categories	n	%
Laterality	Unilateral	45	62.5
	Bilateral	27	37.5
Presenting symptoms*	Redness	49	68.1
	Diminution of vision	36	50.0
	Pain	5	6.9
	Watering	4	5.6

*Multiple presenting symptoms may have been reported by the same patient

Unilateral involvement was observed in 62.5% of patients, while 37.5% had bilateral involvement. Redness was the most common presenting symptom, reported by 68.1% of patients, followed by diminution of vision in 50.0%. Pain and watering were less frequently reported, accounting for 6.9% and 5.6% of patients, respectively. [Table 2]

Among the 72 patients with viral keratitis, epithelial involvement was observed in 52 (72.2%) patients, while stromal involvement was noted in 20 (27.8%) patients. Epithelial keratitis was therefore the predominant clinical presentation in the study population. [Figure 1]

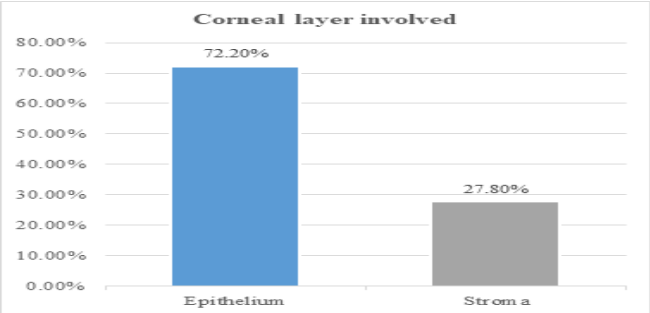

Figure 1: Distribution of patients according to the corneal layer involved (N = 72)

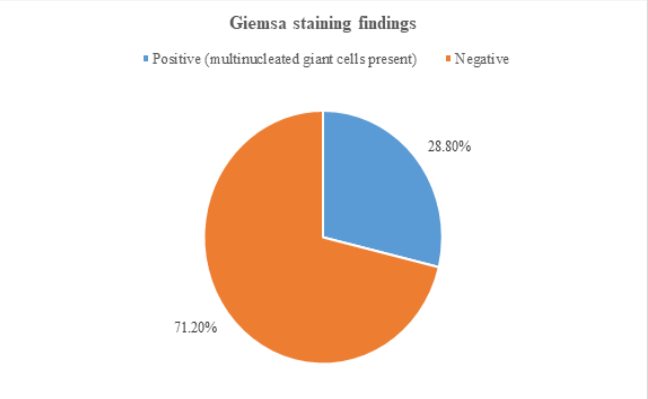

Figure 2: Giemsa staining findings among patients with epithelial viral keratitis (n = 52)

Among the 52 patients with epithelial viral keratitis who underwent Giemsa staining, multinucleated giant cells suggestive of herpes simplex virus infection were identified in 15 (28.8%) patients. The remaining 37 (71.2%) patients had negative Giemsa staining findings. Thus, Giemsa staining demonstrated multinucleated giant cells suggestive of herpes simplex virus infection in 28.8% of clinically diagnosed epithelial viral keratitis cases. [Figure 2]

An identifiable factor preceding the onset of viral keratitis was reported by 12 (16.7%) patients. Fever was the most commonly reported preceding factor, observed in 10 (13.9%) patients, while a history of foreign body was reported by 2 (2.8%). Most patients, comprising 60 (83.3%), had no identifiable factor preceding the onset of viral keratitis. [Table 3]

Table 3: Distribution of reported factors preceding the onset of viral keratitis (N = 72)

Reported preceding factor	n	%
Fever	10	13.9
History of foreign body	2	2.8
No identifiable triggering factor	60	83.3

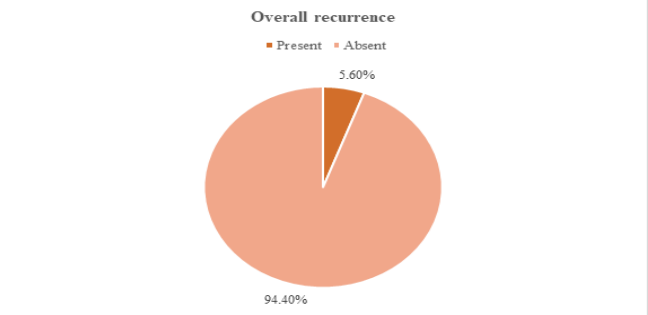

Figure 3: Recurrence pattern among patients with viral keratitis (N = 72)

Recurrence was documented in 4 (5.6%) patients during the study period, while 68 (94.4%) patients had no recurrence. Among the documented recurrent cases, stromal keratitis was observed in 3 (75.0%) patients and epithelial keratitis in 1 (25.0%) patient. [Figure 3 & 4]

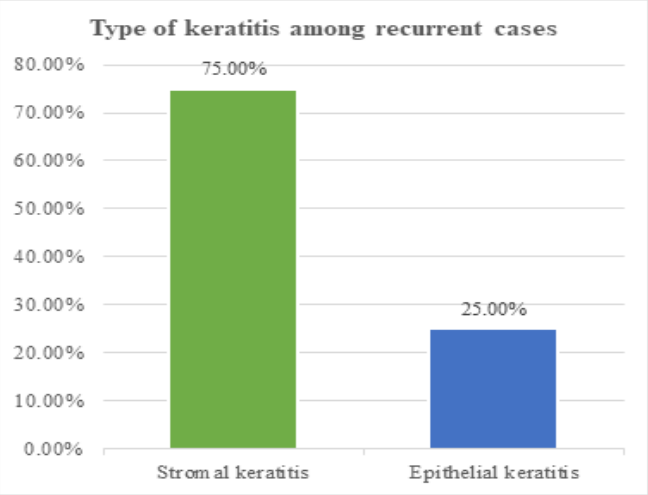

Figure 4: Distribution of recurrent cases according to the type of keratitis (n = 4)

Discussion

Since there are few simple, low-cost diagnostic adjuncts for viral keratitis in resource-limited areas, the utility of Giemsa staining and associated clinical features was assessed in this prospective series. The study was conducted at A.J. Institute of Medical Sciences, Mangalore and 72 clinically diagnosed cases were selected by convenience sampling method after obtaining ethics approval and written consent from October 2015 to September 2016. Each participant had a detailed history, slit lamp examination, visual acuity, fluorescein staining, lacrimal syringing (when indicated), and routine laboratory investigations. Epithelial keratitis cases were Giemsa stained for the presence of multinucleated giant cells in corneal and conjunctival scrapings. Triggering factors and recurrence were recorded.

In the present study, viral keratitis was more common in younger age groups (34.7% were in the age group of 10-20 years and 26.4% were in the age group of 21-30 years). Latha KST et al,[11] (2019) reported similar results with 55.0 % in the first 2 decades. In contrast, Shrestha P et al,[12] (2023) reported a mean age of 47.19 ± 19.14 years, Sinha A et al,[13] (2017) found 62.5% of cases between 20–40 years, Sodani P et al,[14] (2022) reported a mean age of 38.61 years with 79.53% aged 21–50 years, Yousuf M et al,[15] (2018) found 36.0% in the 30–40-year group, Jaffar TT et al,[16] (2016) reported a mean age of 36.2 years with 41.0% aged 41–60 years, and Al Karam M et al,[17] (2026) reported a median age of 48.0 years (IQR: 31.7–66.3). Males constituted 54.2% of cases, consistent with Yousuf M et al,[15] (2018) (60.0%), Sinha A et al,[13] (2017) (57.5%), Latha KST et al,[11] (2019) (61.0%), and Al Karam M et al,[17] (2026) (58.9%), whereas Jaffar TT et al,[16] (2016) reported equal gender distribution and Shrestha P et al,[12] (2023) observed female predominance (60.71%). 87.5% of the participants were urban residents. Similar urban–rural data were not available, however, Yousuf M et al,[15] (2018) reported HSV in both urban and tribal population and Sodani P et al,[14] (2022) reported that manual workers, farmers and labourers accounted for 33.04% of cases,

indicating higher rural exposure.

Sixty-two and a half patients had unilateral disease and thirty-seven and a half patients had bilateral disease. Sinha A et al,[13] (2017) reported 25.0% bilateral involvement and Latha KST et al,[11] (2019) reported 12.0%. Conversely, Jaffar TT et al,[16] (2016) found 86.4% unilateral and 13.6% bilateral disease; Shrestha P et al,[12] (2023) reported only 3.6% bilateral disease; and Al Karam M et al,[17] (2026) found only two cases of bilateral infection in 2,892 patients, further supporting the predominance of unilateral viral keratitis. The most frequent presenting symptom was redness (68.1%), followed by diminution of vision (50.0%), pain (6.9%) and watering (5.6%). Similar findings were reported by Jaffar TT et al (2016),[16] who documented redness (75.0%), blurred vision (65.9%), and pain (52.3%), and by Shrestha P et al (2023),[12] who reported redness (80.35%), decreased vision (47.32%), pain (19.6%), and watering (39.28%). Redness was noted in 83.04% in all cases, decreased vision was the most common sign in subepithelial keratitis (83.0%) and stromal keratitis (80.0%),[14] (2022) Latha KST et al,[11] (2019) on the other hand found that the most common symptoms were photophobia and watering.

The majority (72.2% or 52 cases) had epithelial keratitis and 27.8% (20 cases) had stromal keratitis. Sinha A et al,[13] (2017) also reported the incidence of epithelial keratitis as 60.0% and stromal disease as 23.0%. Epithelial lesions were the most prevalent (11) and dendritic lesions were found in 39.0% to 42.0% and disciform lesions in 24.0% (Latha KST et al,[11] (2019). Sodani P et al,[14] (2022) found 43.04% of cases had epithelial keratitis, 35.22% had subepithelial infiltration and 16.52% had stromal keratitis without ulceration. Shrestha P et al,[12] (2023) on the other hand reported higher stromal involvement with pure stromal disease 33.92%, epithelial-plus-stromal disease 16.94%, stromal-plus-endothelial disease 9.81% and purely epithelial disease 33.91%. Epithelial and stromal keratitis were found to be equal at 38.6% and endothelitis in 22.7% by Jaffar TT et al (2016).[16] In general, epithelial involvement was more common in most of the studies, while stromal and endothelial involvement was more common in the referral centres.

Of 52 cases of epithelial keratitis, 28.8% (n=15) had positive Giemsa staining for multinucleated giant cells and 71.2% (n=37) were negative. Tzanck smear, which is useful for detection of multinucleated giant syncytial cells and acidophilic intranuclear inclusions, has an overall laboratory positivity of 37.0% by culture and immunofluorescence as described by Latha KST et al (2019).[11] Al Karam M et al,[17] (2026) used real-time multiplex PCR and reported positivity rates of 9.8% for HSV-1, 4.6% for VZV, 0.27% for HSV-2, and 1.0% for CMV. Shrestha P et al,[12] (2023), Sinha A et al (2017),[13] Sodani P et al (2022),[14] and Jaffar TT et al,[16] (2016) relied primarily on clinical diagnosis. While the yield is not as high for cytological methods as for PCR, these methods are fast, cheap, and useful in resource-limited settings.

A trigger was identified in 16.7% (n=12) of which 13.9% (n=10) were fever and 2.8% (n=2) were foreign body exposure, and 83.3% (n=60) had no identifiable trigger. Jaffar TT et al,[16] (2016) also reported no precipitating factor in 79.5% and corneal trauma in 13.6% while Yousuf M et al,[15] (2018) reported no precipitating factor in about 80.0% and corneal injury in 13.6%. Sinha A et al,[13] (2017) reported spontaneous onset in 58.75%, fever in 17.5%, trauma in 10.0%, and stress in 8.75%. Spontaneous onset was observed in 51.8%, stress in 10.70%, foreign body exposure in 10.70% and fever in 6.30% as reported by Shrestha P et al (2023).[12] Fever was noted in 39.13%, upper respiratory tract infection in 21.30%, steroid use in 13.48% and spontaneous onset in only 5.65% according to Sodani P et al (2022).[14] Fever was reported in 54.0% and was the most frequent trigger in the recurrent cases by Latha KST et al (2019).[11] Hence fever was the most constant cause, but the percentage of cases of spontaneous origin was very variable.

Recurrence occurred in 5.6% (n=4), while 94.4% (n=68) had no recurrence. Of the recurrent cases, 75.0% (n=3) had stromal keratitis, and 25.0% (n=1) had epithelial keratitis. Sinha A et al,[13] (2017) found a recurrence rate of 20.0% (12.0% stromal and 8.0% epithelial). Higher recurrence rates were documented by Jaffar TT et al,[16] (2016) at 36.4%, Shrestha P et al,[12] (2023) at 33.0%, Yousuf M et al,[15] (2018) at 50.5%, and Latha KST et al,[11] (2019) at 44.0%. The low recurrence rate in the present study may be due to the duration of the observation period (1 year), as compared to other studies that reported a recurrence rate of 20.0% to 50.5% with a longer duration of observation. However, this study and that of Sinha A et al,[13] (2017) showed that the stroma was more frequently involved in recurrent disease.

Strengths and Limitations: The strengths of this study are that it was prospective in design, with standardized clinical evaluation of all patients, and documentation of triggering factors and recurrence patterns over the study period, with an evaluation of the diagnostic utility of Giemsa staining as well as the clinical profile of viral keratitis. The study has some limitations, however, such as the relatively small number of samples, the convenience sampling method, the location of the study (a single tertiary care centre), the absence of confirmatory virological methods (PCR or viral culture), and the descriptive study design, which does not allow for causal inference and generalisation of the results.

Conclusion

The study revealed that viral keratitis mainly occurred in young urban population with slight male predominance and mostly presented with unilateral redness with reduced vision. The most common clinical type was epithelial keratitis. Multinuclear giant cells were found in 28.8% of the epithelial cases by Giemsa staining and this was considered to have limited but supportive diagnostic value. The majority of patients did not have a known precipitant, but the most frequent was fever. Recurrence was rare but if it did occur, stromal keratitis was the most common. In conclusion, the study was successful in describing the clinical profile, triggering factors, recurrence pattern, and diagnostic yield of Giemsa staining in viral keratitis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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