

Temporal trend of microsporidial keratoconjunctivitis and correlation with environmental and air pollution factors in India

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Purpose: The aim of this study was to describe the correlation between the temporal pattern of presentation of acute microsporidial keratoconjunctivitis (MKC) with meteorological parameters such as environmental temperature, rainfall, humidity, windspeed, and air pollution. **Methods:** This cross-sectional hospital-based study included 182,789 patients presenting between January 2016 and December 2019 hailing from the district of Hyderabad. Patients with a clinical diagnosis of MKC in at least one eye with an acute onset (≤ 1 week) of presentation were included as cases. Correlation analysis was performed with the local environmental temperature, rainfall, humidity, and windspeed (Telangana State Development and Planning Society) and air pollution (Central Pollution Control Board, Government of India). **Results:** Overall, 84 (0.05%) patients were diagnosed with acute onset MKC from the district of Hyderabad. The mean monthly prevalence in this cohort was 0.05% with peak prevalence in the months of July (0.08%), August (0.09%), September (0.12%), and October (0.08%). The environmental parameters of rainfall ($r^2 = 0.87/P = < 0.0001$), humidity ($r^2 = 0.78/P = 0.0001$), windspeed ($r^2 = 0.38/P = 0.0321$) were significantly positively correlated and the air pollution parameters such as ground level ozone ($r^2 = 0.89/P = < 0.0001$), particulate matter PM_{10} ($r^2 = 0.65/P = 0.0013$), $PM_{2.5}$ ($r^2 = 0.50/P = 0.0095$), nitrogen dioxide ($r^2 = 0.53/P = 0.0071$), and carbon monoxide ($r^2 = 0.69/P = 0.0008$) were significantly negatively correlated with the temporal pattern of MKC in the population. **Conclusion:** Parasitic infections like MKC show a distinct temporal trend peaking during the monsoon season in the population. An increase in humidity, wind speed, and especially rainfall contributes to a higher prevalence of MKC cases during the year. An increase in ground-level ozone seems to be protective against infection.

Key words: Environmental pollution, India, microsporidial keratoconjunctivitis, trend analysis

Microsporidia are eukaryotic, obligate, spore-forming unicellular parasites. They have been known to infect invertebrates and have increasingly gained prominence in affecting humans over the past few decades.^[1] Microsporidia can cause a host of infections that includes myositis, diarrhea, bronchitis, and keratitis in both immune-compromised or immune-competent individuals. The infection with the microsporidial spores can occur via direct inoculation, inhalation, or ingestion. The ocular manifestations of microsporidial infection include keratoconjunctivitis or stromal keratitis.^[2] A strong correlation with environmental exposure to soil,^[3] sport activities,^[4] and water contamination in swimming pools^[5] have been described in literature. The cases are more commonly acute in nature and can sometimes run a chronic indolent course as well. A large cohort from South India described an increase in incidence of cases from July to December^[6] and others reported cases after a recent rainfall as well.^[3] Due to the rarity of its nature, a high degree of clinical suspicion is warranted to diagnose microsporidial keratoconjunctivitis (MKC) as it often can be misdiagnosed as

a viral ocular infection of epidemic keratoconjunctivitis (EKC) which is more commoner of the two. The understanding of the correlation of environmental factors on the temporal trends of infections affecting the ocular surface like EKC is described in the Indian subcontinent.^[7] The authors wanted to study the relationship between the effect of environmental factors such as temperature, humidity, rainfall, windspeed, and air pollution parameters on the prevalence of MKC in the population. This presents an interesting insight into the temporal trends of MKC and allows us the window of opportunity to learn in a geographic setting like India and presents a new understanding into the complex effect of the environment on the pathophysiology of the infections affecting the ocular surface.

Methods

Study design, period, location, and approval

This cross-sectional observational hospital-based study included all patients hailing from the district of Hyderabad

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presenting between January 2016 and December 2019 to an ophthalmology network located in 210 different geographical locations spread across 4 states (Telangana, Andhra Pradesh, Odisha, and Karnataka) of India.^[8] A written informed consent was obtained a priori from all patients or their parents/guardians for their participation in research, which included a consent form for the protection of electronic data privacy. The study did not include any identifiable parameters of the patient during analysis of the data. The study adhered to the Declaration of Helsinki and was approved by the Institutional Ethics Committee. The clinical data of the comprehensive ophthalmic examination of each patient was entered by uniformly trained ophthalmic personnel using a standardized template and supervised by an ophthalmologist into a browser-based electronic medical records system (eyeSmart EMR).^[9]

Cases

A total of 182,789 patients of all ages hailing from the district of Hyderabad presented to the network during the study period. The standard protocol of managing a case of conjunctivitis in the network involves a high degree of suspicion for MKC that is identified on clinical examination by raised epithelial lesions that are more prominent with fluorescein staining. These cases undergo epithelial scraping for microbiological evaluation.^[10] The eyeSmart EMR was screened for patients with: (i) a microbiologically positive diagnosis of MKC in one or both eyes with an (v) acute onset (≤ 1 week) of presentation. The 84 patients identified with the search strategy were labeled as cases and were included for the trend analysis with the meteorological parameters.

Data retrieval and processing

The data of the patients included in this study were retrieved from the electronic medical record database and segregated in a single Excel sheet. The columns included the data on demographics, clinical presentation, and ocular diagnosis and were exported for analysis. The excel sheet with the required data was then used for analysis using the appropriate statistical software. The weather parameters of 33 districts of the state of Telangana were obtained from the Telangana State Development and Planning Society (TSDPS), Government of Telangana. TSDPS facilitates data collection through 1044 Automated Weather Stations (AWS), 64 global radiation sensors, and 41 soil moisture sensors across the state of Telangana to acquire data on a real-time basis. The AWS measures 6 weather parameters – Rainfall, Windspeed, Wind Direction, Pressure, Humidity, and Temperature in addition to Global Radiation and Soil Moisture at desired locations at every 1 h interval and transmits it in the form of SMS using GSM technology. At least, one AWS for each mandal is installed in a grid of 10 km \times 10 km.^[11] The pollution parameters of Hyderabad of particulate matter (PM₁₀ and PM_{2.5}) $\mu\text{g}/\text{m}^3$, carbon monoxide (CO) mg/m^3 , nitrogen dioxide (NO₂) $\mu\text{g}/\text{m}^3$, sulfur dioxide (SO₂) $\mu\text{g}/\text{m}^3$, and ozone (O₃) $\mu\text{g}/\text{m}^3$ were obtained from the Central Pollution Control Board, Ministry of Environment, Forest and Climate Change, Government of India.^[12] The weather and pollution parameters were then mapped to the cohort of patients from the district of Hyderabad. A comparative analysis of the temporal trends of MKC with EKC^[7] published earlier by the authors was performed to understand the difference in presentation of these cases through the year.

Statistical analysis

Descriptive statistics using mean \pm standard deviation and median with inter-quartile range (IQR) were used to elucidate the demographic data. Prevalence was calculated by dividing the number of MKC cases by the total number of patients presenting to the network from the given geographic location during the study period. The Medcal software (version 19.2.0) was used to perform Pearson's correlation coefficient (r) analysis between the prevalence and individual meteorological parameters. Multiple linear regression analysis was performed using stepwise modeling based on the Akaike information criterion (AIC). The r -square (r^2 , mathematical square of the numerical value of r) value of 0.12 or below indicates low, between 0.13 and 0.25 values indicates medium, 0.26 or above values indicates high effect size.^[13]

Results

Demographics

Of the 182,789 patients who presented from the district of Hyderabad during the study period, 84 (0.05%) patients presented with an acute onset (≤ 1 week) of MKC. The mean age of the patients with EKC was 30.3 ± 12.07 years while the median age was 28.5 (IQR: 23-37) years. There were 46 (55.76%) male and 38 (45.24%) female patients with MKC. Of the 84 patients, 78 (92.85%) presented to the center of excellence in Hyderabad and the remaining 6 (7.15%) patients presented to other centers in and around Hyderabad.

Prevalence

The highest prevalence was seen in the month of September with 0.12% (17/14,129; 95% CI: 0.001 to 0.002) and the lowest was seen in the month of May with 0.01% (1/15,756; 95% CI: 0.000 to 0.000). The weather parameters of rainfall, humidity, temperature, windspeed, and the pollution parameters of PM₁₀, PM_{2.5}, CO, NO₂, SO₂ and O₃ were assessed on a monthly interval. The month-wise distribution of the prevalence of acute cases of MKC from the district of Hyderabad is shown in Fig. 1.

Rainfall

The month-wise rainfall distribution is shown in Fig. 2a. The average rainfall from 2016-2019 was 2.08 mm/day. The highest rainfall was seen in the month of September (6.25 ± 14.49 mm/day) and the lowest in the month of February (0.01 ± 0.11 mm/day). The month-wise prevalence of MKC was positively correlated with the distribution of rainfall ($r = 0.9307$), and this correlation was statistically significant ($r^2 = 0.87/P < 0.0001$).

Humidity

The month-wise relative humidity distribution is shown in Fig. 2b. The average relative humidity from 2016 to 2019 was 57.63%. The highest relative humidity was seen in the month of September ($74.79 \pm 9.09\%$) and the lowest in the month of April ($42.05 \pm 14.55\%$). The month-wise prevalence of MKC was positively correlated with the distribution of humidity ($r = 0.8845$), and this correlation was statistically significant ($r^2 = 0.78/P = 0.0001$).

Windspeed

The month-wise windspeed distribution is shown in Fig. 2c. The average windspeed from 2016 to 2019 was 7.28 km/h. The highest windspeed was seen in the month of August (9.23 ± 4.7 km/h) and the lowest in the month of January (5.06 ± 3.47 km/h). The

month-wise prevalence of MKC was positively correlated with the distribution of windspeed ($r = 0.6185$), and this correlation was statistically significant ($r^2 = 0.38/P = 0.0321$).

Temperature

The month-wise temperature distribution is shown in Fig. 2d. The average temperature from 2016 to 2019 was 28.5°C. The highest average temperature was seen in the month of May ($34.16 \pm 5.62^\circ\text{C}$) and the lowest in the month of December ($24.24 \pm 3.83^\circ\text{C}$). The month-wise prevalence of MKC was negatively correlated with the distribution

of temperature ($r = -0.1948$), and this correlation was not statistically significant ($r^2 = 0.03/P = 0.5441$).

Particulate matter 10 (PM₁₀)

The month-wise particulate matter distribution (PM₁₀) is shown in Fig. 3a. The average particulate matter reading from 2016 to 2019 was 85.88 µg/m³. The highest particulate matter reading was seen in the month of January (125.09 µg/m³) and the lowest in the month of July (41.35 µg/m³). The month-wise prevalence of MKC was negatively correlated with the distribution of particulate matter ($r = -0.8117$), and this correlation was statistically significant ($r^2 = 0.65/P = 0.0013$).

Particulate matter 2.5 (PM_{2.5})

The month-wise particulate matter distribution (PM_{2.5}) is shown in Fig. 3b. The average particulate matter reading from 2016 to 2019 was 43.46 µg/m³. The highest particulate matter reading was seen in the month of January (70.08 µg/m³) and the lowest in the month of August (16.55 µg/m³). The month-wise prevalence of MKC was negatively correlated with the distribution of particulate matter ($r = -0.7114$), and this correlation was statistically significant ($r^2 = 0.50/P = 0.0095$).

Ozone (O₃)

The month-wise O₃ at ground level distribution is shown in Fig. 3c. The average O₃ reading from 2016 to 2019 was 34.09 µg/m³. The highest O₃ reading was seen in the month of May (45.15 µg/m³) and the lowest in the month of August (18.43 µg/m³). The month-wise prevalence of MKC was negatively correlated with

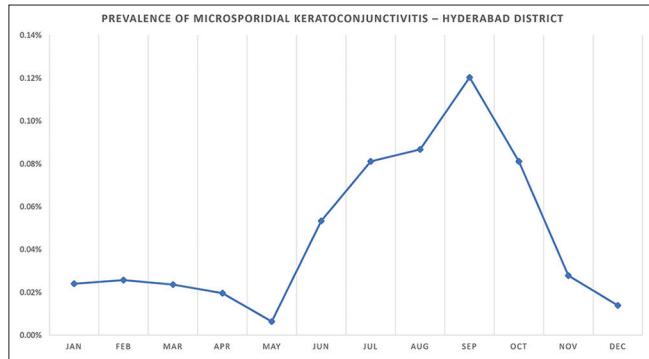


Figure 1: Seasonal variation of microsporidial keratoconjunctivitis (MKC) in the district of Hyderabad. The prevalence of MKC increased steadily from June during the onset of monsoon to peak in the month of September decreasing with the onset of winter

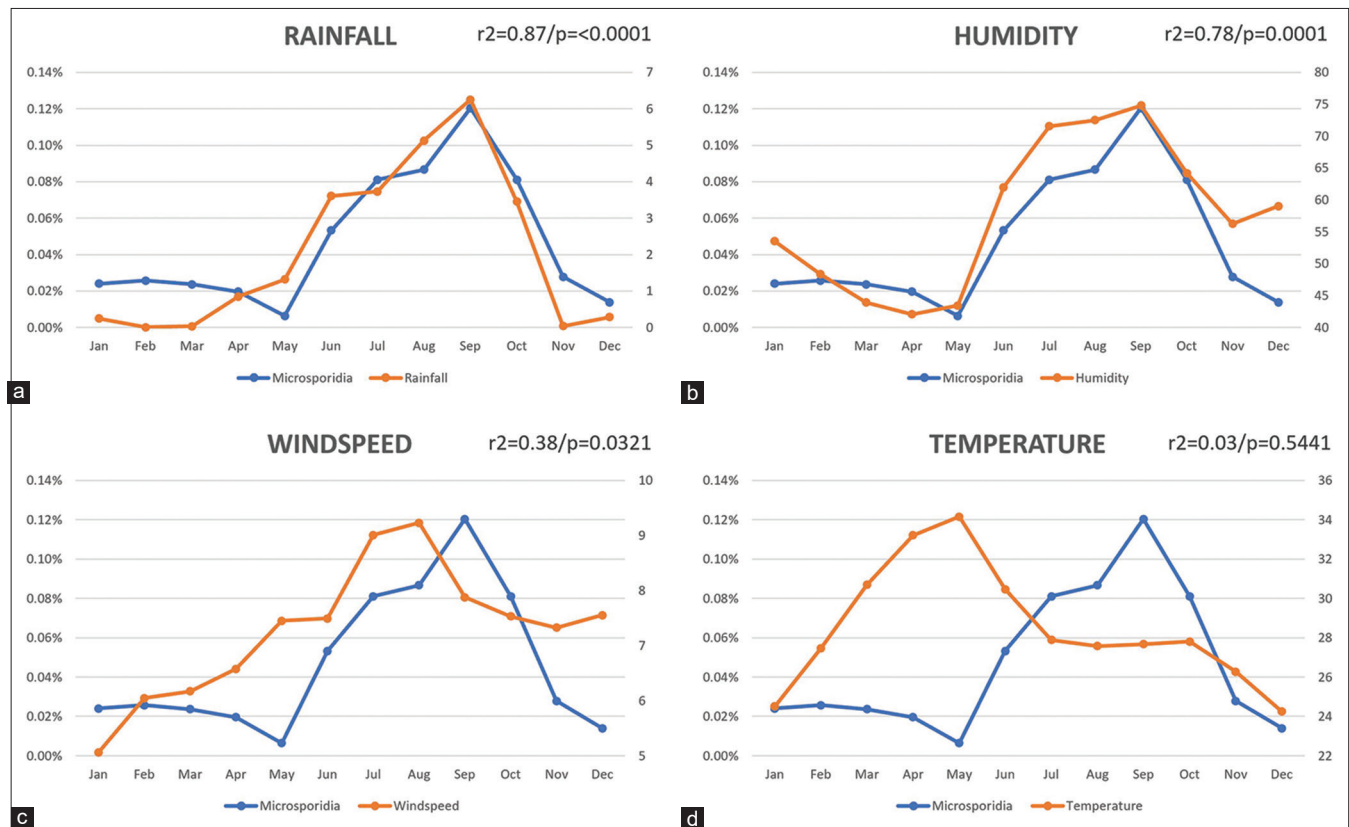


Figure 2: Monthly variation of environmental factors and the prevalence of microsporidial keratoconjunctivitis (MKC) in the district of Hyderabad. The month-wise prevalence of MKC was positively correlated and statistically significant with the distribution of rainfall (a, $r = 0.9307$), humidity (b, $r = 0.8845$), windspeed (c, $r = 0.6185$) but not with temperature (d, $r = -0.1948$)

the distribution of O_3 ($r = -0.9438$), and this correlation was statistically significant ($r^2 = 0.89/P = < 0.0001$).

Carbon monoxide (CO)

The month-wise CO distribution is shown in Fig. 3d. The average CO reading from 2016 to 2019 was 0.53 mg/m^3 . The highest CO reading was seen in the month of December (0.74 mg/m^3) and the lowest in the month of August (0.33 mg/m^3). The month-wise prevalence of MKC was negatively correlated with the distribution of CO ($r = -0.8308$), and this correlation was statistically significant ($r^2 = 0.69/P = 0.0008$).

Nitrogen dioxide (NO_2)

The month-wise NO_2 distribution is shown in Fig. 3e. The average NO_2 reading from 2016 to 2019 was $32.79 \text{ } \mu\text{g/m}^3$. The highest NO_2 reading was seen in the month of January ($46.56 \text{ } \mu\text{g/m}^3$) and the lowest in the month of July ($16.67 \text{ } \mu\text{g/m}^3$). The month-wise prevalence of MKC was negatively correlated with the distribution of NO_2 ($r = -0.7293$), and this correlation was statistically significant ($r^2 = 0.53/P = 0.0071$).

Sulfur dioxide (SO_2)

The month-wise SO_2 distribution is shown in Fig. 3f. The average SO_2 reading from 2016 to 2019 was $10.53 \text{ } \mu\text{g/m}^3$. The highest SO_2 reading was seen in the month of November ($16.58 \text{ } \mu\text{g/m}^3$) and the lowest in the month of October ($8.05 \text{ } \mu\text{g/m}^3$). The month-wise prevalence of MKC was positively correlated with the distribution of SO_2 ($r = -0.5235$), and this correlation was not statistically significant ($r^2 = 0.27/P = 0.0807$).

Multivariate analysis

The environmental variables of rainfall ($r = 0.6962/r^2 = 0.48/P = 0.0253$), windspeed ($r = -0.6894/r^2 = 0.47/P = 0.0274$), and O_3 ($r = -0.8295/r^2 = 0.68/P = 0.0030$) were found to be statistically significant on multivariate analysis ($r^2 = 0.9599$) among all the variables. The details are provided in Supplementary Table 1.

Discussion

This study sought to describe the correlation between the temporal pattern of presentation of MKC with meteorological parameters such as environmental temperature, rainfall, humidity, windspeed, and air pollution parameters in a cohort of patients presenting to a multi-tier hospital network in India using electronic medical records-driven analytics. The findings of this study suggest that there is an interesting correlation with the environmental factors and the temporal pattern of MKC in the population of the district of Hyderabad with a peak prevalence seen during the monsoon season (July to October). An increase in humidity, windspeed, and especially rainfall contributes to a higher prevalence of MKC cases during the year, whereas temperature did not have any bearing in the prevalence of MKC in the population. Intriguingly, many parameters of air pollution, especially O_3 were negatively correlated with the prevalence of MKC. The findings can not only help clinicians have a higher index of suspicion for microsporidial infections during certain months of the year but also help researchers identify potentially novel therapeutic strategies like O_3 .

Agashe *et al.*^[6] reported a large series of 550 patients with MKC from South India. A history of risk factors such as fall of dust and fall of insect was present in a subset of patients. There

was a significant increase in the occurrence of the cases during the last 6 months of the year with a peak seen in the month of November. This is the period of the north-west monsoon in the said geographical region. A similar pattern was seen in our study with the prevalence steadily increasing from June to peak during the monsoon season in India.

Reddy *et al.*^[14] reported an experience of 30 cases of MKC from 2006 to 2009 and their seasonal variation from the same institution as the authors of the current study. The highest percentage of cases (66.3%) presented during the months of Monsoon (June through September). This is similar to our study of 60.71% during the same period. However, the study by Reddy *et al.* did not perform any correlation with the environmental parameters such as rainfall, humidity, windspeed, temperature, and air pollution indicators. The current study further confirms the seasonal nature of MKC cases during the monsoon season and also lends insight into the correlation of rainfall, windspeed, and O_3 to be the significant factors affecting the prevalence in the population. A plausible explanation for this could be the increased transmission of infectious agents through contaminated water sources during this time of the year.

Loh *et al.*^[3] described the prevalence of MKC in Singapore in 124 patients. The mean age of the patients was 31.9 years and the median was 30 years. This is similar to our study as well. They also reported a positive history of soil/mud exposure from various sports activities and recent rainfall contributing to an increased prevalence of cases. Wang *et al.*^[5] reported an outbreak of MKC in 13 patients due to possible contamination in a local swimming pool suggesting spread through contact with contaminated water.

Among the air pollution indicators, ground-level O_3 was found to be strongly negatively correlated with the prevalence of the MKC cases. It is interesting to note that ozonation is used to disinfect the water from potential contamination with microsporidial spores due to its anti-infective properties. Khalifa *et al.*^[15] reported the complete inactivation of the microsporidial spores after a contact time of nine minutes with ozonated water. The concentration of ground-level O_3 in the air and its association with the decreased prevalence of MKC cases is not completely understood and needs to be further explored. We are unable to comment on the effect of the other parameters such as CO, NO_2 , and SO_2 on microsporidia due to lack of similar studies in the literature.

Due to the rarity of the MKC cases, it is possible to misdiagnose this ocular infection with the more common EKC that is more prevalent in the population.^[16] A comparison of the prevalence of these conditions clearly shows two distinct peaks of EKC in April and MKC in September [Fig. 4]. This is an important insight as clinicians should have a high index of suspicion during the months of monsoon to be able to pick up the cases that are due to MKC. This will lead to earlier identification of the disease and enable the patients to be started on the appropriate management for better clinical outcomes. To the best of our knowledge, this is the first paper in India to describe the seasonal variation of MKC along with the correlation with meteorological parameters of rainfall, humidity, windspeed, temperature, and air pollution indicators.

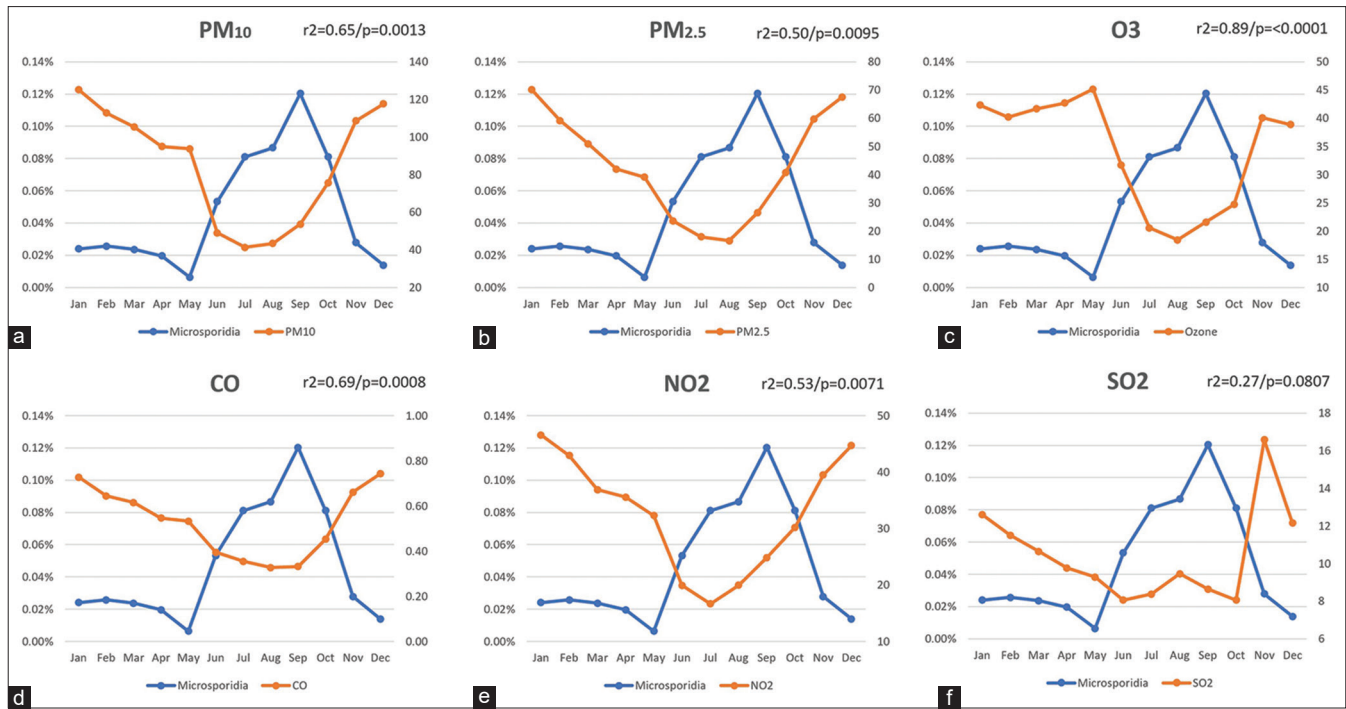


Figure 3: Monthly variation of air pollution indicators and the prevalence of microsporidial keratoconjunctivitis (MKC) in the district of Hyderabad. The month-wise prevalence of MKC was negatively correlated and statistically significant with the distribution of PM₁₀ (a, $r = -0.8117$), PM_{2.5} (b, $r = -0.7114$), ozone (c, $r = -0.9438$), carbon monoxide (d, $r = -0.8308$), nitrogen dioxide (e, $r = -0.7293$) but not with sulfur dioxide (f, $r = -0.5235$)

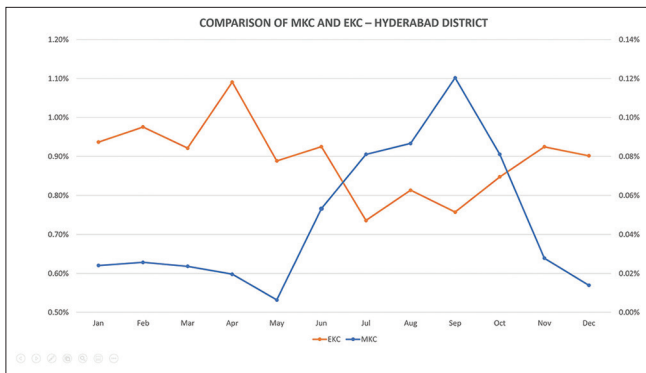


Figure 4: Comparison of the prevalence of microsporidial keratoconjunctivitis (MKC) and epidemic keratoconjunctivitis (EKC) in the district of Hyderabad. MKC showed the highest prevalence in the month of September (0.12%) while EKC showed the highest prevalence in the month of April (1.09%). The data for EKC is derived from Das AV, Basu S. Epidemic Keratoconjunctivitis in India: Trend Analysis and Implications for Viral Outbreaks. Indian J Ophthalmol 2020;68:732-6

The greatest strengths of this study are the use of completely digitized data entry and extraction using electronic medical records and accurate diagnosis of the cases supported by microbiological evaluation where relevant. The principal limitation of the study was its hospital-based method of selection of subjects which may have introduced a certain level of ascertainment bias. Another limitation is that the location provided by the patients at the time of the registration might not be in a geographic zone of an automated weather station. It is understood that there can only be a representation of the parameters of weather and pollution at specific locations in the city at points

designated by the meteorological experts. The exposure of the individuals to the environment is variable in both the severity of the insult and the time duration. Hence we can only have a broad understanding of this association. The environmental factors peculiar to the monsoon season and their effect on the life cycle and pathogenicity of microsporidial infection in humans needs further exploration.

Conclusion

In conclusion, this study aimed to describe the correlation between the temporal pattern of presentation of acute MKC with meteorological parameters such as environmental temperature, rainfall, humidity, windspeed, and air pollution indicators in a cohort of patients from the district of Hyderabad presenting to a multi-tier ophthalmology hospital network in India. The findings show that parasitic ocular infections like MKC show a distinct temporal trend peaking during the monsoon season in the population. An increase in humidity, windspeed, and especially rainfall contributes to a higher prevalence of MKC cases during the year. An increase in ground-level O₃ seems to be protective against infection. A high degree of suspicion is warranted in cases of conjunctivitis presenting during the monsoon season to aid in early diagnosis and management of MKC to enable better outcomes.

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Conflicts of interest

There are no conflicts of interest.

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