# Air pollution and tear lactoferrin among dry eye disease modification by stress and allergy: a case–control study of taxi drivers

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# Abstract

Few studies have explored the possible associations between air pollution and tear lactoferrin (Lf) levels, a non-invasive biological marker of ocular surface diseases, among taxi drivers, while none have explored the modifications by of stress and allergic tendencies in the relationship. We recruited 1,905 taxi drivers with dry eye disease (DED) and 3,803 non-DED controls in Liaoning, China, 2012–2014. After physical examination and questionnaires were recorded, ocular surface were measured and tear Lf was determined by electrophoresis. Air pollutants and humidity were estimated by measured concentrations from monitoring stations. Conditional logistic regression models were employed to examine the associations of air pollutants and humidity with tear Lf levels. Among taxi drivers with stress or allergic tendencies, with an IQR (26 μg/m$^3$, 10 μg/m$^3$) increase in $PM_{10}$ and $NO_2$ levels, adjusted odds ratio was elevated by 1.89 (95% CI, 1.19 to 3.08) or 1.77 (95% CI, 1.06 to 2.90), and 2.87 (95% CI, 1.60 to 3.58) or 2.93 (95% CI, 1.64 to 3.83), respectively. In contrast, humidity was inversely associated for taxi drivers with stress [0.51 (95% CI, 0.38 to 0.64)] or allergic tendencies [0.49 (95% CI, 0.11 to 0.84)] and taxi drivers without stress [0.33 (95% CI: 0.17, 0.39)] or without allergic tendencies [0.39 (95% CI, 0.19 to 0.59)]. Tear Lf was negatively associated with each quartile of $PM_{10}$ or $NO_2$ exposure, and low humidity. $PM_{10}$, $NO_2$, and low humidity were inversely associated with Lf levels, especially for DED taxi drivers with stress and allergic tendencies.


# Introduction

As a major global public health problem, climate change and pollution impact human health and mortality.[1] Recently, the Chinese government has implemented increasingly special policy to reduce air pollution,[2,3] and affected people have established self-help to prevent respiratory illnesses and cardiovascular ailments.[4] Unfortunately, less protection measures are provided regarding ocular surface health, despite ocular surface is lastingly exposed to climate change and pollution all the time.[4]

As the most common ocular surface disease, dry eye disease (DED) causes ocular discomfort due to the abnormal amount or quality of tear fluid involving the mechanisms in toxicity, oxidative stress, and inflammation.[5] Although DED doesn't cause life-threatening, it seriously interferes vision-related quality of life.[5] Especially in China, the prevalence of DED continues to increase and was as high as 17–21%.[6] However, studies on the association of air pollution with DED face main challenges: (1) There is no uniform diagnostic standard for DED.[7] The questionnaires of symptoms can induce under-reporting due to homeostatic mechanisms of the ocular surface, or over-reporting due to individual sensitive.[8] The clinical tests are not specific for one subtype.[9] (2) Most are cross-sectional studies, not established the dose-response relationship between DED and exposure, and the modification of potential factors between air pollution and DED are not evaluated.[10]

Thus, this study selected a specific occupation (taxi drivers) under long-term outdoors exposure, collected air pollution data two years before diagnosis, and matched age and sex through a case-control study to explore whether covariates (especially stress and allergic tendencies) can conduct the modifications in the relationship between air pollution and DED (aggravating DED risk). In addition, lactoferrin (Lf), an iron-bound multifunctional cationic glycoproteinn in different human fluids and secretions, exerts antibacterial, antioxidant, anti-inflammatory and immunomodulatory activities.[11] Tear Lf is an important marker to diagnose various types of ocular surface diseases. Next, we wanted to further analyze whether Lf was a non-invasive biological marker of chronic exposure to specific pollution and DED risk, stratified by covariates.

# Materials And Methods

## Study participants

First, based on the 2012–2014 total gross domestic product (GDP) of each city provided by the Liaoning Provincial Bureau of Statistics, we divided all of 14 cities into three socio-economic zones: low (a total annual GDP of less than RMB 100 billion), medium (a total annual GDP of RMB 100 billion to RMB 600 billion), and high (a total annual GDP of more than RMB 600 billion). So, there are eight cities (Benxi, Chaoyang, Liaoyang, Fushun, Dandong, Huludao Tieling, Fuxin) in Liaoning that belong to low socio-economic zones, four cities (Anshan, Yingkou, Panjin, Jinzhou) that belong to middle socio-economic zones, and two cities (Shenyang, Dalian) that belong to high socio-economic zones. To maximise the inter-city gradients of interest, and to minimise the correlation between pollutants, based on 2012–2014 air pollution measurements, we selected 6 cities from these three socio-economic regions as sample selection regions, including low zone (Benxi, Dandong), medium zone (Anshan, Jinzhou), and high zone (Dalian, Shenyang).

Second, we randomly invited 11,322 taxi drivers from these six cities by mobile number between Jan 1 and Dec 31, 2014. Of these, 2,564 people refused to participate in the study or did not respond to it. A total of 8,758 individuals agreed to participate in this study and signed the informed consent form. They provided information about physical examination and standardised questionnaires of sociodemographics, socioeconomic status, behavioural habits, and Ocular Surface Disease Index (OSDI).[8] Then, we excluded individuals younger than 20 years old and older than 50 years old, individuals who had less than 1 year of service, and excluded patients with Sjögren's syndrome or rheumatoid arthritis, individuals who have dry eye symptoms before joining the job. In fact, 7,611 individuals without medical treatment of DED were included in the study, so these participants were invited to the hospital for further free examination or treatment. Cases had to have at least two of the following three signs in at least one eye: a) corneal fluorescein staining (CFS),[12] b) tear break-up time (TBUT),[13] c) Schirmer's test[14] with anesthesia. Controls were selected from enrolled taxi drivers without DED, were matched to cases by age and sex.

In the end, we recruited 1,905 participants with DED (case group) and 3,803 participants without DED (control group) from 5,706 taxi drivers. After examinations of visual acuity and slit lamp were measured, we collected unstimulated tears of these taxi drivers by using a capillary tube, and quantified Lf levels by electrophoresis afterwards.[15] All study procedures and protocols were approved by the medical ethics committee of China Medical University [Approval number: SCXK_LN CMU 2013 – 0222].

# Air pollution exposure assessment

The average ambient concentrations of $PM_{2.5}$, $PM_{10}$, $O_3$, $SO_2$, CO and $NO_2$ were obtained as previously described.[16] For CO and $O_3$ exposure, we calculated the average 8-hour concentration, and calculated the average 24-hour concentration for other pollutants. We obtained the average daily concentrations of these pollutants from 2012 to 2014 from the database of the Liaoning Provincial Meteorological Bureau by the use of AirData (http://www.zhb.gov.cn/). In the selected 6 cities, each district of each city had a municipal air monitoring station, which was used to record the concentration of daily ambient air pollutants and relative humidity. All data was collected by the hour. Since taxi drivers work outdoors all year round, we used the air pollutant concentration in each district to estimate the daily average concentration of air pollutants in the city where the participants were located, and then added these data together to calculate the monthly average concentration of aggregate exposures for each taxi driver.

# Covariates

We collected the characteristics of taxi drivers (gender, age, BMI, chronic diseases, allergies, smoking, drinking, eye surface status), socioeconomic status (education, income, business style, work intensity, and family pressure). Chronic stress was obtained through Occupational Stress Scale[17] and Life Stress Interview.[18]

# Statistical analysis

We used Q-Q plots to test data for normality, and used Bartlett's test for unequal variances to detect data for homogeneity. For continuous variables with normal distribution, we calculated the mean ± standard deviation (SD) by *t*-test; for non-normal distribution variables, we calculated the median and interquartile range (IQR) by Wilcoxon rank-sum test. For categorical variables, we used contingency tables and the Chi-square test to describe the difference in distribution. Because tear Lf levels belonged to a skewed distribution, a logarithmic transformation on tear Lf was performed. Then we calculated the geometric means and their 95% confidence intervals (CI) for the ln-transformed Lf (Ln-Lf). In our data, $PM_{2.5}$ was highly correlated with $PM_{10}$ ($r = 0.825$), which increase some potential variance inflation and deviation in the statistical model. In the multivariable models, logistic regression was used to evaluate whether selected covariates interfered with the association between air pollution and DED risk. If the covariate is significantly related to the effect ($p < 0.05$), or the adjusted odds ratio (aOR), which is calculated by increasing an IQR of each air pollutant concentration, has changed by more than 10%. The covariate is included into the model analysis. In the conditional logistic regression model, likelihood ratio test is used to compare the effects of including and excluding the moderating variables, so as to analyze whether there is a potential moderating effect. If the *p*-value of the difference level of likelihood ratio test is less than 0.1, then this latent variable can be considered to have a moderating effect. In this study, only stress and allergies were found to meet this standard, so only these two adjustment variables were used for stratification and OR (95% CI) was calculated. We used exposure (pollutants or humidity) level below the 25th percentile as the referent category (baseline level), and used logistic regression to estimate associations with Lf in exposure quartiles, and adjusted for covariates. Data were analyzed by SPSS 20.0 (IBM SPSS, Inc., Chicago, IL).

# Results

Table 1 showed the physical examination and basic work statuses of 8,758 taxi drivers who agreed to participate in this study. However, considering the influence of working age and participant age on the incidence of DED, we deleted 13% (1,147) of taxi drivers. Compared with the included 7,611 taxi drivers, there was no statistical difference in demographic variables. Figure 1 was a flow chart of case and control selection.

Table 2 showed the distribution of the annual mean pollutant concentration in the included taxi drivers before DED diagnosis date from the 6 cities of Liaoning. The concentration range of these pollutants varied widely between district gradients for $PM_{2.5}$ (34-119 μg/$m^3$), $PM_{10}$ (25-155 μg/$m^3$), $O_3$ (29-256 μg/$m^3$), $SO_2$ (5-70 μg/$m^3$), CO (512-2433 μg/$m^3$) and $NO_2$ (12-61 μg/$m^3$). Compared with Chinese National Ambient Air Quality Standards or WHO standards, PM levels exceed WHO guidelines in all districts of these 6 cities. Other pollutants, such as $O_3$ levels ($SO_2$ and $NO_2$) exceed exceed WHO guidelines 43.1% (76.1% and 52.5%). The variation range of relative humidity was from 45% to 90%.

Table 3 showed Spearman's correlation air-pollutant averages. The correlation coefficients showed only moderate correlations observed between air pollutants. The strongest correlation among coefficients was seen for $PM_{2.5}$ and $PM_{10}$ ($r$ = 0.825).

Table 4 showed the distributions of sociodemographics, socioeconomic characteristics, habits and clinical examination of taxi drivers with DED compared to controls. The 25.0% (1,905/7,611) among enrolled taxi drivers was diagnosed by clinicians. The 1,905 DED were matched 1:2 to 3,803 controls by age and gender. Among variables, the distributions of shift drivers (day-time) (59% vs 49%), allergies (43% vs 32%), non-self-employment (96% vs 91%), occupational and family stresses (90% vs 77%) were higher in taxi drivers with DED than those in taxi drivers without DED, there were statistical differences in other categorical variables between cases and controls. In addition, tear Lf levels (0.69, 95%CI: 0.67-0.71) in taxi drivers with DED were significantly higher than those (0.41, 95%CI: 0.39-0.43) in taxi drivers without DED. The TBUT values (2.65 ± 1.94 sec) in cases were significantly lower than those (4.51 ± 2.36 sec) in controls, moreover the mean values of both groups were below 7 seconds in both eyes. There was no statistically significant difference in mean (SD) values of OSDI score, CFS and Schirmer's test between cases and controls.

Table 5 showed the aORs and 95% CI for associations between air pollutants, humidity (an increase in IQR) and the occurrence of DED in the single-factor and multi-factor models. In the single-factor model, $PM_{10}$ (1.32, 95% CI: 1.15, 1.70) and $NO_2$ (2.61, 95% CI: 1.67, 3.56) were positively correlated with DED under adjusting other covariates. Humidity (0.44, 95%CI: 0.54, 0.34) was inversely correlated with DED. In the multi-factor model, it also showed the same related trend as in the single-factor model, and the CI range was increased. Therefore, there was a strong association between pollutants ($PM_{10}$ or $NO_2$), humidity and DED, apart from affecting the changes in the effect estimation.

Table 6 showed the relationship between $PM_{10}$, $NO_2$, humidity and DED after adjusting for other variables, stratified by the four factors of stress, allergies, self-employment or shift work, on the basis of each IQR increase in conditional logistic regression. The stratifications were based on the variables in Table 4 that had statistically different distributions between DED and non-DED. Among taxi drivers with stressful events, or with allergic tendencies, each IQR (26 $\mu g/m^3$) increase in $PM_{10}$ was positively statistically associated with the risk of DED with aOR = 1.89 (95% CI, 1.19 to 3.08) or 1.77 (95% CI, 1.06 to 2.90). Similarly, each IQR (10 $\mu g/m^3$) increase in $NO_2$ was positively associated with DED with aOR = 2.87(95% CI, 1.60 to 3.58) or 2.93 (95% CI, 1.64 to 3.83). On the contrary, an inversely association of DED with an IQR (15%) increase in humidity were showed in taxi drivers without stress, or without allergies, respectively, with aOR = 0.33(95% CI, 0.17 to 0.39) or 0.39 (95% CI, 0.19 to 0.59).

Table 7 showed the possible associations between $PM_{10}$, $NO_2$, or humidity and tear Lf levels, a prominent biomarker of DED.

Among non-DED taxi drivers without stress, tear Lf was significantly negatively associated with above the 75$^{th}$ percent of quartiles (vs. lowest) of $NO_2$ exposure (adjusted $\beta$ = -0.525 mg/ml, 95% CI: -0.997, -0.049). On the contrary, Lf was significantly positively associated with above the 75$^{th}$ percent of quartiles of humidity (adjusted $\beta$ = 1.897 mg/ml, 95% CI: 0.494, 3.020).

Among non-DED taxi drivers with stress, tear Lf was significantly negatively associated with above the 75$^{th}$ percent of quartiles of $PM_{10}$ exposure (adjusted $\beta$ = -0.586 mg/ml, 95% CI: -0.888, -0.300), and $NO_2$ exposure (adjusted $\beta$ = -0.329 mg/ml, 95% CI: -0.613, -0.039). On the contrary, Lf was significantly positively associated with above the 75$^{th}$ percent of quartiles of humidity (adjusted $\beta$ = 1.734 %, 95% CI: 0.872, 2.659).

Among DED taxi drivers with stress, tear Lf was significantly negatively associated with the 25$^{th}$ to 50$^{th}$ percent of quartiles (adjusted $\beta$ = -0.512 mg/ml, 95% CI: -1.065, -0.041), above the 75$^{th}$ percent of quartiles of $PM_{10}$ exposure (adjusted $\beta$ = -0.912mg/ml, 95% CI: -1.374, -0.413), and each quartile of $NO_2$ exposure (adjusted $\beta$ = -0.445 mg/ml, 95% CI: -0.744, -0.151), (adjusted $\beta$ = -0.495 mg/ml, 95% CI: -1.089, -0.094), (adjusted $\beta$ = -0.668 mg/ml, 95% CI:-1.141, -0.199). On the contrary, Lf was significantly positively associated with the 25$^{th}$ to 50$^{th}$ quartile (adjusted $\beta$ = 1.309 %, 95% CI: 0.095, 2.507), above the 75$^{th}$ quartile of humidity (adjusted $\beta$ = 1.994%, 95% CI: 1.572, 2.303). There were inverse dose-response relationships between $PM_{10}$, $NO_2$ and DED odds, and positive dose-response relationship between humidity and DED odds. Similar associations were observed among taxi drivers with allergic tendencies.

# Discussion

The main strength of this study included (1) A positive association of $PM_{10}$ or $NO_2$ with DED, and an inverse association of lower humidity with DED. (2) Potential factors including day-time, allergies, non-self-employment, stress were positively correlated with DED, moreover, stress or allergic tendencies increased DED risk of $PM_{10}$, $NO_2$ exposure or lower humidity to DED. (3) An inverse relationship between $PM_{10}$, $NO_2$, low humidity and tear Lf levels. The above associations between above ambient factors on tear Lf levels were more pronounced among taxi drivers with stress or allergic tendencies than taxi drivers without stress or allergic tendencies.

Although epidemiological studies provided that acute and chronic exposures to air pollution cause negative impact on ocular surface health, there are statistical differences in the association of each pollutant on DED, due to the different sources, intensity, exposure time, and dose calculation methods of air pollutants from different regions, and study participants' characteristics.[8,19,20] This study only included taxi drivers with long-term exposure to outdoor pollution, who are low-income, high-stress, and disadvantaged groups in China, so the partial demographic bias could be avoided during the statistical analysis. Of course, this also limited the popularization of our results to the public, but at least further proved that air pollution is partly to blame for the increasing DED.

We used a single-pollutant model to provide that long-term $PM_{10}$ and $NO_2$ exposure increased DED risk by 32% and 161%, while high humidity decreased DED risk by 56%. Although the results of the single-pollutant models were similar with the multi-pollutant models, we chose single-pollutant models to avoid multicollinearity due to high or moderate correlations between air pollutants, which may induce potential variance inflation and bias. Although the complex composition of pollution exposure can lead to $PM_{2.5}$ and $PM_{10}$ being non-specific pollutants and weaken the correlation between them, the present study showed a high correlation between PMs, because automobile exhaust is currently the main source of air pollution in China.[21] Moreover, the enhanced correlations ($PM_{10}$, $NO_2$ and humidity) from the multi-pollution models compared to those from the single-pollution models, and other reduced correlations ($O_3$, $SO_2$ and CO) were noted. These ranges of confidence interval were wider, which also proved that the multi-pollution models were not appropriate in this study.

In one study of taxi drivers from Sao Paulo (Brazil), a negative association between $PM_{2.5}$ or $NO_2$ levels and tear film osmolarity levels (the increasing levels is an indicator of DED) within normal limits (316 mOsm/L),[22] which indicated the existence of adaptive response when the ocular surface in individuals exposed to air pollution. It should be noted that the average $PM_{2.5}$ levels (40 $\mu g/m^3$) in Sao Paulo is higher than the WHO levels (10 $\mu g/m^3$), but the value is lower than the Chinese standard (50 $\mu g/m^3$). In Liaoning, the average $PM_{2.5}$ levels is 80 $\mu g/m^3$.

If individuals initially are exposed to high levels of $PM_{2.5}$, tear osmolarity will increase and be prone to DED.[10] But, we didn't find this relationship between $PM_{2.5}$ exposure and DED in this study. This may be related to $PM_{2.5}$ is unlikely to directly affect tear secretion and epithelial barrier function compared with $PM_{10}$,[22] which may be due to the fact that heavy metals not only adsorb on the surface of PM, but also penetrate into the structure of PM.[23,24] In China, $PM_{10}$ contains more heavy metal elements (S, Zn, Cu and Pb) than $PM_{2.5}$ (S and Pb).[25] Thereby these can exacerbate allergic reactions of PM as allergen carrier. In addition, under long-term exposure to high concentrations, tear film osmotic pressure may be normalized to a certain extent.[8]

As for $PM_{10}$, the data were not available in the study from Brazil. But, we demonstrated that $PM_{10}$ was associated with DED, which was inconsistent with the results of a South Korean study. Although reflex tear may flush PM from the ocular surface, the annual $PM_{10}$ level (84 μg/$m^3$) in China was higher than that in Korea (40 μg/$m^3$). Such high $PM_{10}$ levels may be enough to induce DED.[8]

In China, CO and $NO_2$ mainly come from automobile exhaust emissions due to incomplete combustion inside motor engines.[26] Among Chinese taxi drivers, higher CO and $NO_2$ levels were positively associated with DED, but there was not statistically significant between CO and DED. This problem may be related to CO assessment, because ambient CO concentrations have considerable spatial variability.[27] Different from the change of symptoms after $NO_2$ exposure, inhalation of CO can increase the diameter of arteries and veins, retinal blood flow velocity and fundus pulsation amplitude.[28] The indirect symptoms of ocular surface present in a delayed fashion.[29] For $NO_2$, this pollutant can acidify tears and reduce tear break-up time.[30,31] In this study, a negative correlation was found between exposure levels and DED. However, in the Brazilian taxi driver survey, there is no statistically significance. A possible explanation is the difference in $NO_2$ exposure levels between the two places (Brazil: 175 μg/$m^3$, China: 31 μg/$m^3$). The sensory receptor reactivity might adapt to high levels of air pollution and be reduced.[32] When $NO_2$ levels (less than 40 mg/$m^3$ in a hospital in São Paul) were similar to our values, the results of significantly negative correlation were consistent with this study.[31]

Also, we found a negative correlation between $O_3$ and DED, but no statistical difference. However, in a Korean study, increased $O_3$ levels were associated with DED.[8] This inconsistent result was due to different $O_3$ background levels (Korea: 250 μg/$m^3$, China: 92 μg/$m^3$), participants' characteristics (Korea: population, China: taxi drivers) and different diagnostic criteria for DED (Korea: symptoms or diagnosis, China: OSDI, TBUT, CFS, Schirmer's test and Lf levels). Another possible explanation was that $O_3$ and $NO_2$ in the environment can be are coupled by chemical bonds, and the formation of $O_3$ needs to deplete nitrogen with the help of sunlight.[33] Thus, when exposed to high levels of $NO_2$, individuals typically experience lower $O_3$ exposure, especially in areas with heavy traffic.[34]

There also are many controversies about the relationship between $SO_2$ and DED. Some studies reported positive association,[7,20] the others reported null association.[8,35] Our study also showed null association, which was related to the decreasing $SO_2$ levels, due to the worldwide use of fuel with low sulfur content.[36]

As we know, one of the most noteworthy findings of this study is stress and allergies may aggravate the effects of $PM_{10}$, $NO_2$ exposure or low ambient humidity on DED among taxi drivers. To reduce the influence of confounding factors, we matched the age and gender of the participants before analysis. In fact, among the covariates listed in this study, four factors (including stress, allergies, driving during the day, and non-self-employment) were highly correlated with DED and may be risk factors for DED. After taking each stratum with a single potential factor,[37] we found stress and allergies modified the effects of $PM_{10}$, $NO_2$ exposure or lower humidity on DED.

Similar to other countries,[38,39] in Chinese today, taxi drivers are assigned to disadvantaged groups. Due to their low income (less than 5,000 RMB/month) and high work intensity (more than 10 hours/day), they feel stressed (business license 300,000 RMB, Didi Private Car) and irritable (traffic congestion), which increase traffic risks and the occurrence of various chronic diseases (anemia, hypertension, hypercholesterolemia, hypertriglyceridemia, high blood sugar) based on our study. Even if these taxi drivers pay attention to health protection, they are unavoidably exposed to air pollution for a long time due to occupational characteristics.[22]

As for identification of susceptible individuals,[10] we collected through questionnaires whether participants had experienced asthma, allergic rhinitis, eczema and food allergy before working as taxi drivers. Although there was a certain recall bias here, we partially proved that underlying allergic tendencies increased DED risk in taxi drivers with chronic exposure to $PM_{10}$, $NO_2$, and low humidity.

Another major contribution of this study is to identify the inverse associations between above ambient factors ($PM_{10}$, $NO_2$, and low humidity) and tear Lf, especially for taxi drivers with stress or allergies. There were the negative dose-response relationships between these ambient factors and Lf. Up to now, there are few literatures on the relationship between air pollution and Lf. Regardless of animal experiments and epidemiological studies,[40–42] the existing literatures showed that air pollution transiently elevated Lf levels in the lung and nasal airway of healthy subjects, who faced to acute inflammatory reactions caused by short-term exposure. Whereas our data is inconsistent with theirs, we found more chronic exposures to airborne particulates and low humidity may be associated with decreased Lf levels in tear. As we know, there is no report similar to ours. Because healthy subjects will escape from the polluted environment after acute exposure, the body's compensation mechanism can restore Lf to normal.[41,42] Here, these taxi drivers had to be exposed outdoors for more than 10 hours on average every day for their livelihoods, and they rarely took breaks unless they were ill or had major issues at home.

A possible explanation is that the iron and inflammatory homeostasis may cause changes in tear Lf, a natural glycoprotein, in acute and chronic exposures.[11] In the process of infection or inflammation caused by acute exposure eventually leading to oxidative damage, Lf levels may in turn increase in tear due to a transient recruitment of neutrophils, increased production of Lf.[43] During irritation/cytotoxicity caused by chronic exposure, Lf may decrease due to low production of epithelial cells. Once the self-adjusting adaptive mechanism fails as a result of chronic exposure, it may induce tear hyperosmolarity and loss of goblet cells, and dry eyes to occur.[22] Another reason may be that the average Hb levels of taxi drivers (male: 118g/L; female: 108g/L) in this study was slightly lower than the standard levels of Chinese (male: 151g/L; female: 129g/L).[44]

Our results also showed the DED status modified the effect of air pollution exposure on the biomarkers (Lf). The difference of mean tear Ln-Lf levels between DED (0.41mg/mL) and non-DED (0.69 mg/mL) taxi drivers, may reflect the activity of DED after air pollution exposure. But, among other symptoms and clinical tests in this study, the results of OSDI score, CFS and Schirmer's test were similar between DED and non-DED taxi drivers, except for TBUT. This again reflected these taxi drivers' insensitivity to dry eye symptoms and the specificity of Lf as a non-traumatic biomarker of DED.

When the examination was stratified by the potential role of stressful or allergic status, effect estimates for the ambient factors (per IQR increase in $PM_{10}$, $NO_2$, and low humidity) - tear Lf associations were stronger for taxi drivers with stressful or allergic status, which suggested that the dose-response relationship may be more intense in stressful or allergic status. Through further stratification, we found that DED may amplify the effect of ambient factors on Lf levels, but similar relationships were not founded for non-DED, which suggested DED taxi drivers with stressful or allergic status may be more sensitive to Lf level change after ambient factors exposure.

Our study had the followed limitations. First, because of the lack of multiple time points, we couldn't claim to obtain the causal relationship between ambient factors and the results of concern. Whereas, robust associations of ambient factors with ocular surface injury indicated that there may be causal relationship. Second, the results of this study were limited to taxi drivers, and different results may be obtained from other professionals. So, it cannot be extended to the general population.

# Conclusions

This case-control study supports that stress and allergic tendencies may increase the susceptibility of DED caused by long-term exposure to $PM_{10}$, $NO_2$, and low humidity in taxi drivers. Moreover, the aggravatation in $PM_{10}$, $NO_2$, and low humidity may decrease tear Lf levels, especially for DED taxi drivers with stress and allergic tendencies.

# Declarations

### Declaration of competing interest

The authors declare no competing interest.

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### Author Contribution

F. Y., W. H. and F.X. K. wrote the main manuscript text. W. S. and L. Z. collected the data. Z.J. R. and X.Y. X. analyzed the data. J. L. and F.X. K. prepared clinical tests.

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# Tables

**Table 1**

**Baseline characteristics of taxi drivers included (*n* =7,611) and not included (*n* = 1,147)**

| Baseline characteristics | Included *n* (%) | Not included *n* (%) | *p*-Value |
|---|---|---|---|
| Male sex | 7,276 (95.6) | 1,092 (95.2) | 0.92 |
| Daytime drivers | 4,156 (54.6) | 617 (53.8) | 0.78 |
| < CNY 5,000/month | 5,480 (72.0) | 848 (73.9) | 0.80 |
| ≧ 10 hours/day | 4,353 (57.2) | 630 (54.9) | 0.53 |
| Smoking | 6,667 (87.6) | 1,015 (88.5) | 0.98 |
| Alcohol | 4,818 (63.3) | 760 (66.3) | 0.83 |
| Allergic tendencies* | 2,658 (35.1 ) | 413 (37.6 ) | 0.89 |
| Obesity | 4,346 (57.1) | 650 (56.7) | 0.59 |
| Iron deficiency anemia | 2,679 (35.2) | 414 (36.1) | 0.77 |
| Hypertension | 3,889 (51.1) | 571 (49.8) | 0.65 |
| Hypercholesterolemia | 2,839 (37.3) | 472 (41.2) | 0.70 |
| Hypertriglyceridemia | 3,311 (43.5) | 535 (46.6) | 0.72 |
| High blood sugar | 1,659 (21.8) | 221 (19.3) | 0.42 |
| Self-employed | 571 (7.5) | 83 (7.2) | 0.97 |
| Education (college) | 175 (2.3) | 7 (0.6) | 0.50 |
| Stress (occupation and family)* | 6,439 (84.6) | 1,000 (87.2) | 0.90 |

*Total numbers were not equal to 7,611 and 1,147 for these characteristics due to missing data.

**Table 2**

**Descriptive Statistic of air pollution concentrations (μg/m$^3$) and humidity (%) in the six cities of Liaoning, China, from 2012 to 2014 .**

| | $PM_{2.5}$ | $PM_{10}$ | $O_3$ | $SO_2$ | CO | $NO_2$ | Humidity |
|---|---|---|---|---|---|---|---|
| City (mean ± SD) | | | | | | | |
| Shenyang | 92 ± 11 | 110 ± 7 | 88 ± 21 | 54 ± 8 | 1211 ± 841 | 40 ± 6 | 68 ± 8 |
| Dalian | 80 ± 24 | 92 ± 46 | 72 ± 17 | 67 ± 3 | 1314 ± 570 | 49 ± 17 | 65 ± 10 |
| Anshan | 101 ± 13 | 107 ± 15 | 174 ± 72 | 71 ± 22 | 2020 ± 324 | 37 ± 9 | 56 ± 8 |
| Jinzhou | 89 ± 38 | 99 ± 52 | 108 ± 36 | 63 ± 14 | 1995 ± 628 | 43 ± 10 | 57 ± 10 |
| Benxi | 59 ± 7 | 76 ± 11 | 45 ± 5 | 26 ± 13 | 408 ± 119 | 21 ± 15 | 64 ± 8 |
| Dandong | 62 ± 15 | 71 ± 6 | 49 ± 21 | 24 ± 6 | 511 ± 45 | 23 ± 13 | 70 ± 11 |
| Mean ± SD | 78 ± 19 | 83 ± 35 | 94 ± 36 | 33 ± 18 | 1197 ± 480 | 33 ± 12 | 63 ± 10 |
| Minimum | 34 | 25 | 29 | 5 | 512 | 12 | 45 |
| $P_{25}$ | 72 | 74 | 81 | 19 | 880 | 27 | 56 |
| $P_{50}$ | 80 | 84 | 92 | 26 | 1080 | 31 | 64 |
| $P_{75}$ | 89 | 100 | 99 | 38 | 1386 | 37 | 71 |
| Maximum | 119 | 155 | 256 | 70 | 2433 | 61 | 90 |
| IQR* | 17 | 26 | 18 | 19 | 506 | 10 | 15 |
| NS† | 50 | 70 | 160 | 60 | 4000 | 40 | - |
| % of >NS | 72.2 | 69.2 | 23.3 | 21.6 | 0 | 52.5 | - |
| WHO guideline‡ | 10 | 20 | 100 | 20 | - | 40 | - |
| % of >WHO | 100 | 100 | 41.3 | 76.1 | - | 52.5 | - |

*IQR: Interquartile range

†NS: national standard. China national ambient air quality standard in 2012 [25].

‡ WHO 2005 air quality guidelines [26].

**Table 3**

**Spearman correlation coefficients* between air pollutants**

| Pollutant | $PM_{2.5}$ | $PM_{10}$ | $O_3$ | $SO_2$ | CO | $NO_2$ |
|---|---|---|---|---|---|---|
| $PM_{2.5}$ | 1 | 0.825 | 0.170 | 0.331 | 0.409 | 0.578 |
| $PM_{10}$ | | 1 | 0.171 | 0.439 | 0.572 | 0.420 |
| $O_3$ | | | 1 | - 0.261 | - 0.277 | - 0.235 |
| $SO_2$ | | | | 1 | 0.428 | 0.330 |
| CO | | | | | 1 | 0.367 |
| $NO_2$ | | | | | | 1 |

*All coefficients were statistically significant ($p < 0.05$).

**Table 4**

**Characteristics of taxi drivers with or without DED ($n$ = 5,708)**

| Characteristics | Cases ($n$ = 1,905), $n$ (%) or mean ± SD | Controls ($n$ = 3,803), $n$ (%) or mean ± SD | $p$-Value |
|---|---|---|---|
| Sex | | | 0.940 |
| Males<br>Females | 1,822 (95.6)<br>83 (4.4) | 3,636 (95.6)<br>167 (4.4) | |
| Age | | | 0.948 |
| 21-30 | 446 (23.4) | 887 (23.3) | |
| 31-40 | 882 (46.3) | 1,740 (45.8) | |
| 41-50 | 577 (30.3) | 1,176 (30.9) | |
| BMI | | | 0.213 |
| < 18.5 | 130 (6.8) | 422 (11.1) | |
| 18.5 ~ 23.9 | 689 (36.2) | 1,213 (31.9) | |
| 24 ~ | 1,086 (57.0) | 2,168 (56.9) | |
| Shift drivers | | | 0.033 |
| Day-time | 1,128 (59.2) | 1,871 (49.2) | |
| Night-time | 777 (40.8) | 1,932 (50.8) | |
| Myopia | | | 0.904 |
| No | 1,775 (93.2) | 3,567 (93.8) | |
| Yes | 130 (6.8) | 236 (6.2) | |
| CNY/month | | | 0.500 |
| < 5,000 | 1,393 (73.1) | 2,722 (71.6) | |
| 5,000 ~ 8,000 | 396 (20.8) | 757 (19.9) | |
| 8,000 ~ | 116 (6.1) | 324 (8.5) | |
| Working hours/day | | | |
| 8 ~10 | 853 (45.8) | 1,468 (38.6) | 0.206 |
| ≥10 | 1,052 (55.2) | 2,335 (61.4) | |
| Smoking | | | |
| Never | 236 (12.8) | 354 (9.3) | 0.236 |
| Former | 1,667 (23.7) | 3,449 (26.3) | |

| | | | |
|---|---|---|---|
| Current | 1,667 (63.5) | 3,449 (64.4) | |
| Alcohol | | | 0.320 |
| No | 607 (31.9) | 1,373 (36.1) | |
| Yes | 1,296 (68.1) | 2,430(63.9) | |
| Allergic tendencies | | | 0.011 |
| No | 1,090 (57.3) | 2,578 (67.8) | |
| Yes | 813 (42.7) | 1,225 (32.2) | |
| Hypertension | | | 0.514 |
| No | 942 (49.5) | 1,784 (46.9) | |
| Yes | 961 (50.5) | 2,019 (53.1) | |
| Hypercholesterolemia | | | 0.143 |
| No | 1,106 (58.1) | 2,426 (63.8) | |
| Yes | 798 (41.9) | 1,377 (36.2) | |
| Hypertriglyceridemia | | | 0.069 |
| No | 961 (50.5) | 2232 (58.7) | |
| Yes | 942 (49.5) | 1571 (41.3) | |
| High blood sugar | | | 0.276 |
| No | 1,448 (76.1) | 3,039 (79.9) | |
| Yes | 455 (23.9) | 764 (20.1) | |
| Self-employed | | | 0.026 |
| No | 1,821 (95.7) | 3,442 (90.5) | |
| Yes | 82 (4.3) | 361 (9.5) | |
| Education | | | 0.151 |
| ≤ High school | 1,869 (98.2) | 3,712 (97.6) | |
| College | 34 (1.8) | 91 (2.4) | |

| | | | |
|---|---|---|---|
| Stress events (occupation and family) during the 2 years [a] | | | 0.000 |
| No | 183 (9.6) | 870 (22.9) | |
| Yes | 1,720 (90.4) | 2,932 (77.1) | |
| Ln-Lf (mg/mL) [b] | 0.41 (0.39-0.43) | 0.69 (0.67-0.71) | 0.000 |
| OSDI score | 9.11 ± 8.05 | 8.40 ± 12.11 | 0.206 |
| CFS | 1.06 ± 0.98 | 0.98 ± 0.42 | 0.152 |
| TBUT (sec) | 2.65 ± 1.94 | 4.51 ± 2.36 | 0.021 |
| Schirmer's test (mm/5 min) | 12.10 ± 7.66 | 10.85 ± 7.09 | 0.089 |
| Hb (g/L) | | | |
| male | 116 ± 12 | 119 ± 13 | 0.081 |
| female | 105 ± 11 | 110 ± 12 | 0.069 |

[a] Among decrease in income, severe traffic penalties, separation/divorce, offspring loss of school or job, serious health problems, or death of family member or close relative.

[b] Values are presented as median (Q1-Q3).

OSDI = Ocular Surface Disease Index, CFS = corneal fluorescein staining, TBUT = tear break-up time

**Table 5**

**Adjusted ORs (95% CI) for DED associated with an IQR increase of air pollutants and humidity**

| | Single | Multi[b] |
|---|---|---|
| $PM_{2.5}$ | 1.01 (0.98, 1.02) | — |
| $PM_{10}$ | 1.32 (1.15, 1.70)* | 1.33 (1.07, 1.90)* |
| $O_3$ | 0.98 (0.78, 1.19) | 0.95 (0.76, 1.21) |
| $SO_2$ | 1.10 (0.63, 1.81) | 1.08 (0.54, 1.72) |
| $NO_2$ | 2.61 (1.67, 3.56)* | 2.64 (1.30, 4.37)* |
| CO | 1.08 (0.78, 1.82) | 1.04 (0.62, 2.16) |
| Humidity | 0.44 (0.54, 0.34) * | 0.40 (0.83, 0.27) * |

OR (95%CI) was estimated for an IQR increase in $PM_{2.5}$, $PM_{10}$, $O_3$, $SO_2$, $NO_2$, CO and humidity.

* $p < 0.05$

*a* Single-pollutant model: adjustment for all the potential covariates in Table 4.

*b* Multi-pollutant model: $PM_{10} + O_3 + SO_2 + NO_2$ + CO + humidity. Further adjustment for the effects of the other air pollutants on the base of single-pollutant model.

**Table 6**

**Association of $PM_{10}$, $NO_2$ or humidity (per IQR increase[a]) and DED, stratified by significant covariates.**

| | *n* | $PM_{10}$ (26 μg/m$^3$ increase) aOR (95% CI) *p*-Value[a] | $NO_2$ (10 μg/m$^3$ increase) aOR (95% CI) *p*-Value[a] | Humidity (15% increase) aOR (95% CI) *p*-Value[a] |
|---|---|---|---|---|
| Stress events | | | | |
| No | 1053 | 1.01 (0.50, 2.04) | 1.63 (0.77, 3.47) | 0.33 (0.17, 0.39) |
| Yes | 4652 | 1.89 (1.19, 3.08)* 0.023 | 2.87 (1.60, 3.58)** 0.003 | 0.51 (0.38, 0.64)* 0.039 |
| Allergic tendencies | | | | |
| No | 3688 | 1.12 (0.31, 2.01) | 1.23 (0.56, 2.68) | 0.39 (0.19, 0.59) |
| Yes | 2038 | 1.77 (1.06, 2.90)* 0.038 | 2.93 (1.64, 3.83)** 0.002 | 0.49 (0.11, 0.84) ** 0.010 |
| Self-employed | | | | |
| No | 5263 | 0.95 (0.37, 1.52) | 1.39 (0.49, 3.94) | 0.47 (0.11, 0.93) |
| Yes | 443 | 0.94 (0.59, 1.59) 0.415 | 1.40 (0.78, 2.53) 0.098 | 0.52 (0.23, 1.02) 0.108 |
| Shift drivers | | | | |
| Day-time | 2999 | 1.11 (0.69, 1.78) | 1.40 (0.64, 2.83) | 0.63 (0.42, 1.14) |
| Night-time | 2709 | 1.06 (0.92, 1.21) 0.870 | 1.79 (0.54, 3.42) 0.553 | 0.58 (0.34, 1.08) 0.316 |

[a] *p*-Value are presented as hypothetical test of likelihood ratio test to compare model fit including or excluding interactive items.

* $p < 0.05$

** $p < 0.01$.

Adjustment for all the potential covariates in Table 4 including characteristics of taxi drivers except for the stratification variables.

Table 7 is available in the Supplementary Files section

# Figures


**Figure 1**

flow chart of case

# Supplementary Files

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- table7.docx