The effects of short-term and long-term air pollution exposure on meibomian gland dysfunction

Ran Hao1,2,13, Yu Wan1,2,13, Liming Zhao3,13, Yang Liu4,13, Min Sun5,13, Jing Dong6,13, Yanhui Xu7,13, Feng Wu8,13, Jinwen Wei9,13, Xiangyang Xin10,13, Zhongping Luo11,13, Shuxuan Lv12,13 & Xuemin Li1,2,13*

We aim to assess the effects of different air pollutants on meibomian gland dysfunction (MGD). As a prospective multicenter study, 864 patients were recruited from four different regions (i.e., coal, oil, steel, and living). The oil region had a significantly lower temperature and higher SO2 concentrations than other regions. Notably, participants in oil region presented with more frequent and serious MGD signs and higher cytokine levels (median interleukin 6 [IL-6] in oil: 2.66, steel: 0.96, coal: 0.38, living: 0.56; IL-8 in oil: 117.52, steel: 46.94, coal: 26.89, living: 33; vascular endothelial growth factor [VEGF] in oil: 25.09, steel: 14.02, coal: 14.02, living: 28.47). The short-term fluctuations of cytokine levels were associated with the changes in gas levels (PM2.5 and IL-8: β = 0.016 [0.004–0.029]; O3 and IL-8: β = 0.479 [0.369–0.890]; SO2 and VEGF: β = 0.021 [0.001–0.047]). After long-term exposure, lid margin neovascularization (r = 0.402), meibomian gland (MG) expression (r = 0.377), MG secretion (r = 0.303), MG loss (r = 0.404), and tear meniscus height (r = −0.345) were moderately correlated with air quality index (AQI). Individuals in oil region had more serious MGD signs and higher cytokine levels. MGD is susceptible to long-term exposure to high AQI.

Air pollution poses a serious global threat to public health1–5. According to World Health Organization (WHO), around 4.2 million deaths per year are due to ambient air pollution, and about 91% of the world’s population lives in areas where air quality levels are over WHO guidelines. Particulate matter (PM), ozone (O3), nitrogen dioxide (NO2), and sulfur dioxide (SO2) have been reported to endanger health and jointly constitute the dimensions of WHO air quality assessment.

Due to the rise in industrialization in the past decades, such as the growing steel casting, coal burning, oil extraction, and vehicle production, environmental pollution has increased in China. NO2 is produced by incomplete combustion inside motor engines, and traffic emission may be the primary source1,2. PM2.5 (PM with a diameter ≤ 2.5 µm) is part of PM10 (PM with a diameter ≤ 10 µm), and both of them originate from a variety of sources, including combustion, photochemical smog reactions, and sandstorms1,2. In China, people benefit from a high level of social development but suffer from the adverse health effects associated with air pollution. These

1Department of Ophthalmology, Peking University Third Hospital, No. 49, North Garden Street, Beijing, China. 2Beijing Key Laboratory of Restoration of Damaged Ocular Nerve, Peking University Third Hospital, Beijing, China. 3Department of Ophthalmology, Beijing Fengtai Hospital, Beijing, China. 4Department of Ophthalmology, Daqing Oilfield General Hospital, Heilongjiang, China. 5Department of Ophthalmology, Huabei Petroleum General Hospital, Hebei, China. 6Department of Ophthalmology, The First Affiliated Hospital of Baotou Medical College, Inner Mongolia, China. 7Department of Ophthalmology, Hebei Provincial Eye Hospital, Hebei, China. 8Department of Ophthalmology, Fuyang Hospital Affiliated to Anhui Medical University, Anhui, China. 9Department of Ophthalmology, Inner Mongolia Autonomous Region Xilingol League Hospital, Inner Mongolia, China. 10Department of Ophthalmology, Inner Mongolia Baogang Hospital, Inner Mongolia, China. 11Department of Ophthalmology, Tongliao City Ke’erqin Zuoyi Zongqi People’s Hospital, Inner Mongolia, China. 12Department of Ophthalmology, Yongqing People’s Hospital, Hebei, China. 13These authors contributed equally: Ran Hao, Yu Wan, Liming Zhao, Yang Liu, Min Sun, Jing Dong, Yanhui Xu, Feng Wu, Jinwen Wei, Xiangyang Xin, Zhongping Luo and Shuxuan Lv.

*email: 13911254862@163.com
pollutants are attributed to a range of cardiovascular diseases1–8, pulmonary diseases9, metabolic diseases10, strokes11, and even sudden infant death syndrome12.

Increasing organ impairment has been associated with air pollution, and the ocular surface is not an exception since it is constantly exposed to the external environment. Symptoms of ocular surface pathologies tend to increase in a poor air environment, manifesting as irritation, foreign body sensation, redness, and blurred vision, among others. Air pollutants have been shown to have adverse effects on dry eye disease (DED)13–15. Many studies have been conducted to investigate the changes in tear stability, tear volume, and corneal fluorescein staining (CFS) after exposure to air pollution. Chronic inflammation, allergy, and oxidative stress are involved in the pathogenesis of ocular surface diseases after air pollution exposure13. However, limited studies have assessed the changes in eyelid margin and meibomian gland dysfunction (MGD), which are supposed to exist before clinical manifestations are observed. The eyelid margin and MGD play key roles in the development of evaporative dry eye (DE). Additional, different regions may produce different pollutants and result in different ocular surface injury, which has not been mentioned in previous studies.

Because of the paucity of evidence regarding the effects of air pollutants on MGD in different locations in China, this study aimed to assess the changes in the ocular surface, particularly the lid margin, meibomian gland (MG) and tear cytokines, upon exposure to different air pollutants across different regions.

Materials and methods

Study design and participants. In this multicenter prospective cohort study, individuals were recruited from five provinces across China, including 11 hospitals in Beijing, Hebei, Heilongjiang, Anhui, and Inner Mongolia from February 2020 to February 2021, covering four different regions where factories mainly dedicated to steel, coal or oil production, and densely populated (living) are predominant in the area. Participants aged 20–80 years who spend 3–4 h outdoor activities per day (average) in the corresponding zone and met diagnostic criteria of MGD were recruited in the study. Individuals with histories of another ocular surface abnormality, contact lens use, ocular surgeries, glaucoma medications use were excluded. Participants in each hospital were examined by the same trained doctors. Only first visit participants were included and divided into four groups according to their sampling regions (i.e., steel, coal, oil, and living). The study was performed in accordance with the Declaration of Helsinki and was approved by the Peking University Third Hospital Ethics Committee (Research Ethics Number M2019101). Informed consent was obtained from all participants.

Outdoor air pollutants and meteorology data. During the observation period, the daily mean temperature, relative humidity, and wind speed were provided by the meteorological administrations of Beijing, Hebei, Heilongjiang, Anhui, and Inner Mongolia. The concentrations of daily air quality index (AQI), PM, PM, O, NO, and SO were obtained from open-access government air-quality monitoring data. Since the air pollutants exposure are constant, the 24-h average concentrations of PM, PM, NO, NO, and SO as well as the 8-h maximum value of O were collected as daily exposures according to previous studies. AQI is determined by monitoring the five major air pollutants, i.e., PM, O, NO, SO and carbon monoxide (CO), and the maximum value among each of the pollutants is assigned as the date- and location-specific AQI value20,21. As Gope et al. and Mirabelli et al. reports, we recorded 24-h average AQI as daily AQI. According to different AQI levels (level I: 0–50; level II: 51–100; level III: 101–150), participants were divided into three groups. The daily exposure is considered as short-term exposure. And we recorded the 1-month average AQI (AQI mean) before the first observation as long-term exposure.

Ocular surface health assessment. Symptoms were assessed using the ocular surface disease index (OSDI) questionnaire22. Lid margin morphology, MG morphology/function, tear meniscus height (TMH), Schirmer’s test (ST), tear breakup time (TBUT), and CFS were examined in the individuals’ right eyes, following previously reported methods23–26. Palpebral margin (hyperemia/telangiectasia, debris, edema/thickening, irregularity, and neovascularization) and MG (plugging, stenosis, expression secretion, and loss) were examined with the slit-lamp microscope. The MG loss was graded as follows26: 0 (no dropout), 1 (< 1/3 total area dropout), 2 (1/3–2/3 total area dropout), and 3 (> 2/3 total area dropout). The MG secretion was scored as follows26: 0 (clear meibum), 1 (cloudy meibum), 2 (granular meibum with debris), and 3 (inspissated meibum and toothpaste-like). MG expression was evaluated in five glands on the temporal, central, and nasal eyelid using the following standard: 0 (all glands expressible), 1 (three to four glands expressible), 2 (one to two glands expressible), and 3 (no glands expressible)27.

Tear film collection and cytokine measurement. Tear samples were collected from the right eyes of participants. Without any anesthetic, non-irritating tear collection was conducted using 5-μL capillary pipettes. A plastic head was used to squeeze tears into 0.2 mL Eppendorf tubes, and tears were immediately frozen at −80 °C. The cytokine levels in the tear samples were measured by a flow cytometer (BD FACs Canto II, Becton Dickinson, Franklin Lakes, NJ, USA) and a bead array system (BD Cytometric Bead Array system, Becton Dickinson) following the instructions of the manufacturer. At least 50 μL undiluted tear samples were analyzed for cytokines including interleukin 1 beta (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 17 (IL-17), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), vascular endothelial growth factor (VEGF), and B-cell activating factor (BAFF).

Statistical analysis. Continuous variables were presented as the mean ± standard deviation (SD)/median (25% quantile, 75% quantile). Categorical variables were expressed as frequencies and percentages. Continuous variables were compared using analysis of variance or Kruskal–Wallis tests among four groups. Continuous
data between two groups were compared using independent t tests or Mann–Whitney nonparametric U tests. Categorical variables were compared using the Chi-square test. Crosstabs analyses were used to compare the status of the lid margin and MG morphology/function. Spearman correlation analysis was conducted to assess the associations between different atmospheric environments, various indices of ocular surface, and cytokine levels. Logistic regression analysis and multivariate linear regression analysis were used to evaluate the changes in ocular surface parameters and tear cytokines according to each air pollutant and meteorological variables. After variables collinearity checking and covariates (such as age and sex) being adjusted, regression models were developed. Since the MG function, conjunctivochalasis, OSDI, CFS and cytokine concentrations did not show a normal distribution, normality transition was performed before analysis. Statistical analysis was performed using SPSS version 23.0 (IBM Corp., Armonk, New York, USA). A P value <0.05 was considered significant for all comparisons.

**Results**

Between February 2020 and February 2021, 864 individuals were included, including 198 patients from steel region, 78 from coal region, 276 from living region, and 312 from oil region. The demographic characteristics in the four regions are shown in Table 1. The gender (P = 0.067) and age (P = 0.743) among the four regions showed no significant difference.

**Different regions presented varying air quality.** The meteorological factors and ambient pollutants varied greatly and presented large disparity across the four regions, as shown in Table 1 and Fig. 1. The temperature (°C) in oil region was significantly lower than other regions (median temperature in oil: -3, coal: 17, living: 21, P < 0.001), but the concentrations of O₃ (μg/m³) and SO₂ (μg/m³) were significantly higher than other regions (median O₃ in oil: 66, coal: 17, living: 43.5; median SO₂ in oil: 16, coal: 15, steel: 9, living: 9; all P < 0.001). Relative humidity (%), AQI, PM2.5 (μg/m³), and PM10 (μg/m³) in coal region were significantly higher than the other regions (median relative humidity: 81.5, AQI: 78, PM₂.₅: 107, PM₁₀: 160, all P < 0.001). The wind speed (level) in steel and oil region was significantly higher than coal and living regions (median in steel: 3, oil: 3.5, coal: 1, living: 2, P < 0.001), but few suffered from MG stenosis (oil: 6.4%, living: 23.2%, steel: 41.4%, coal: 56.4%, P < 0.001), but few suffered from MG stenosis (oil: 6.4%, living: 23.2%, steel: 41.4%, coal: 56.4%, P < 0.001)

**Individuals in four regions presented different ocular surface status and cytokine levels.** The ocular surface status and cytokine levels of individuals according to the four regions are shown in Table 1 and Fig. 1. More than half of the participants demonstrated a variety of lid margin abnormalities. Individuals in coal and oil regions exhibited a high proportion of lid margin debris (coal: 27.6%, oil: 13.8%, living: 8.0%, steel: 4.5%, P = 0.000). Individuals in oil and steel regions exhibited a high proportion of eyelid neovascularization (oil: 33.0%, steel: 7.6%, living: 1.1%, coal: 0%, P = 0.000). Participants in steel region had a relatively high proportion of lid edema/thickening (38.4%, P = 0.000) and irregularity (15.2%, P = 0.026). Individuals in oil region exhibited a high proportion of eyelid hyperemia (living: 26.8%, coal: 34.6%, oil: 36.7%, steel: 42.7%, P = 0.028).

The abnormalities of MG morphology in the four regions demonstrated a difference, particularly in oil region. Compared with other regions, participants in oil region had a high incidence of MG plugging (oil: 41.4%, living: 24.8%, steel: 10.2%, coal: 5.4%, P = 0.000). Regarding MG expression, MG secretion, MG loss, and conjunctivochalasis, individuals in oil region mainly exhibited higher levels than other regions (all P < 0.001).

**The diversity of ocular surface status in different regions was associated with air quality indicators.** The associations between air quality and MGD signs are shown in Table 2. In this logistic regression model, PM₂.₅, O₃ and SO₂ exposure showed an odd ratio (OR) 1.016 [95% confidence interval (CI) 1.004–1.038], OR 1.024 (95% CI 1.004–1.045), OR 1.037 (95% CI 1.015–1.059) for lid margin neovascularization, respectively (P = 0.043, P = 0.016, P = 0.001). PM₁₀ exposure had an OR 1.017 (95% CI 1.002–1.031) for lid margin debris (P = 0.025). Temperature presented an OR 1.060 (95% CI 1.041–1.080) for lid margin hyperemia (P = 0.000), OR 1.037 (95% CI 1.007–1.068) for debris (P = 0.016), OR 1.040 (95% CI 1.009–1.073) for edema (P = 0.012), OR 0.934 (95% CI 0.904–0.965) for neovascularization (P = 0.000) and OR 0.965 (95% CI 0.948–0.982) for MG plugging (P = 0.000). Relative humidity had an OR 0.967 (95% CI 0.942–0.993) for lid margin irregularity (P = 0.012) and OR 0.977 (95% CI 0.963–0.992) for MG stenosis (P = 0.002). Age presented an OR 1.017 (95% CI 1.001–1.032) for lid margin hyperemia (P = 0.039), OR 1.097 (95% CI 1.051–1.146) for debris (P = 0.000), OR 1.107 (95% CI 1.045–1.171) for edema (P = 0.000), OR 1.094 (95% CI 1.044–1.147) for neovascularization (P = 0.000) and OR 1.016 (95% CI 1.003–1.029) for MG plugging (P = 0.015). No significant association of gender, wind speed, AQI and NO₂ with MGD signs was observed.
|                           | Steel | Coal | Living | Oil   | P value |
|---------------------------|-------|------|--------|-------|---------|
| Numbers                   | 198   | 78   | 276    | 312   |         |
| Gender                    | 1:2.09| 1:2.25| 1:1.97 | 1:2.39| 0.067   |
| Male                      | 64    | 24   | 93     | 92    |         |
| Female                    | 134   | 54   | 183    | 220   |         |
| Age (years)               | 64.85 ± 6.70 | 65.82 ± 7.57 | 63.81 ± 9.93 | 67.64 ± 8.85 | 0.743   |
| Temperature (°C)          | 20 (± 5, 25) | 17 (13, 22) | 21 (1, 35) | – 3 (– 13, 28) | 0.000*  |
| Relative humidity (%)     | 55 (35, 60) | 81.5 (65, 92) | 55 (43, 66) | 68 (29, 80) | 0.000*  |
| Wind speed (level)        | 3 (2, 3) | 1 (0, 3) | 2 (1, 3) | 3.5 (0, 4) | 0.000*  |
| AQP6                      | 66 (44, 71) | 78 (60, 90) | 54 (33, 69) | 60 (50, 84) | 0.000*  |
| PM2.5 (μg/m³)             | 23 (12.75, 43) | 107 (7, 113) | 45 (11.25, 95.75) | 43 (23, 55) | 0.000*  |
| PM10 (μg/m³)              | 45 (32, 72) | 160 (18, 177) | 64 (27, 85) | 61 (50.25, 104) | 0.000*  |
| O₃ (μg/m³)                | 38 (12.90) | 17 (7.71) | 43.5 (11, 56) | 66 (39, 79) | 0.000*  |
| NO₂ (μg/m³)               | 26 (16, 44) | 36 (26, 54) | 36 (17, 50) | 28 (21, 45) | 0.000*  |
| SO₂ (μg/m³)               | 9 (6, 15) | 15 (12, 19) | 9 (5, 12) | 16 (12, 23) | 0.000*  |
| Lid margin                |       |      |        |       |         |
| Hyperemia                 | 76 (42.7%) | 27 (34.6%) | 74 (26.8%) | 115 (36.7%) | 0.028*  |
| Debris                    | 9 (4.5%) | 21 (27.6%) | 22 (8.0%) | 43 (13.8%) | 0.000*  |
| Edema/thickening          | 76 (38.4%) | 9 (11.8%) | 34 (12.3%) | 40 (12.8%) | 0.000*  |
| Irregularity              | 30 (15.2%) | 3 (3.8%) | 33 (12.0%) | 27 (8.7%) | 0.026*  |
| Neovascularization        | 15 (7.6%) | 0 (0.0%) | 3 (1.1%) | 103 (33.0%) | 0.000*  |
| Meibomian gland           |       |      |        |       |         |
| Plugging                  | 57 (28.8%) | 28 (35.9%) | 165 (59.8%) | 279 (89.4%) | 0.000*  |
| Stenosis                  | 82 (41.4%) | 44 (56.4%) | 64 (23.2%) | 20 (6.4%) | 0.000*  |
| Meibomian gland expression|       |      |        |       |         |
| 0                         | 92 (46.5%) | 6 (7.7%) | 53 (19.2%) | 2 (0.6%) | 0.000*  |
| 1                         | 44 (22.2%) | 39 (50.0%) | 134 (48.6%) | 42 (13.5%) | 0.000*  |
| 2                         | 58 (29.3%) | 33 (42.3%) | 84 (30.4%) | 184 (59.0%) | 0.000*  |
| 3                         | 4 (2.0%) | 0 (0.0%) | 5 (1.8%) | 84 (26.9%) | 0.000*  |
| Meibomian gland secretion |       |      |        |       |         |
| 0                         | 79 (40.3%) | 9 (11.5%) | 87 (31.5%) | 1 (0.3%) | 0.000*  |
| 1                         | 45 (23.0%) | 52 (66.7%) | 90 (32.6%) | 68 (21.8%) | 0.000*  |
| 2                         | 43 (21.9%) | 16 (20.5%) | 52 (18.9%) | 129 (41.4%) | 0.000*  |
| 3                         | 29 (14.8%) | 1 (1.3%) | 47 (17.0%) | 114 (36.5%) | 0.000*  |
| Meibomian gland loss       |       |      |        |       |         |
| 0                         | 142 (71.7%) | 36 (46.2%) | 149 (54.0%) | 1 (0.3%) | 0.000*  |
| 1                         | 41 (20.7%) | 33 (42.3%) | 102 (37.0%) | 113 (36.2%) | 0.000*  |
| 2                         | 11 (5.6%) | 9 (11.5%) | 23 (8.3%) | 110 (35.3%) | 0.000*  |
| 3                         | 4 (2.0%) | 0 (0.0%) | 2 (0.7%) | 88 (28.2%) | 0.000*  |
| Conjunctivochalasis       |       |      |        |       |         |
| 0                         | 81 (41.3%) | 28 (35.9%) | 136 (49.3%) | 93 (29.8%) | 0.000*  |
| 1                         | 72 (36.7%) | 41 (52.6%) | 135 (48.9%) | 163 (52.2%) | 0.000*  |
| 2                         | 39 (19.9%) | 9 (11.5%) | 5 (1.8%) | 38 (12.2%) | 0.000*  |
| 3                         | 4 (2.1%) | 0 (0.0%) | 0 (0.0%) | 18 (5.8%) | 0.000*  |
| OSDI (score)              | 22.55 (13.60, 32.39) | 20.45 (14.77, 31.80) | 15.91 (6.82, 22.15) | 19.32 (14.77, 28.15) | 0.000*  |
| TMH (mm)                  | 0.25 ± 0.11 | 0.43 ± 0.16 | 0.28 ± 0.14 | 0.16 ± 0.06 | 0.000*  |
| ST (mm)                   | 6.88 ± 3.25 | 8.70 ± 3.81 | 7.85 ± 3.35 | 6.49 ± 3.79 | 0.000*  |
| TRUT (s)                  | 8.12 ± 4.51 | 7.85 ± 3.36 | 4.86 ± 2.17 | 4.10 ± 1.95 | 0.000*  |
| CFS (score)               | 1 (0, 2.5) | 0 (0, 0.0) | 0 (0, 0.0) | 0 (0, 0.0) | 0.000*  |
| IL-1β (pg/ml)             | 0 (0, 1.25) | 0 (3, 3.26) | 0 (0, 0) | 0 (0, 1.35) | 0.198  |
| IL-6 (pg/ml)              | 0.86 (0, 3.5) | 0.36 (0, 8.54) | 0.56 (0, 3.27) | 2.66 (0, 36, 13.67) | 0.012*  |
| IL-8 (pg/ml)              | 46.94 (20.39, 87.94) | 26.89 (5.02, 115.82) | 33 (15.57, 75.16) | 117.52 (24,17,247.38) | 0.001*  |
| VEGF (pg/ml)              | 14.02 (5.25, 25.97) | 14.02 (1.92, 77.93) | 28.47 (11.43, 59.21) | 25.09 (14.31, 46.25) | 0.002*  |
The effects of air quality on ocular surface and tear cytokines are shown in Table 3. Multicollinearity between all air pollution variables was examined by ensuring that the variance inflation factors did not exceed 10. Significant associations were found between increased OSDI scores and higher PM$_{2.5}$ \( \beta = 0.146 \) (95% CI 0.094–0.198), \( P = 0.000 \), per 1 $\mu g/m^3$ increase, higher O$_3$ \( \beta = 0.233 \) (95% CI 0.179–0.353), \( P = 0.020 \), per 1 $\mu g/m^3$ increase, and higher SO$_2$ \( \beta = 0.137 \) (95% CI 0.093–0.210), \( P = 0.000 \), per 1 $\mu g/m^3$ increase. Significant associations were shown between decreased TMH and higher PM$_{2.5}$ \( \beta = -0.074 \) (95% CI -0.110 to -0.012), \( P = 0.031 \) and higher O$_3$ \( \beta = -0.075 \) (95% CI -0.127 to -0.010), \( P = 0.000 \). Higher O$_3$ exposure also associated with decreased ST \( \beta = -0.112 \) (95% CI -0.145 to -0.100), \( P = 0.043 \). Decreased TBUT showed associations with higher PM$_{2.5}$ \( \beta = -0.023 \) (95% CI -0.032 to -0.014), \( P = 0.000 \), higher O$_3$ \( \beta = 0.024 \) (95% CI -0.039 to -0.010), \( P = 0.001 \) and higher SO$_2$ \( \beta = -0.077 \) (95% CI -0.092 to -0.051), \( P = 0.000 \). Significant associations were shown between increased CFS scores and higher PM$_{2.5}$ \( \beta = 0.021 \) (95% CI 0.018–0.039), \( P = 0.000 \), higher PM$_{10}$ \( \beta = 0.012 \) (95% CI 0.007–0.018), \( P = 0.000 \), higher O$_3$ \( \beta = 0.028 \) (95% CI 0.018–0.039), \( P = 0.001 \) and higher SO$_2$ \( \beta = 0.089 \) (95% CI 0.054–0.123), \( P = 0.000 \). MG loss was associated with increased PM$_{2.5}$ \( \beta = 0.015 \) (95% CI 0.011–0.020), \( P = 0.000 \) and higher O$_3$ \( \beta = 0.015 \) (95% CI 0.010–0.020), \( P = 0.000 \). However, no association was found between air pollutants and MG expression and secretion. PM was associated with tear cytokines, such as PM$_{2.5}$ and IL-6 \( \beta = 0.015 \) (95% CI 0.001–0.028), \( P = 0.035 \), IL-8 \( \beta = 0.016 \) (95% CI 0.004–0.029), \( P = 0.013 \) and VEGF \( \beta = 0.012 \) (95% CI 0.002–0.022), \( P = 0.044 \). PM$_{10}$ and IL-6 \( \beta = 0.018 \) (95% CI 0.006–0.032), \( P = 0.006 \). Higher O$_3$ exposure was associated with increased IL-1$\beta$ \( \beta = 0.011 \) (95% CI 0.000–0.022), \( P = 0.045 \), IL-6 \( \beta = 0.057 \) (95% CI 0.036–0.072), \( P = 0.007 \) and IL-8 \( \beta = 0.479 \) (95% CI 0.369–0.589), \( P = 0.022 \). However, was not associated with VEGF concentrations. Increased VEGF was associated with higher NO$_2$ \( \beta = 0.012 \) (95% CI 0.003–0.022), \( P = 0.012 \) and higher SO$_2$ \( \beta = 0.021 \) (95% CI 0.001–0.047), \( P = 0.047 \). Decreased temperature was associated with lower TBUT \( \beta = 0.035 \) (95% CI 0.019–0.051), \( P = 0.001 \) and serious MG secretion \( \beta = -0.010 \) (95% CI -0.016 to -0.004), \( P = 0.000 \). Because of collinearity, the AQIs were not included in the analysis model.

The correlations between cytokine concentrations and ocular surface status during short-term exposure are shown in Table 4. Notably, the IL-6 concentration was moderately correlated with conjunctivochalasis \( r = 0.312 \), \( P = 0.000 \), CFS \( r = 0.307 \), \( P = 0.038 \), and TBUT \( r = -0.323 \), \( P = 0.000 \). The VEGF concentration was moderately correlated with CFS \( r = 0.323 \), \( P = 0.028 \).

### Air quality index monthly average was related to MGD.

Participants were divided into three groups according to the average AQI 1 month before the study, resulting in 144 individuals in level I, 574 in level II, and 146 in level III. The 1-month average AQI (AQI mean) of levels I, II, and III were 38.95 ± 8.86, 77.34 ± 10.59, and 108.99 ± 8.76, respectively. The ocular surface status and cytokine levels of the three groups, and the correlations between the ocular surface and AQI mean during long-term exposure are shown in Table 5 and Fig. 2.

Individuals in Level II group exhibited a high proportion of lid hyperemia (II: 38.5%, III: 21.5%, I: 21.5%), \( P = 0.000 \). Individuals in Level I group exhibited a high proportion of eyelid edema (I: 39.6%, III: 19.2%, II: 21.5%), \( P = 0.000 \). Participants in Level III group demonstrated a relatively high proportion of eyelid neovascularization (III: 30.8%, II: 16.5%, I: 0%, \( P = 0.000 \)). Regarding MG expression, MG secretion, MG loss, and conjunctivochalasis, individuals in level III group exhibited higher levels than those in other groups (all \( P = 0.000 \). Similar with eyelid and MG evaluation, individuals in Level III group had a significantly higher OSDI (median in III: 20.45, II: 20.45, I: 13.62, \( P = 0.005 \)), lower TMH (III: 0.21 ± 0.08, II: 0.23 ± 0.13, I: 0.33 ± 0.16, \( P = 0.000 \) and ST (III: 5.55 ± 3.38, II: 8.52 ± 3.35, I: 8.57 ± 3.69, \( P = 0.000 \), shorter TBUT (III: 4.65 ± 2.49, II: 5.88 ± 3.74, I: 5.84 ± 2.40, \( P = 0.000 \)), and higher CFS score (median in III: 1, II: 0, I: 0, \( P = 0.000 \)). Individuals in Level III group exhibited the highest concentrations of IL-1$\beta$, IL-6, IL-8, and VEGF. However, the difference was not significant.

### Discussion

In this study, MGD signs (eyelid neovascularization, MG plugging, MG expression, MG secretion, and MG loss) in the participants from oil region were more frequent and severe compared with other regions. At the same time, individuals in oil region showed a significant reduction in the TMH, ST, and TBUT, and a significant increase in CFS, IL-1$\beta$, IL-6, IL-8, and VEGF concentration. In the long term, individuals exposed to higher AQI levels presented with MGD more commonly.
At present, many studies have reported that DED is related to air pollution. As Mo et al. reported, PM2.5, PM10, SO2, NO2, and CO are strongly related with DED, while O3 was not. However, Hwang et al. suggests that higher O3 and lower humidity were associated with DED in Korean, while PM10 was not. NO2 and PM2.5 were also found to cause tear film stability and osmolality reduction. Another study by Um et al. found that only SO2 was associated with DED in South Korea, while NO2, O3, CO, and PM10 were not. Nevertheless, the impact of air pollutants on ocular surface remains controversial. In our study, lower temperature, higher O3, and higher SO2 levels in oil region are theorized to be contributing factors. Our result suggested that reduced temperatures might have favored the lower TBUT, more serious lid margin neovascularization, MG plugging and MG secretion. A possible reasonable explanation is that low temperatures may cool down the MG orifice surface, which results in the transient blockages of orifices, premature condensation of secretions and an increase in the viscosity after being discharged from MG orifices, leading to tear film instability and serious MGD signs. However, the
### Table 2. Logistic regression: effects of air quality on meibomian gland dysfunction signs. PM particulate matter, O₃ ozone, NO₂ nitrogen dioxide, SO₂ sulfur dioxide. The odds ratio (95% confidence interval) is shown for all significant correlations in bold. *P<0.05, **P<0.01.

| Variable          | Odds Ratio (95% CI) |
|-------------------|---------------------|
| Age (per 1 year)  | 0.995 (0.992–0.997) |
| Temperature (per 1 °C) | 0.992 (0.987–0.997) |
| Relative humidity (per 1%) | 0.992 (0.987–0.997) |
| Wind speed (per 1 level) | 0.992 (0.987–0.997) |
| PM₁₀ (per 1 μg/m³) | 0.992 (0.987–0.997) |
| PM₁₅ (per 1 μg/m³) | 0.992 (0.987–0.997) |
| PM₂₅ (per 1 μg/m³) | 0.992 (0.987–0.997) |
| O₃ (per 1 μg/m³)  | 0.992 (0.987–0.997) |
| NO₂ (per 1 μg/m³) | 0.992 (0.987–0.997) |
| SO₂ (per 1 μg/m³) | 0.992 (0.987–0.997) |

### Table 3. Multiple linear regression analysis: effects of air quality on ocular surface and tear cytokines. PM particulate matter, O₃ ozone, SO₂ sulfur dioxide, NO₂ nitrogen dioxide, O₃Sstitial ocular surface disease index, ST Schirmer’s I test, TMH tear meniscus height, TBUT tear film break-up time, CFS corneal fluorescein staining, MG meibomian gland, IL-1β interleukin 1 beta, IL-6 interleukin 6, IL-8 interleukin 8, VEGF vascular endothelial growth factor. The β (95% confidence interval) is shown for all significant correlations in bold. *P<0.05, **P<0.01.

| Variable          | β (95% CI) |
|-------------------|------------|
| VEGF              | 0.00 (0.00–0.00) |
| IL-1β             | 0.00 (0.00–0.00) |
| IL-6              | 0.00 (0.00–0.00) |
| IL-8              | 0.00 (0.00–0.00) |
| O₃Sstitial        | 0.00 (0.00–0.00) |

more serious lid margin hyperemia, debris and edema were seen in a relatively higher temperatures, suggested that elevated temperatures might favor the lid margin abnormality and inflammation. The results seemed to be a paradox. A human study was similar with our results that showing increased lipid layer thickness and TBUT but a high tear evaporation rate at a higher temperature. Therefore, appropriate temperature is recommended for ocular surface health. O₃ possesses the property of powerful oxidation and could affect the eye, skin, and
Table 4. Correlations between the cytokine concentrations and ocular surface status during short-term exposure. *OSDI ocular surface disease index, **TMH tear meniscus height, ST Schirmer’s I test, TBUT tear film break-up time, CFS corneal fluorescein staining, IL-1β interleukin 1 beta, IL-6 interleukin 6, IL-8 interleukin 8, VEGF vascular endothelial growth factor. *P < 0.05, two-tail, **P < 0.01, two-tail for Spearman correlation analysis.

|          | IL-1β | IL-6 | IL-8 | VEGF |
|----------|-------|------|------|------|
| Lid margin hyperemia |       |      |      |      |
| r        | 0.128 | 0.194** | 0.197** | 0.046 |
| P        | 0.069 | 0.005 | 0.005 | 0.512 |
| Lid margin debris |       |      |      |      |
| r        | 0.148* | 0.274** | 0.264** | 0.237** |
| P        | 0.033 | 0.000 | 0.000 | 0.000 |
| Lid margin edema |       |      |      |      |
| r        | 0.047 | 0.079 | 0.081 | −0.013 |
| P        | 0.496 | 0.259 | 0.243 | 0.847 |
| Lid margin irregularity |       |      |      |      |
| r        | −0.079 | −0.108 | −0.001 | −0.063 |
| P        | 0.259 | 0.121 | 0.986 | 0.363 |
| Lid margin vascularization |       |      |      |      |
| r        | 0.061 | −0.034 | −0.042 | −0.055 |
| P        | 0.378 | 0.623 | 0.548 | 0.429 |
| Meibomian gland plugging |       |      |      |      |
| r        | 0.055 | 0.005 | 0.048 | 0.024 |
| P        | 0.431 | 0.945 | 0.487 | 0.730 |
| Meibomian gland stenosis |       |      |      |      |
| r        | −0.018 | 0.089 | 0.071 | 0.044 |
| P        | 0.794 | 0.206 | 0.317 | 0.536 |
| Meibomian gland expression |       |      |      |      |
| r        | 0.208** | 0.255** | 0.294** | 0.129 |
| P        | 0.000 | 0.000 | 0.000 | 0.062 |
| Meibomian gland secretion |       |      |      |      |
| r        | 0.078 | 0.271** | 0.282** | 0.108 |
| P        | 0.263 | 0.000 | 0.000 | 0.120 |
| Meibomian gland loss |       |      |      |      |
| r        | 0.079 | 0.203** | 0.266** | 0.059 |
| P        | 0.270 | 0.000 | 0.000 | 0.410 |
| Conjunctivochalasis |       |      |      |      |
| r        | 0.151** | 0.312** | 0.288** | 0.053 |
| P        | 0.000 | 0.000 | 0.000 | 0.445 |
| OSDI     |       |      |      |      |
| r        | 0.283* | 0.045 | 0.014 | −0.010 |
| P        | 0.028 | 0.518 | 0.838 | 0.891 |
| TMH      |       |      |      |      |
| r        | 0.090 | −0.002 | 0.055 | 0.063 |
| P        | 0.196 | 0.978 | 0.428 | 0.365 |
| ST       |       |      |      |      |
| r        | 0.056 | −0.185* | −0.126 | −0.057 |
| P        | 0.477 | 0.018 | 0.107 | 0.470 |
| TBUT     |       |      |      |      |
| r        | 0.013 | −0.323* | −0.039 | −0.076 |
| P        | 0.850 | 0.038 | 0.580 | 0.273 |
| CFS      |       |      |      |      |
| r        | 0.099 | 0.307** | 0.245** | 0.323* |
| P        | 0.155 | 0.000 | 0.000 | 0.028 |
Even at low concentrations, exposure to environmental O₃ directly can be hazardous. A cross-sectional study from Korea suggested that higher O₃ levels were strongly related to DED. According to Lee et al., long-term exposure to a high concentration of O₃ induces oxidative damage, enhances inflammatory cytokine levels in tears, and decreases tear production and conjunctival goblet cell density, known to be via the NF-κB pathway in vitro. Additionally, in SOD1 (−/−) mice, increased periglandular inflammatory infiltrates, increased fibrosis, decreased glandular acinar density, and apoptosis were found in MGs. Those previous researches in agreement with our results, the increased O₃ concentration might contribute to the elevated

| Level I | Level II | Level III | F   | P Value | r   | P Value |
|---------|----------|-----------|-----|---------|-----|---------|
| Average AQI (1-month) | 38.95 ± 8.86 | 77.34 ± 10.59 | 108.99 ± 8.76 | 17.87 | 0.000* | 0.108 | 0.002* |
| Numbers | 144 | 574 | 146 |

| Lid margin |
|-----------|
| Hyperemia/telangiectasia | 31 (21.5%) | 221 (38.5%) | 40 (27.6%) | 17.87 | 0.000* | 0.108 | 0.002* |
| Debris | 17 (11.8%) | 59 (10.3%) | 19 (13.0%) | 1.01 | 0.604 | 0.366 |
| Edema/thickening | 57 (39.6%) | 75 (13.1%) | 18 (19.2%) | 53.62 | 0.000* | −0.164 | 0.006* |
| Irregularity | 16 (11.1%) | 69 (12.0%) | 9 (6.2%) | 3.77 | 0.152 | −0.049 | 0.151 |
| Neovascularization | 0 (0.0%) | 78 (13.6%) | 45 (30.8%) | 57.64 | 0.000* | 0.402 | 0.000* |

| Meibomian gland |
|-----------------|
| Plugging | 86 (59.7%) | 354 (61.7%) | 89 (61.0%) | 0.19 | 0.910 | 0.132 | 0.221 |
| Stenosis | 29 (20.1%) | 133 (23.2%) | 48 (33.6%) | 7.76 | 0.051 | 0.066 | 0.055 |

| Meibomian gland expression |
|---------------------------|
| 0 | 53 (36.8%) | 93 (16.2%) | 7 (4.8%) | 61.27 | 0.000* | 0.377 | 0.000* |
| 1 | 48 (33.3%) | 170 (29.6%) | 39 (26.7%) |
| 2 | 40 (27.8%) | 239 (41.6%) | 81 (55.5%) |
| 3 | 3 (2.1%) | 72 (12.6%) | 19 (13.0%) |

| Meibomian gland secretion |
|---------------------------|
| 0 | 48 (33.3%) | 118 (20.6%) | 9 (6.2%) | 34.01 | 0.000* | 0.303 | 0.000* |
| 1 | 41 (28.5%) | 177 (30.8%) | 39 (26.7%) |
| 2 | 28 (19.4%) | 163 (28.4%) | 50 (34.2%) |
| 3 | 27 (18.8%) | 116 (20.2%) | 48 (32.9%) |

| Meibomian gland loss |
|----------------------|
| 0 | 112 (77.8%) | 192 (33.4%) | 25 (17.1%) | 108.07 | 0.000* | 0.404 | 0.000* |
| 1 | 24 (16.7%) | 204 (35.6%) | 59 (40.4%) |
| 2 | 6 (4.2%) | 104 (18.1%) | 43 (29.5%) |
| 3 | 2 (1.3%) | 74 (12.9%) | 19 (13.0%) |

| Conjunctivochalasis |
|---------------------|
| 0 | 76 (52.8%) | 218 (38.0%) | 44 (30.1%) | 26.03 | 0.000* | 0.163 | 0.000* |
| 1 | 47 (32.6%) | 308 (53.6%) | 57 (39.1%) |
| 2 | 21 (14.6%) | 39 (6.8%) | 32 (21.9%) |
| 3 | 0 (0.0%) | 9 (1.6%) | 13 (8.9%) |

**Table 5.** Correlations between the ocular surface status and air quality index (AQI) during long-term exposure. OSDI ocular surface disease index, TBUT tear film break-up time, ST Schirmer’s I test, CFS corneal fluorescein staining, TMH tear meniscus height, IL-1β interleukin 1 beta, IL-6 interleukin 6, IL-8 interleukin 8, VEGF vascular endothelial growth factor. *Crosstab analyses were compared and results were expressed as number (percentage); *Kruskal–Wallis tests were compared among three groups, independent t tests between two groups and results were shown as mean ± standard deviation (SD); *Kruskal–Wallis tests were compared among three groups, Mann–Whitney nonparametric U tests between two groups and results were shown as median (25% quantile, 75% quantile); Spearman correlation analysis was conducted to assess the associations between different atmospheric environments, various indices of ocular surface, and cytokine levels. *P<0.05. Respiratory system. Even at low concentrations, exposure to environmental O₃ directly can be hazardous. A cross-sectional study from Korea suggested that higher O₃ levels were strongly related to DED. According to Lee et al., long-term exposure to a high concentration of O₃ induces oxidative damage, enhances inflammatory cytokine levels in tears, and decreases tear production and conjunctival goblet cell density, known to be via the NF-κB pathway in vitro. Additionally, in SOD1 (−/−) mice, increased periglandular inflammatory infiltrates, increased fibrosis, decreased glandular acinar density, and apoptosis were found in MGs. Those previous researches in agreement with our results, the increased O₃ concentration might contribute to the elevated
Figure 2. The ocular surface status and cytokine levels of the AQI level groups during long-term exposure. Individuals in Level II group exhibited a high proportion of lid hyperemia (A), Level I group exhibited a high proportion of eyelid edema (C), Level III group demonstrated a relatively high proportion of eyelid neovascularization (E), higher levels MG expression (H), MG secretion (I), MG loss (J), and conjunctivochalasis (K) than those in other groups. Individuals in Level III group had a significantly higher OSDI (L), lower TMH (M) and ST (N), shorter TBUT (O), and higher CFS score (P). The concentrations of IL-1β, IL-6, IL-8, and VEGF in three groups showed no difference. The results were expressed as percentage in A-G; as median with interquartile range in (H-L) and (O-T); as mean ± standard deviation (SD) in (M,N). AQI levels I: 0–50, II: 51–100, III: 101–150; OSDI ocular surface disease index, TMH tear meniscus height, ST Schirmer’s I test, TBUT tear film break-up time, CFS corneal fluorescein staining, IL-1β interleukin 1 beta, IL-6 interleukin 6, IL-8 interleukin 8, VEGF vascular endothelial growth factor, *P<0.05.
cytokine levels (IL-1β, IL-6, IL-8), decreased ST, TMB and TBUT, higher OSDI and CFS scores, and serious MGD signs (MG loss and margin vascularization). Ozone is such a small molecule that it may approach the ocular surface and lacrimal glands, then induce inflammation and tear secretion abnormality. According to the studies of Wolkoff et al., aggressive aerosols and combustion products, such as SO2, could alter the structural composition of the outermost lipid layer of the precorneal tear film and cause eye burning, drying, and itching, resulting in DED in most cases. The results of this study demonstrated SO2 concentrations were associated with more serious MGD (lid vascularization), lower TBUT, higher OSDI and CFS scores, and higher VEGF concentration, which is in accordance with the previously reported findings. Therefore, it is speculated that lower temperature induces temporary blockages of orifices, SO2 alters the lipid layer of the tear film, and excessive oxidative stress exacerbates inflammation and the chance of microbial infection.

In this study, individuals in oil regions presented more frequent and severe MGD, along with an increased level of cytokines, including IL-1β, IL-6, IL-8, and VEGF. Cytokine fluctuations were significant associated with air pollutants, such as PM, O3, and SO2 levels. The fluctuations in the cytokine concentrations showed a strong correlation with ocular surface changes, including conjunctivochalasis, TBUT and CFS, among others. These indicated that the expression of proinflammatory cytokines was sensitive to air pollutant changes, especially gases. Solomon et al. have detected an increase in the proinflammatory forms of IL-1 (IL-1α and mature IL-1β) in the tear fluid of patients with DED, suggesting that IL-1 may play a key role in the pathogenesis of keratoconjunctivitis sicca. Barton et al. demonstrated an increased expression of IL-1, IL-6, and IL-8, and decreased epidermal growth factor levels in eyes with Sjögren’s syndrome. Lee et al. demonstrated that the expression of IL-6, IL-8, IL-17, and IFN-γ increased after exposure to O3 in human conjunctival epithelial cells not pretreated with IL-1α. Generally, IL-1β, IL-6, and IL-8 are considered to induce an oxidative stress response. IL-1 and IL-6 jointly promote Th-17 cell differentiation, and IL-6 is also associated with the B-cell activation, proliferation, and differentiation. The chronic inflammation is associated with high tear cytokines concentrations, and both of them are important in the pathogenesis of MGD. Therefore, the tear cytokines may play key roles in the pathogenesis of MGD exposed on air pollution.

According to the WHO, PM concentration in China have reached “bad” or even “very bad” levels, particularly in those industrial and densely populated areas. PM is identified as a crucial indicator of environment pollution and should draw public attention. Previous studies demonstrated that high levels of PM2.5 and PM10 were associated with decreased TBUT and conjunctival goblet cells density and increased pro-inflammatory cytokines in mice, which were in accordance with our results. There were some correlations between PM concentration fluctuations and MG morphology/function and tear quantity/stability. These results suggested that PM2.5 and PM10 were positively related to the severity of ocular surface pathologies and MGD, we suspect that the PM may first affect the MG, leading to abnormal morphology and secretory function, thus affecting the tear film stability and ocular surface, resulting in ocular surface discomfort. Fu et al. reported that PM2.5 has a time- and dose-dependent effect on cytotoxicity in cultured human corneal epithelial cells. Thus, it may be difficult to detect ocular surface differences when the short-term changes in PM are not obvious. Yoon et al. have found that the effects of collected road dust on cellular responses are strongly dependent on their concentration and solubility, indicating that atmospheric PM concentrations cannot be equal to PM concentrations in tears. Rather than PM concentrations, the components of PM are more important. The specific compositions of PM, such as polyaromatic hydrocarbons, elemental/organic carbon, heavy metals, nitrate and sulfate, change according to different zones and time. And the chemical characteristics of the compounds adsorbed to the particle surface will definitely affect the PM toxic effects. In our study, the concentrations of PM2.5 and PM10 in the oil region were higher than those in the living and steel regions but lower than those in the coal region. However, the ocular surface presented more serious signs, and cytokine concentrations are higher than the three regions. We suspect that the air pollutants in the oil region are more soluble and complex, especially when affected by weather conditions, atmospheric chemistry, and complicated interactions with multiple air pollutants (such as O3 and SO2).

This study attempts to reflect the long-term pollution status by using the average preliminary AQI. Previous studies reported dose–response relationships in the constant concentration of air pollutants. However, the exposure on the ocular surface is continuous, and we used the mean AQI in the present study inevitably. Symptoms and signs of MGD gradually increased with the 1-month average AQI, especially the lid margin neovascularization, MG expression, MG secretion, and MG loss. A certain transition period between the onset of DED and MGD signs is suspected, and long-term air quality likely plays an important role in the process. However, the AQI reflects the pollution levels of the most influential pollutants daily, which the 1-month AQI may not have captured.

The study had several limitations. First, the study’s sample size was not large enough, making it difficult to stratify the differences in temperature and relative humidity for further analysis. Second, the study was a prospective cohort study, so the results did not definitively provide causal evidence between MGD and air pollutants. Third, the air pollutants exposure on the ocular surface is continuous. However, it is hard to monitor air quality personally and constantly in a large amount of people. We used the daily basis in the present study inevitably. There were differences between the indoor and outdoor activities of individual. However, our participants were asked to do 3–4 h outdoor activities to eliminate this difference. Despite some limitations, we believe this study is meaningful because it is a well-designed multicenter prospective study with organized statistical analysis. We investigate different air pollutants, ocular surface parameters and inflammation cytokines in different areas across China, and found it necessary to make a protocol to handle those air pollutants as much as possible to decrease their harmful effects.

In conclusion, individuals in the oil region presented more frequent and severe MGD, along with higher cytokine levels. MGD is likely in long-term exposure to relatively high AQI. The significant association between air pollution and ocular surface discomfort indicates the urgency to accelerate the environmental protection.
Author contributions
R.H. and Y.W. setup the protocol and recruited the participants. R.H. collected and analyzed the data, created the figures, and contributed to the writing. Y.W. and L.Z. discussed the data and participated in writing manuscript. X.L., M.S., J.D., Y.X., F.W., J.W., X.X., Z.L. and S.L. recruited the participants. X.L. setup the protocol and institutional affiliations.

Funding
This work was supported by “National natural science foundation of Beijing” (No. 7202229). The funding organization had no role in the design or conduct of this research.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence
Reprints and permissions information
Publisher’s note
Open Access

© The Author(s) 2022