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Short-term effects of ambient air pollution on stroke hospitalizations in Shenzhen, China

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Abstract

Objective: To investigate the association between ambient air pollution and stroke morbidity in different subgroups and seasons.

Methods: We collected data on 67,078 stroke hospitalizations in Shenzhen, China, during January 1, 2014, and December 31, 2016. We performed a time-series analysis based on generalized linear models to study the short-term exposure-response relationships between air pollution and stroke hospitalizations, and conducted subgroup analyses to identify possible sensitive populations.

Results: For every 10 μg/m^3 increase in the concentration of air pollutant on average from lag 0-3, the relative risk (RR) of stroke hospitalization was 1.029 (95% CI: 1.013-1.045) for $PM_{2.5}$, 1.054 (95% CI: 1.031-1.077) for NO_2, and 1.012 (1.002-1.022) for O_3. Subgroup analyses showed that short-term ambient air pollution influenced both men and women, middle-aged and older populations, and both cerebral infarction and intracerebral hemorrhage. Harmful effects mainly occurred in the winter.

Conclusions: Our study indicates that short-term exposure to $PM_{2.5}$, NO_2 and O_3 have harmful effects on stroke morbidity, and the government should take actions to mitigate air pollution and protect sensitive populations.

Keywords: Air pollution; Stroke morbidity; Time-series analysis; Season

Article Summary

Strengths and limitations of this study

► This study investigated the association between air pollution and subtypes of stroke morbidity, and explored the modification effects of different demographic characteristics and seasons.
► This study included more than 67,000 cases, ensured the robust of statistical power.
► Single-city study limited the generalization of the findings.
► Associations between air pollution and stroke hospitalizations did not prove causality.

Introduction

Stroke is one of the leading causes of disability-adjusted life years (DALYs) and mortality, accounting for approximately 132 million DALYs and 6.2 million deaths globally in 2017.[1 2] It is also the leading cause of long-term adult disability and leading cause of death in China; 20.19% of total deaths were caused by stroke in 2017,[3 4] and the age-standardized incidence and mortality rates were 246.8 and 114.8 per 100,000 person-years, respectively.[5] Well-known risk factors of stroke include history of hypertension, current smoking status, and alcohol intake.[6]

With the deterioration of global air quality, concerns have been raised about the relationship between ambient air pollution and stroke. According to the Global Burden of Disease, approximately 12.75% of stroke cases in China in 2017 were linked to air pollution.[3] Possible mechanisms linking ambient air pollution to stroke include inflammation, oxidative stress, atherosclerosis and autonomic dysregulation.[7]

To date, most of the relevant epidemiological studies have focused on stroke mortality instead of morbidity,[8 9] but it should be noted that morbidity is more indicative of the early stages of stroke; early prevention of stroke has an important public health significance. Studies that examined the relationship between ambient air pollution and stroke morbidity obtained inconsistent results, and most of them studied the association over a full year but failed to consider the seasonal differences in pollutant concentration and their health effects.[10-12]

In this study, a time-series analysis was conducted to investigate the association between ambient air pollution and stroke morbidity in different seasons; we also researched possible sensitive populations by conducting subgroup analyses by sex, age group, education level and stroke subtypes.

Materials and Methods

Study area

Shenzhen is located within the Pearl River Delta, China, is one degree south of the Tropic of Cancer, and has a permanent population of approximately 12.5 million people[13]. Because of the Siberian anticyclone, it has a warm and humid subtropical climate. Six kinds of air pollutants, including ozone (O_3), fine particulate matter ($PM_{2.5}$), nitrogen dioxide (NO_2), inhalable particulate matter (PM_{10}), sulfur dioxide

(SO_2) and carbon monoxide (CO), are monitored in Shenzhen, and the main ambient air pollutants are O_3, $PM_{2.5}$ and NO_2.

Data collection

Stroke data, containing 67,078 cases from 1 Jan 2014 to 31 Dec 2016, were collected from the Shenzhen Center for Chronic Disease Control. Sixty-nine hospitals from 10 administrative districts reported their stroke case information (including patient age and sex, clinical diagnosis and classification, onset date, diagnosis date, and major risk factors) to the Shenzhen Center for Chronic Disease Control. All the cases were coded according to the World Health Organization's International Classification of Diseases, 10th revision (ICD-10), ranging from I60-I64: (1) Subarachnoid hemorrhage (SAH), which was coded as I60; (2) intracerebral hemorrhage (ICH), which was coded as I61; (3) other nontraumatic intracranial hemorrhage (ONIH), which was coded as I62; (4) cerebral infarction (CBI), which was coded as I63; (5) stroke not specified as hemorrhage or infarction (SNSHI), which was coded as I64. All cases were diagnosed in the hospital with computed tomography, magnetic resonance imaging or neurological examination. In the subgroup analysis for stroke subtypes, we focused on ICH and CBI, which accounted for 94.8% of all stroke cases.

Hourly concentrations of O_3, $PM_{2.5}$ and NO_2 were obtained from the National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035/). The daily mean concentration of each pollutant was computed using the centering method. Daily meteorological data were obtained from the National Meteorological Data Sharing Platform (http://data.cma.cn/), including the daily mean, maximum and minimum temperatures, the mean relative humidity, the mean wind speed, and the mean atmospheric pressure.

Statistical modeling

The time-series analysis was based on a distributed lag linear model [14]. In this study, a cross-basis matrix was established for the daily mean concentration of air pollutants ($PM_{2.5}$, NO_2, or O_3) and the delayed effects (lags); the number of daily hospital admissions for stroke was the dependent variable. Generalized linear Poisson models (GLMs) were used to estimate the association between these exposures and stroke hospitalizations.[15] Meteorological variables (i.e., temperature, relative humidity and air pressure), long-term trends, seasonal patterns and the day of the week (DOW)

were used as adjustment variables. The influence of other pollutants was examined in a two-pollutant model.

The model to represent the relationship between the daily mean concentration of air pollutants and hospital admissions for stroke is as follows:

$$\log\left[\mathrm{E}(Y_t)\right] = \alpha + \beta_1 POL_{t,l} + ns(TEM_t, df = 3) + ns(RH_t, df = 3) + ns(PRE_t, df = 3) + ns(TIME_t, df = 7/\text{year} * No.\ of\ years) + \beta_2 DOW_t$$

Here, $\mathrm{E}(Y_t)$ indicates the expected number of hospital admissions for stroke on day t; $POL_{t,l}$ is the cross-basis matrix of the air pollutants ($PM_{2.5}$, NO_2, or O_3), with β_1 as the corresponding vector of coefficients; and $ns()$ is the natural cubic spline function. DOW_t is a dummy variable for the day of week. We used 7 degrees of freedom (df) per year for the time trend ($TIME_t$), 3 df for the previous 14 days' moving average temperature (TEM_t) [16], and 3 df for both the relative humidity (RH_t) and the air pressure (PRE_t) on day t; the choice of df for each variable was based on the Akaike information criterion for quasi-Poisson models.

Subgroup analyses were conducted for different sexes, age groups (<40, 40-64, ≥65), education levels (primary school or below, junior high school, high school or above) and stroke subtypes (CBI, ICH). When comparing seasonal differences, we defined spring as the period from 1 March to 31 May, summer as the period from 1 June to 31 August, autumn as the period from 1 September to 30 November, and winter as the period from 1 December to 28 February of the next year. Sensitive analyses were performed by adjusting the df of $TIME_t$ and TEM_t. All results were reported as relative risks (RRs) and 95% confidence intervals (CIs) of hospital admissions for stroke for every 10 μg/m^3 increase in air pollutant exposure. Main analyses were performed using the "dlnm" and "splines" packages in R version 3.5.1.

Results

Data description

In total, during 2014-2016, 67,078 hospital admissions for stroke were recorded, of which 77.8% were first-time cases, and 61.9% were men. Patients aged 40-64 years accounted for 49.3% of the total, and 44.7% were older (age≥65 years old). In total, 27.7% of the cases had an education level of primary school or below, 39.8% completed junior high school, and 32.5% had a high school education or higher. Among the

subtypes of stroke, CBI and ICH accounted for 52,168 (77.8%) and 11,415 (17.0%), respectively. The other three subtypes accounted for approximately 5% of the total (Table 1).

On average, there were approximately 61 hospital admissions for stroke per day during our study period, and the 24-hour mean concentrations of $PM_{2.5}$, NO_2 and O_3 were 30.1 μg/m^3, 33.2 μg/m^3 and 56.9 μg/m^3, respectively. The daily mean temperature was 23.5 °C, the mean relative humidity was 74.7%, and the daily mean air pressure was 1005.7 hPa (Table 2). Correlations among the air pollutants, weather factors and stroke admissions are in Table 3.

Table 1. Summary statistics of stroke hospitalizations in Shenzhen

Variables	Number	Percentage (%)
Stroke cases	67,078	100.0
Sex		
Male	41,546	61.9
Female	25,526	38.1
Age (years)		
<40	4,051	6.0
40-64	33,053	49.3
≥65	29,968	44.7
Education		
Primary school or below	18,558	27.7
Junior high school	26,686	39.8
High school or above	21,792	32.5
Stroke subtypes		
CBI	52,168	77.8
ICH	11,415	17.0
SAH	2,369	3.5
SNSHI	219	0.3
ONIH	783	1.2

Table 2. Distribution of daily air pollution concentrations, meteorological factors and stroke hospitalizations in Shenzhen

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Air Pollutants							
$PM_{2.5}$, μg/m^3	30.1	16	6.3	16.8	27	39.9	96.1
NO_2, μg/m^3	33.2	11.1	11.3	25	31	39.5	84.8
O_3, μg/m^3	56.9	22.1	16.9	38.8	52.9	71.9	147.8
Meteorological Factors							
Temperature, ℃	23.5	5.7	3.5	19	25.1	28.2	33
Relative humidity, %	74.7	12.2	19	69	76	83	99
Air pressure, hPa	1005.6	6.6	986.8	1000.7	1005.3	1010.6	1027.3
Stroke cases							
All cases	61.2	15.7	23.0	51.0	59.0	70.0	182.0
ICH	10.4	4.2	1.0	7.0	10.0	13.0	40.0
CBI	47.6	13.1	17.0	39.0	46.0	54.0	143.0
Sex							
Male	37.9	10.2	15.0	31.0	37.0	43.0	113.0
Female	23.3	7.4	7.0	18.0	22.0	27.0	77.0
Age (years old)							
<40	3.7	2.1	0.0	2.0	4.0	5.0	12.0
40-64	30.2	8.4	9.0	24.0	30.0	35.0	90.0
≥65	27.3	8.7	9.0	21.0	26.0	32.0	101.0
Education							
Primary school or below	16.9	5.4	3.0	13.0	16.0	20.0	51.0
Junior high school	24.4	7.9	7.0	19.0	23.0	29.0	85.0
High school or above	19.9	7.6	5.0	14.0	19.0	24.0	67.0

Table 3. The correlation between air pollutants, meteorological factors and stroke hospitalizations

Variables	Stroke	$PM_{2.5}$	NO_2	O_3	Temperature	Relative humidity	Air pressure
Stroke	1.00						
$PM_{2.5}$	0.02	1.00					
NO_2	0.11*	0.55*	1.00				
O_3	0.05	0.56*	0.02	1.00			
Temperature	-0.03	-0.43*	-0.27*	-0.09*	1.00		
Relative humidity	0.05	-0.53*	-0.1*	-0.51*	0.27*	1.00	
Air pressure	0.08*	0.50*	0.26*	0.21*	-0.85*	-0.45*	1.00

*p<0.05: Spearman correlation analysis was adopted

Regression analysis

When the lag length was set as 7 days, the results showed that the lag effect mainly lasted for 3 days (Figure S1). Therefore, we used 3 days as the lag length in later analyses. Positive associations and lag effects were observed between stroke morbidity and air pollution. Every 10 μg/m^3 increase in $PM_{2.5}$ concentrations on average from lag 0-3 corresponded to a 2.9% (95% CI: 1.3%-4.5%) increase in daily stroke hospitalizations; it was a 5.3% (3.1%-7.7%) increase in the case of NO_2, and a 1.2% (0.2%–2.2%) increase for O_3.

Table 4 showed that, for $PM_{2.5}$, an association was found in both men and women, populations older than 40 years old, and people with education levels of junior high school and above, and the CBI stroke subtype. For NO_2, the association was consistently significant in both men and women, populations in all age groups and all education levels, and subtypes CBI and ICH. For O_3, the association was significant in women, the middle-aged population, populations with an education level of junior high school and the CBI stroke subtype. The associations mainly occurred in the winter.

For the two-pollutant model (Table 5), the results did not vary substantially compared to those in the single-pollutant models, especially for NO_2. The results were stable after adjusting the df of $TIME_t$ and TEM_t in the model, indicating the stability of the estimated effects (Table S1).

Table 4. Relative risks and 95% confidence intervals for stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2, and O_3 in the single-pollutant model.

Subgroups	RR (95% CI)		
	$PM_{2.5}$	NO_2	O_3
All strokes	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
Sex			
Male	1.029(1.011-1.046)	1.056(1.032-1.080)	1.011(0.999-1.022)
Female	1.030(1.009-1.052)	1.050(1.020-1.080)	1.013(1.000-1.027)
Age			
<40	1.030(0.990-1.070)	1.084(1.027-1.145)	0.998(0.974-1.023)
40-64	1.025(1.008-1.044)	1.058(1.033-1.084)	1.013(1.002-1.024)
>65	1.033(1.012-1.055)	1.046(1.017-1.076)	1.012(0.999-1.025)
Education			
Primary and below	1.013(0.991-1.036)	1.050(1.019-1.081)	1.007(0.993-1.021)
Junior high school	1.043(1.021-1.065)	1.072(1.041-1.104)	1.023(1.009-1.037)
Senior high school and above	1.028(1.006-1.049	1.036(1.007-1.066)	1.003(0.990-1.016)
Stroke subtype			
ICH	1.021(0.995-1.048)	1.058(1.021-1.096)	1.008(0.991-1.025)
CBI	1.029(1.011-1.047)	1.050(1.026-1.075)	1.012(1.001-1.023)

Season			
Spring	1.031(0.989-1.076)	1.034(0.989-1.081)	1.012(0.989-1.035)
Summer	1.031(0.989-1.075)	0.992(0.944-1.044)	1.015(0.990-1.041)
Autumn	1.021(0.993-1.050)	1.040(0.994-1.089)	1.009(0.992-1.025)
Winter	1.073(1.022-1.126)	1.095(1.024-1.170)	1.033(0.983-1.086)

Table 5. Relative risks and 95% confidence intervals of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2 and O_3 in the two-pollutant model.

Pollutants and model	RR	95% CI
$PM_{2.5}$		
Single-pollutant model	1.029	(1.013-1.045)
+NO_2	1.020	(1.002-1.038)
+O_3	1.030	(1.012-1.048)
+PM_{10}	0.996	(0.960-1.033)
+SO_2	1.028	(1.010-1.046)
+CO	1.026	(1.009-1.043)
NO_2		
Single-pollutant model	1.054	(1.031-1.077)
+$PM_{2.5}$	1.054	(1.029-1.080)
+O_3	1.053	(1.031-1.076)
+PM_{10}	1.050	(1.024-1.077)
+SO_2	1.059	(1.034-1.085)
+CO	1.053	(1.029-1.077)
O_3		
Single-pollutant model	1.012	(1.002-1.022)
+NO_2	1.011	(1.001-1.021)
+$PM_{2.5}$	1.009	(0.997-1.020)
+PM_{10}	1.007	(0.996-1.018)
+SO_2	1.011	(1.001-1.022)
+CO	1.011	(1.000-1.021)

Discussion

In this study, a positive association was found between the daily stroke hospitalizations and the concentration of ambient $PM_{2.5}$. Previous studies showed inconsistent results when exploring the short-term effect of $PM_{2.5}$ on stroke morbidity. Some studies observed a nonsignificant association, while other studies found a significant positive association[10 17]. For example, a 2014 meta-analysis of nonfatal strokes found an RR of 0.7-0.8% for short-term effects per 10 μg/m^3 $PM_{2.5}$.[18] Exposure to particulate matter increases inflammation, antioxidant activity and circulating blood platelet activation, and decreases vascular endothelial functions and enzyme activity, the latter of which may increase peripheral thrombosis and blood clotting.[19-21] Furthermore, with a small size and large surface area, $PM_{2.5}$ could carry more toxicity and harmful substances and penetrate deep into the human body,[22] which may induce a stroke.

We found that daily stroke hospitalizations increased by 5.4% for every 10 μg/m^3 increase of NO_2. These results are similar to those of previous studies, but some of the previous studies obtained different effect scales.[23-25] For example, a study in Wuhan, China showed a 2.9% increase in stroke hospitalizations per 10 μg/m^3 increase in NO_2 in the cold season, which was smaller than the result (9.5%) from our study.[26] Evidence has shown that NO_2 is related to plasma fibrinogen[27] and is also correlated with fine particulate matter, which leads to increased coagulability through the release of cytokines.[28] Gaseous NO_2 can also be mixed with water to form acid aerosols after inhalation, which damages health through a series of oxidation mechanisms.[29]

Our study found stronger effects of NO_2 on stroke than $PM_{2.5}$. Previous studies showed similar results;[30 31] gaseous pollutants appeared to have a stronger effect on stroke mortality,[32 33] and some studies indicated that, after adjusting for other pollutants, only NO_2 showed consistent results with a single-pollutant model.[34 35] Concern has been raised that the observed health effects due to NO_2 might be a result of exposure to traffic-related emissions or fine particles.[36]

A weak positive association between O_3 and hospital admissions for stroke was found in our study. Previous studies exploring this relationship showed different results. For example, a 10-year study conducted in France showed a significant positive relationship between O_3 exposure and ischemic stroke in men over 40 years of age, and

the result was observed to a 1-day lag (OR 1.133, 95% CI 1.052 to 1.220).[37] In the United States, where air pollution levels are low, the incidence of CBI/TIA was also associated with O_3 exposure.[38] However, there was no statistically significant association found between O_3 pollution and the incidence of stroke in South Carolina and Nueces County, Texas.[39] [40] Some studies even showed a significant or nonsignificant negative association between O_3 and CBI.[41] [42] The mechanisms linking O_3 and stroke need to be further studied.

The effects of air pollution were found in both men and women; no clear trends were found across age groups and education levels. The seasonal analyses showed that statistically significant correlations between ambient air pollutant concentrations and hospital admissions for stroke were only found in the winter; Dong et al. also found that the harmful effects of NO_2 and SO_2 were more robust in the cold season[31]. One possible reason for this result is that the air pollution in Shenzhen in the winter is much heavier than in the summer. The substances carried by particles may vary between seasons, which would influence the effect. Furthermore, the interaction between air pollutants and temperature that has been observed by some epidemiological studies should be considered, and the strongest effect exists in winter and on low-temperature days.[43] [44]

Our study found that $PM_{2.5}$, NO_2 and O_3 were related to hospitalizations for CBI but found different results for ICH. Similarly, previous studies showed consistent associations between CBI and air pollution, while the association between ICH was more variable and had a higher imprecision.[45] One reason is that the prevalence of ICH was much lower than CBI. In our study, 77.8% of stroke hospitalizations were for CBI, and ICH only accounted for 17.0% of stroke hospitalizations. Therefore, the risk estimates for ICH were more imprecise, and the confident interval of the RR for ICH was wider. Another reason may be the differential mechanisms linking air pollution exposure to CBI and ICH. $PM_{2.5}$ exposure is conducive to atherosclerosis and the oxidative stress of the heart through an increase in malondialdehyde, which upregulates visfatin, reduces heart rate variability and induces CD36-dependent 7-ketocholesterol accumulation in macrophages,[46-49] which is more likely to cause a CBI.

There are several limitations in this study. First, the air pollution data from fixed monitoring stations may not be representative of actual exposure. For example, the air pollution exposure in places near the main trunk would be heavier than places far away from it, and the air pollution monitoring stations were usually set places far away from the main trunk. Second, it is difficult to completely eliminate the possibility of

ecological fallacy in time-series studies. Moreover, associations between air pollution and stroke hospitalizations did not prove causality.

Conclusions

$PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, especially in the winter. All three air pollutants were associated with higher risks of hospital admission due to cerebral infarction, the most prevalent stroke subtype in the study location. The government should take actions to address air pollution issues and develop warning systems for sensitive populations during high-risk periods.

Contributions: The study was conceived and designed by Bao J, Peng J and Guo Y; Xie X and Guo Y conducted statistical analysis and drafted the manuscript; Lei L, Zhou H, and Deng S contributed to data collection and processing; Xu Y and Liu Z helped in study management and the interpretation of the results; Bao J, Peng J and Huang C reviewed the manuscript for important intellectual content; All authors read and approved the final version.

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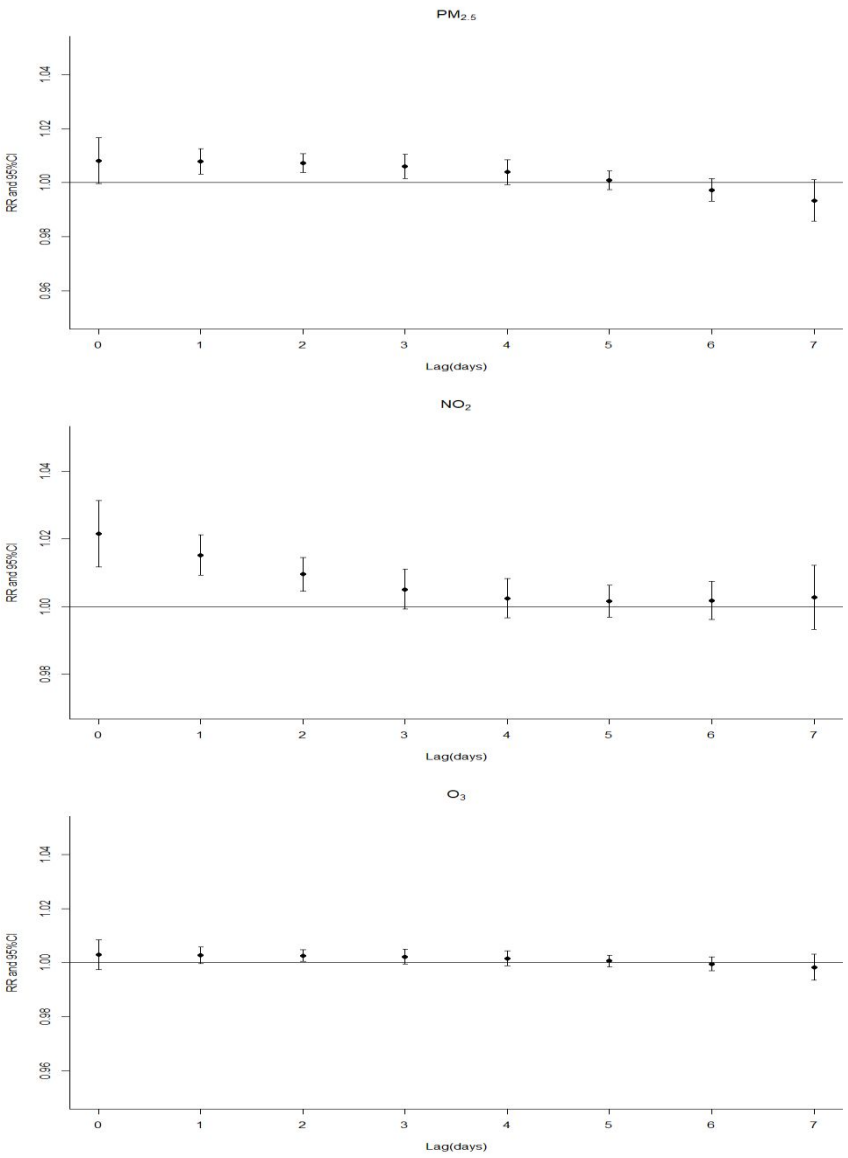

Figure S1. The lag effect of $PM_{2.5}$, NO_2 and O_3 on stroke when setting the largest lag day to 7.

Table S1. The adjustments of the df of $TIME_t$ and TEM_t and the corresponding changes in the RR and 95% CI of stroke in the whole population for every 10 μg/m^3 increase in air pollutant concentration.

df of $TIME_t$	df of TEM_t	RR(95% CI) for $PM_{2.5}$	RR(95% CI) for NO_2	RR(95% CI) for O_3
5x3	2	1.023(1.007-1.039)	1.054(1.032-1.076)	1.004(0.994-1.014)
	3	1.022(1.006-1.038)	1.052(1.030-1.075)	1.005(0.995-1.015)
	4	1.022(1.006-1.039)	1.053(1.031-1.076)	1.004(0.995-1.015)
6x3	2	1.028(1.012-1.044)	1.053(1.031-1.075)	1.010(0.999-1.020)
	3	1.027(1.011-1.044)	1.054(1.031-1.076)	1.010(1.000-1.020)
	4	1.028(1.012-1.044)	1.054(1.032-1.077)	1.010(0.999-1.020)
7x3	2	1.029(1.013-1.046)	1.054(1.032-1.077)	1.011(1.001-1.021)
	3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
	4	1.029(1.013-1.046)	1.056(1.033-1.080)	1.011(1.001-1.022)

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Short-term effects of ambient air pollution on stroke hospitalizations: A time-series study in Shenzhen, China.

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Abstract

Objective: To investigate the association between ambient air pollution and stroke morbidity in different subgroups and seasons.

Methods: We performed a time-series analysis based on generalized linear models to study the short-term exposure-response relationships between air pollution and stroke hospitalizations, and conducted subgroup analyses to identify possible sensitive populations.

Results: For every 10 μg/m^3 increase in the concentration of air pollutant, across lag 0-3 days, the relative risk (RR) of stroke hospitalization was 1.029 (95% CI: 1.013-1.045) for $PM_{2.5}$, 1.054 (95% CI: 1.031-1.077) for NO_2, and 1.012 (1.002-1.022) for O_3. Subgroup analyses showed that short-term ambient air pollution influenced both men and women, middle-aged and older populations, and both cerebral infarction and intracerebral hemorrhage. Harmful effects mainly occurred in the winter.

Conclusions: Our study indicates that short-term exposure to $PM_{2.5}$, NO_2 and O_3 have harmful effects on stroke morbidity, and the government should take actions to mitigate air pollution and protect sensitive populations.

Keywords: Air pollution; Stroke morbidity; Time-series analysis; Season

Article Summary

Strengths and limitations of this study

► This study investigated the association between air pollution and subtypes of stroke morbidity.
► This study included more than 67,000 cases, ensured the robust of statistical power.
► The modification effects of different demographic characteristics and seasons were explored.
► Single-city study limited the generalization of the findings.
► Associations between air pollution and stroke hospitalizations did not prove causality.

Introduction

Stroke is one of the leading causes of disability-adjusted life years (DALYs) and mortality, accounting for approximately 132 million DALYs and 6.2 million deaths globally in 2017.[1 2] It is also the leading cause of long-term adult disability and leading cause of death in China; 20.19% of total deaths were caused by stroke in 2017,[3 4] and the age-standardized incidence and mortality rates were 246.8 and 114.8 per 100,000 person-years, respectively.[5] Well-known risk factors of stroke include history of hypertension, current smoking status, and alcohol intake.[6]

With the deterioration of global air quality, concerns have been raised about the relationship between ambient air pollution and stroke. According to the Global Burden of Disease, approximately 12.75% of stroke cases in China in 2017 were linked to air pollution.[3] Possible mechanisms linking ambient air pollution to stroke include inflammation, oxidative stress, atherosclerosis and autonomic dysregulation.[7] To date, most of the relevant epidemiological studies have focused on stroke mortality instead of morbidity,[8 9] but it should be noted that morbidity is more indicative of the early stages of stroke; early prevention of stroke has an important public health significance. Studies that examined the relationship between ambient air pollution and stroke morbidity obtained inconsistent results, and most of them studied the association over a full year but failed to consider the seasonal differences in pollutant concentration and their health effects.[10-12]

In this study, a time-series analysis was conducted to investigate the association between ambient air pollution and stroke morbidity in different seasons; we also researched possible sensitive populations by conducting subgroup analyses by sex, age group, education level and stroke subtypes.

Materials and Methods

Study area

Shenzhen is located within the Pearl River Delta, China, is one degree south of the Tropic of Cancer, and has a permanent population of approximately 12.5 million people.[13] Because of the Siberian anticyclone, it has a warm and humid subtropical climate. Six kinds of air pollutants, including ozone (O_3), fine particulate matter ($PM_{2.5}$), nitrogen dioxide (NO_2), inhalable particulate matter (PM_{10}), sulfur dioxide (SO_2) and carbon monoxide (CO), are monitored in Shenzhen, and the main ambient air pollutants are O_3, $PM_{2.5}$ and NO_2.

Data collection

Stroke data, containing 67,078 cases from Jan 1st 2014 to Dec 31st 2016, were collected from the Shenzhen Center for Chronic Disease Control. Sixty-nine hospitals from 10 administrative districts reported their stroke case information (including patient age and sex, clinical diagnosis and classification, onset date, diagnosis date, and major risk factors) to the Shenzhen Center for Chronic Disease Control. All the cases were coded according to the World Health Organization's International Classification of Diseases, 10th revision (ICD-10), ranging from I60-I64: (1) Subarachnoid hemorrhage (SAH), which was coded as I60; (2) intracerebral hemorrhage (ICH), which was coded as I61; (3) other nontraumatic intracranial hemorrhage (ONIH), which was coded as I62; (4) cerebral infarction (CBI), which was coded as I63; (5) stroke not specified as hemorrhage or infarction (SNSHI), which was coded as I64. All cases were diagnosed in the hospital with computed tomography, magnetic resonance imaging or neurological examination. In the subgroup analysis for stroke subtypes, we focused on ICH and CBI, which accounted for 94.8% of all stroke cases.

Hourly concentrations of O_3, $PM_{2.5}$ and NO_2 were obtained from the National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035/). The daily mean concentrations for each air pollutant were averaged from all the 11 fixed-site air pollution monitoring stations in Shenzhen.[14] Daily meteorological data were obtained from the National Meteorological Data Sharing Platform (http://data.cma.cn/), including the daily mean, maximum and minimum temperatures, the mean relative humidity, the mean wind speed, and the mean atmospheric pressure.

Statistical modeling

This time-series analysis adopted generalized linear Poisson models (GLMs) to estimate the association between air pollutants and stroke hospitalizations.[15] The daily mean concentrations of air pollutant ($PM_{2.5}$, NO_2, or O_3) were the independent variables, and the number of daily hospital admissions for stroke was the dependent variable. Meteorological variables (i.e. temperature, relative humidity and air pressure), long-term trends and seasonal patterns, as well as day of the week (DOW) were used as adjustment variables. The influence of other pollutants was examined in two-pollutant models.

Considering the possible delay (lag) effects of air pollution on stroke morbidity, we created cross-basis matrix for air pollutants, within the framework of distributed lag linear model (DLM).[16] The selection of lag days was justified by the polynomial lag models.

The model to represent the relationship between the daily mean concentration of air pollutants and hospital admissions for stroke is as follows:

$$\log\left[\mathrm{E}(Y_t)\right] = \alpha + \beta POL_{t,l} + ns(TEM_t, df = 3) + ns(RH_t, df = 3) + ns(PRE_t, df = 3) + ns(TIME_t, df = 7/\text{year} * No.\ of\ years) + \gamma DOW_t$$

Here, $\mathrm{E}(Y_t)$ indicates the expected number of hospital admissions for stroke on day t; $POL_{t,l}$ is the cross-basis matrix of the air pollutants ($PM_{2.5}$, NO_2, or O_3), with β as the corresponding vector of coefficients; and $ns()$ is the natural cubic spline function. DOW_t is a dummy variable for the day of week. We used 7 degrees of freedom (df) per year for the time trend ($TIME_t$), 3 df for the previous 14 days' moving average temperature (TEM_t),[17] and 3 df for both the relative humidity (RH_t) and the air pressure (PRE_t) on day t; the choice of df for each variable was based on the Akaike Information Criterion for quasi-Poisson models.

Subgroup analyses were conducted for different sexes, age groups (<40, 40-64, ≥65), education levels (primary school or below, junior high school, high school or above) and stroke subtypes (CBI, ICH). When comparing seasonal differences, we defined spring as the period from Mar 1st to May 31st, summer as the period from Jun 1st to Aug 31st, autumn as the period from Sept 1st to Nov 30th, and winter as the period from Dec 1st to Feb 28th of the next year. The differences among the effect estimates between different subgroups were tested via the Z-test.[18] Sensitivity analyses were performed by adjusting the df values of $TIME_t$ and TEM_t, as well as the lag days of air pollution. All results were reported as relative risks (RRs) and 95% confidence intervals (CIs) of hospital admissions for stroke for every 10 μg/m^3 increase in air pollutants exposure. Main analyses were performed using the "dlnm" and "splines" packages in R version 3.5.1.

Patient and Public Involvement

This study belongs to ecological research and does not reveal any personal information. Therefore, patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research.

Results

Data description

In total, during 2014-2016, 67,078 hospital admissions for stroke were recorded, of which 77.8% were first-time cases, and 61.9% were men. Patients aged 40-64 years

accounted for 49.3% of the total, and 44.7% were older (age≥65 years old). In total, 27.7% of the cases had an education level of primary school or below, 39.8% completed junior high school, and 32.5% had a high school education or higher. Among the subtypes of stroke, CBI and ICH accounted for 52,168 (77.8%) and 11,415 (17.0%), respectively. The other three subtypes accounted for approximately 5% of the total (Table 1).

On average, there were approximately 61 hospital admissions for stroke per day during our study period, and the 24-hour mean concentrations of $PM_{2.5}$, NO_2 and O_3 were 30.1 μg/m^3, 33.2 μg/m^3 and 56.9 μg/m^3, respectively. The daily mean temperature was 23.5 °C, the mean relative humidity was 74.7%, and the daily mean air pressure was 1005.7 hPa (Table 2). In general, the pollutant concentrations of $PM_{2.5}$ and NO_2 were higher in winter than in other seasons (Table S1). Correlations between air pollutants and meteorological factors are in Table 3.

Table 1. Summary statistics of stroke hospitalizations in Shenzhen

Variables	Number	Percentage (%)
Stroke cases	67,078	100.0
Sex		
Male	41,546	61.9
Female	25,526	38.1
Age (years)		
<40	4,051	6.0
40-64	33,053	49.3
≥65	29,968	44.7
Education		
Primary school or below	18,558	27.7
Junior high school	26,686	39.8
High school or above	21,792	32.5
Stroke subtypes		
CBI	52,168	77.8
ICH	11,415	17.0
SAH	2,369	3.5
SNSHI	219	0.3
ONIH	783	1.2

Table 2. Distribution of daily air pollution concentrations, meteorological factors and stroke hospitalizations in Shenzhen

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Air Pollutants							
$PM_{2.5}$, μg/m^3	30.1	16	6.3	16.8	27	39.9	96.1
NO_2, μg/m^3	33.2	11.1	11.3	25	31	39.5	84.8
O_3, μg/m^3	56.9	22.1	16.9	38.8	52.9	71.9	147.8
Meteorological Factors							
Temperature, ℃	23.5	5.7	3.5	19.0	25.1	28.2	33.0
Relative humidity, %	74.7	12.2	19.0	69.0	76.0	83.0	99.0
Air pressure, hPa	1005.6	6.6	986.8	1000.7	1005.3	1010.6	1027.3
Stroke cases							
All strokes	61.2	15.7	23.0	51.0	59.0	70.0	182.0
Type							
ICH	10.4	4.2	1.0	7.0	10.0	13.0	40.0
CBI	47.6	13.1	17.0	39.0	46.0	54.0	143.0
Sex							
Male	37.9	10.2	15.0	31.0	37.0	43.0	113.0
Female	23.3	7.4	7.0	18.0	22.0	27.0	77.0
Age (years old)							
<40	3.7	2.1	0.0	2.0	4.0	5.0	12.0
40-64	30.2	8.4	9.0	24.0	30.0	35.0	90.0
≥65	27.3	8.7	9.0	21.0	26.0	32.0	101.0
Education							
Primary school or below	16.9	5.4	3.0	13.0	16.0	20.0	51.0
Junior high school	24.4	7.9	7.0	19.0	23.0	29.0	85.0
High school or above	19.9	7.6	5.0	14.0	19.0	24.0	67.0

Table 3. Spearman's rank coefficients between daily mean air pollutants and meteorological variables

Variables	$PM_{2.5}$	NO_2	O_3	Temperature	Relative humidity	Air pressure
$PM_{2.5}$	1.00					
NO_2	0.55*	1.00				
O_3	0.56*	0.02	1.00			
Temperature	-0.43*	-0.27*	-0.09*	1.00		
Relative humidity	-0.53*	-0.1*	-0.51*	0.27*	1.00	
Air pressure	0.50*	0.26*	0.21*	-0.85*	-0.45*	1.00

*$p<0.05$.

Regression analysis

When the lag length was set as 7 days, the results showed that the lag effect mainly lasted for 3 days (Figure S1). Therefore, we used 3 days as the lag length in later analyses. Positive associations and lag effects were observed between stroke morbidity and air pollution. The cumulative effect of a 10 μg/m^3 increase in $PM_{2.5}$ concentrations corresponded to a 2.9% (95% CI: 1.3%-4.5%) increase in daily stroke hospitalizations, across lag 0-3 days.; it was a 5.3% (3.1%-7.7%) increase in the case of NO_2, and a 1.2% (0.2%–2.2%) increase for O_3.

Table 4 showed that, for $PM_{2.5}$, an association was found in both men and women, populations older than 40 years old, and people with education levels of junior high school and above, and the CBI stroke subtype. For NO_2, the association was consistently significant in both men and women, populations in all age groups and all education levels, and subtypes CBI and ICH. For O_3, the association was significant in women, the middle-aged population, populations with an education level of junior high school and the CBI stroke subtype. The associations mainly occurred in the winter. Comparing the effect estimates between different subgroups, we found none of the differences were statistically significant.

For the two-pollutant model (Figure 1), the results did not vary substantially compared to those in the single-pollutant models, especially for NO_2. The results were stable after

adjusting the df values of $TIME_t$ and TEM_t, as well as the lag days in the model, indicating the stability of the estimated effects (Table S2-S3).

Table 4. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2, and O_3 in the single-pollutant model, across lag 0-3 days.

Subgroups	RR(95% CI)		
	$PM_{2.5}$	NO_2	O_3
All strokes	1.029 (1.013-1.045)	1.054 (1.031-1.077)	1.012 (1.002-1.022)
Sex			
Male	1.029 (1.011-1.046)	1.056 (1.032-1.080)	1.011 (0.999-1.022)
Female	1.030 (1.009-1.052)	1.050 (1.020-1.080)	1.013 (1.000-1.027)
Age			
<40	1.030 (0.990-1.070)	1.084 (1.027-1.145)	0.998 (0.974-1.023)
40-64	1.025 (1.008-1.044)	1.058 (1.033-1.084)	1.013 (1.002-1.024)
>65	1.033 (1.012-1.055)	1.046 (1.017-1.076)	1.012 (0.999-1.025)
Education			
Primary and below	1.013 (0.991-1.036)	1.050 (1.019-1.081)	1.007 (0.993-1.021)
Junior high school	1.043 (1.021-1.065)	1.072 (1.041-1.104)	1.023 (1.009-1.037)
Senior high school and above	1.028 (1.006-1.049)	1.036 (1.007-1.066)	1.003 (0.990-1.016)
Stroke subtype			
ICH	1.021 (0.995-1.048)	1.058 (1.021-1.096)	1.008 (0.991-1.025)
CBI	1.029 (1.011-1.047)	1.050 (1.026-1.075)	1.012 (1.001-1.023)
Season			
Spring	1.031 (0.989-1.076)	1.034 (0.989-1.081)	1.012 (0.989-1.035)
Summer	1.031 (0.989-1.075)	0.992 (0.944-1.044)	1.015 (0.990-1.041)
Autumn	1.021 (0.993-1.050)	1.040 (0.994-1.089)	1.009 (0.992-1.025)
Winter	1.073	1.095	1.033

	(1.022-1.126)	(1.024-1.170)	(0.983-1.086)

Discussion

In this study, a positive association was found between the daily stroke hospitalizations and the concentration of ambient $PM_{2.5}$. Previous studies showed inconsistent results when exploring the short-term effect of $PM_{2.5}$ on stroke morbidity. Some studies observed a nonsignificant association, while other studies found a significant positive association.[10 19] For example, a 2014 meta-analysis of nonfatal strokes found an RR of 0.7-0.8% for short-term effects per 10 μg/m^3 $PM_{2.5}$.[20] Exposure to particulate matter increases inflammation, antioxidant activity and circulating blood platelet activation, and decreases vascular endothelial functions and enzyme activity, the latter of which may increase peripheral thrombosis and blood clotting.[21-23] Furthermore, compared with PM_{10}, $PM_{2.5}$ has a smaller size and larger surface area; it could carry more toxicity and harmful substances and penetrate deep into the human body,[24] finally induce a stroke.,

We found that daily stroke hospitalizations increased by 5.4% for every 10 μg/m^3 increase of NO_2. These results are similar to those of previous studies, but some of the previous studies obtained different effect estimates.[25-27] For example, a study in Wuhan, China showed a 2.9% increase in stroke hospitalizations per 10 μg/m^3 increase in NO_2 in the cold season, which was smaller than the result (9.5%) from our study.[28] Evidence has shown that NO_2 is related to plasma fibrinogen[29] and is also correlated with fine particulate matter, which leads to increased coagulability through the release of cytokines.[30] Gaseous NO_2 can also be mixed with water to form acid aerosols after inhalation, which damages health through a series of oxidation mechanisms.[31]

This study found that the harmful effect estimates for every 10 μg/m^3 increase of NO_2 was slightly higher than that of $PM_{2.5}$. Previous studies showed similar results;[14 32] gaseous pollutants appeared to have a stronger effect on stroke mortality,[33 34] and some studies indicated that, after adjusting for other pollutants, only NO_2 showed consistent results with a single-pollutant model.[35 36] Concern has been raised that the observed health effects associated with NO_2 might be a result of exposure to traffic-related emissions or fine particles.[37]

A weak positive association between O_3 and hospital admissions for stroke was found in our study. Previous studies exploring this relationship showed different results. For example,

a 10-year study conducted in France showed a significant positive relationship between O_3 exposure and ischemic stroke in men over 40 years of age, and the result was observed for a 1-day lag (OR 1.133, 95% CI 1.052 to 1.220).[38] In the United States, where air pollution levels are low, the incidence of CBI/TIA was also associated with O_3 exposure.[39] However, there was no statistically significant association found between O_3 pollution and the incidence of stroke in South Carolina and Nueces County, Texas.[40 41] Some studies even showed a significant or nonsignificant negative association between O_3 and CBI.[42 43] The mechanisms linking O_3 and stroke need to be further studied.

The effects of air pollution were found in both men and women; no clear trends were found across age groups and education levels. We found that the associations between air pollution and stroke morbidity had no statistically significant differences between different age groups. Similar results have been found in some previous studies.[14 44] Elderly people may spend more time indoors, and they are likely to wear masks when air pollution is severe, thus their individual exposure may be less.[45] On the other hand, Tian et al. found that the associations between air pollution and stroke morbidity were stronger in the elderly.[32] Therefore, the modification effect of age on the associations between air pollution and stroke morbidity needs further study. In addition, vascular risk factors, such as hypertension and hyperlipidemia, may also modify their associations, relevant researches need to be carried out in the future.

The seasonal analyses showed that statistically significant associations between ambient air pollutant concentrations and hospital admissions for stroke were only found in the winter; Dong et al. also found that the harmful effects of NO_2 and SO_2 were more robust in the cold season[14]. One possible reason for this result is that the air pollution concentrations in Shenzhen are higher in winter than in summer (Table S1). The substances carried by particles may vary between seasons, which would influence the effect. Furthermore, the interaction between air pollutants and temperature should be considered, and the strongest effect exists in winter and on low-temperature days.

Our study found that $PM_{2.5}$, NO_2 and O_3 were related to hospitalizations for CBI but found different results for ICH. Similarly, previous studies showed consistent associations between CBI and air pollution, while the association between ICH was more variable and had a higher imprecision.[46] One reason is that the prevalence of ICH was much lower than CBI. In our study, 77.8% of stroke hospitalizations were for CBI, and ICH only accounted for 17.0% of stroke hospitalizations. Therefore, the risk estimates for ICH were more imprecise, and the confidence interval of the RR for ICH was wider. Another reason may be

the differential mechanisms linking air pollution exposure to CBI and ICH. $PM_{2.5}$ exposure is conducive to atherosclerosis and the oxidative stress of the heart. These conditions may upregulate visfatin, reduce heart rate variability and induce CD36-dependent 7-ketocholesterol, which accumulate in macrophages[47-50]. All of these factors are more likely to induce CBI than ICH.

There are several limitations in this study. First, the air pollution data from fixed-site monitoring stations may not be representative of individual exposure. For example, the air pollution concentrations near the main roads with heavy traffic were often higher than that near the parks with few vehicles, and the air pollution monitoring stations were usually located far away from the main roads with heavy traffic. Second, it is difficult to completely eliminate the possibility of ecological fallacy in time-series studies, and we cannot provide absolute risks in this study. Moreover, associations between air pollution and stroke hospitalizations did not prove causality.

Conclusions

$PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, especially in the winter. All three air pollutants were associated with higher risks of hospital admission due to cerebral infarction, the most prevalent stroke subtype in the study location. The government should take actions to address air pollution issues and develop warning systems for sensitive populations during high-risk periods.

Contributors: The study was conceived and designed by Bao J, Peng J and Guo Y; Xie X and Guo Y conducted statistical analysis and drafted the manuscript; Lei L, Zhou H, and Deng S contributed to data collection and processing; Xu Y and Liu Z helped in study management and the interpretation of the results; Bao J, Peng J and Huang C reviewed the manuscript for important intellectual content; All authors read and approved the final version.

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Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2 and O_3 in the two-pollutant model, across lag 0-3 days

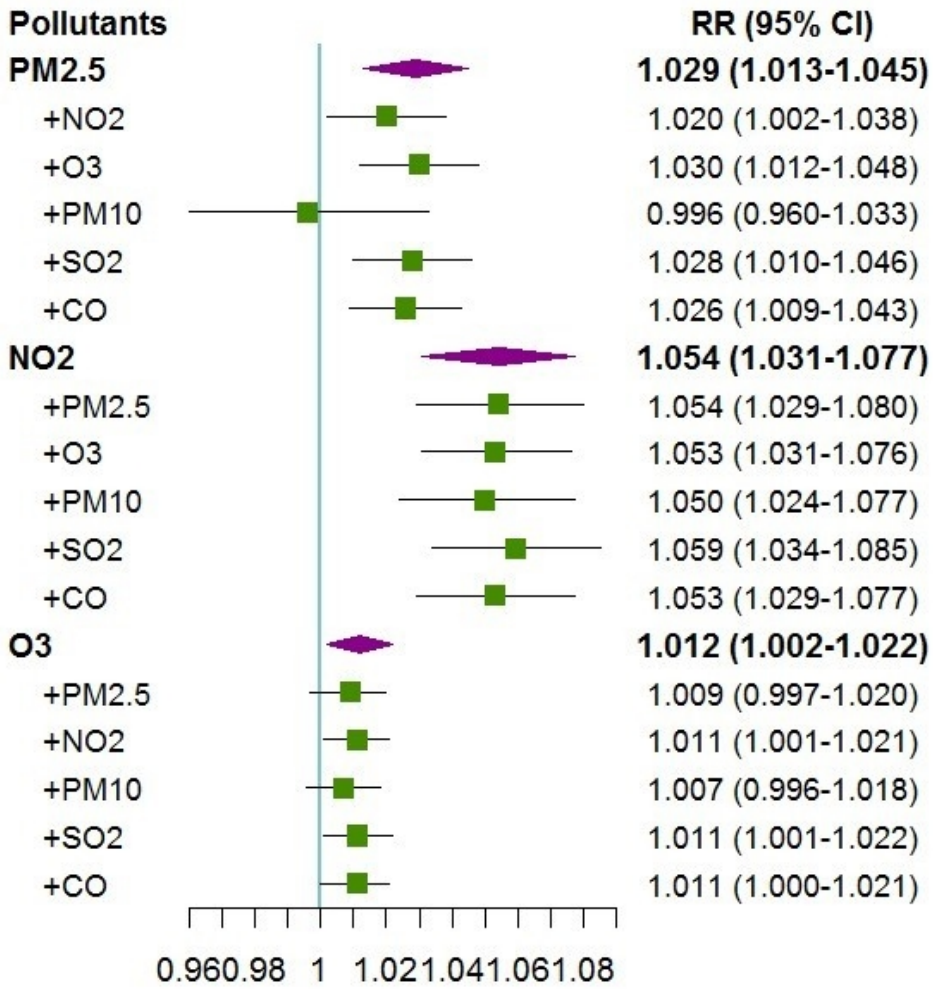

Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m3 increase in ambient PM2.5, NO2 and O3 in the two-pollutant model, across lag 0-3 days

153x160mm (96 x 96 DPI)

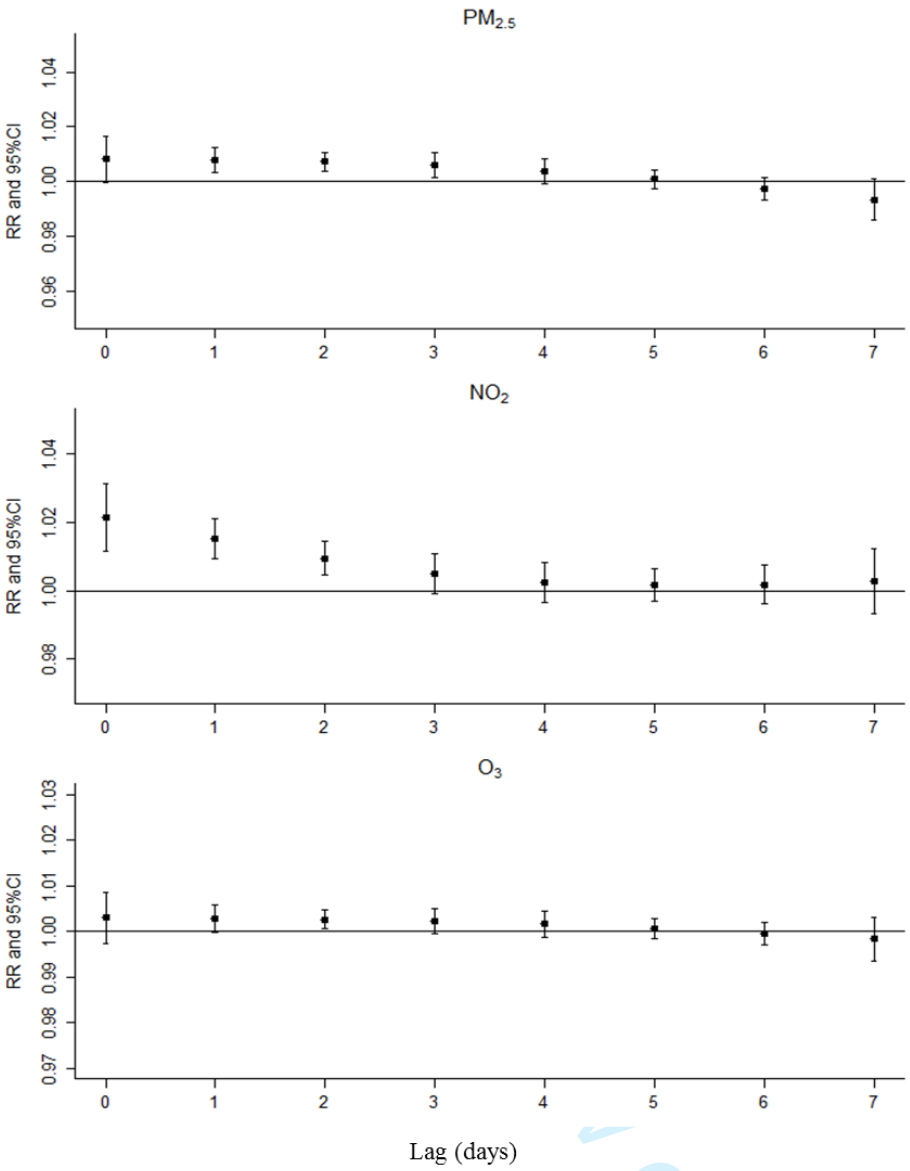

Figure S1. The distribution of lagged associations (RR, 95% CI) between air pollutants ($PM_{2.5}$, NO_2 and O_3) and stroke hospitalizations

Table S1. The distribution of daily air pollutant concentrations and temperature in four seasons of Shenzhen.

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Spring							
$PM_{2.5}$, μg/m^3	27.3	11.8	6.3	17.9	25.1	33.8	72.5
NO_2, μg/m^3	33.9	11.0	17.6	25.0	31.0	40.1	81.0
O_3, μg/m^3	53.7	22.8	17.0	37.0	47.2	68.9	130.7
Temperature	22.9	4.2	9.6	20.2	23.5	26.2	30.3
Summer							
$PM_{2.5}$, μg/m^3	18.4	10.3	7.1	11.4	14.7	18.4	59.5
NO_2, μg/m^3	28.7	8.4	12.0	22.4	27.5	33.4	61.2
O_3, μg/m^3	47.6	18.7	19.9	33.4	42.7	56.6	115.4
Temperature	29.1	1.3	25.0	28.17	29.4	30.0	33.0
Autumn							
$PM_{2.5}$, μg/m^3	33.2	14.8	8.5	22.5	31.2	43.0	84.0
NO_2, μg/m^3	32.3	9.1	11.3	25.3	30.9	38.4	60.0
O_3, μg/m^3	67.8	23.8	23.8	49.5	66.0	83.2	147.8
Temperature	25.5	3.2	14.5	24.2	26.1	27.9	30.7
Winter							
$PM_{2.5}$, μg/m^3	40.4	16.7	7.3	27.7	39.1	51.8	84.1
NO_2, μg/m^3	38.9	13.0	12.8	30.1	37.7	46.9	84.8
O_3, μg/m^3	58.3	17.1	26.6	44.6	56.4	70.2	113.0
Temperature	16.2	3.5	3.5	14.2	16.3	18.8	23.5

Table S2. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adjusting the df values of $TIME_t$ and TEM_t.

df of $TIME_t$	df of TEM_t	RR (95% CI)		
		$PM_{2.5}$	NO_2	O_3
5x3	2	1.023(1.007-1.039)	1.054(1.032-1.076)	1.004(0.994-1.014)
	3	1.022(1.006-1.038)	1.052(1.030-1.075)	1.005(0.995-1.015)
	4	1.022(1.006-1.039)	1.053(1.031-1.076)	1.004(0.995-1.015)
6x3	2	1.028(1.012-1.044)	1.053(1.031-1.075)	1.010(0.999-1.020)
	3	1.027(1.011-1.044)	1.054(1.031-1.076)	1.010(1.000-1.020)
	4	1.028(1.012-1.044)	1.054(1.032-1.077)	1.010(0.999-1.020)
7x3	2	1.029(1.013-1.046)	1.054(1.032-1.077)	1.011(1.001-1.021)
	3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
	4	1.029(1.013-1.046)	1.056(1.033-1.080)	1.011(1.001-1.022)

Table S3. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adopting different lag days.

Lag days	RR (95% CI)		
	$PM_{2.5}$	NO_2	O_3
1	1.023(1.009-1.037)	1.039(1.022-1.056)	1.009(0.999-1.017)
2	1.029(1.013-1.044)	1.052(1.032-1.072)	1.010(1.000-1.020)
3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
4	1.028(1.012-1.046)	1.050(1.025-1.075)	1.012(1.001-1.023)
5	1.028(1.010-1.046)	1.054(1.027-1.081)	1.010(0.999-1.021)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page/line
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2, 10-16
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2, 18-27
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4, 6-36
Objectives	3	State specific objectives, including any prespecified hypotheses	4, 38-43
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4, 51-60 5, 6-39
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	NA
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5, 43-55 5, 13-24
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5, 6-39
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	5, 6-14
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6, 3-43
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5, 43-60 6, 3-43
		(*b*) Describe any methods used to examine subgroups and interactions	6, 25-36
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	6, 35-43
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6, 58-60
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, 58-60 7, 3-22 Table 1, Table2
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10, 8-60 Table 4 18, 7-43 Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	10, 8-60 Table 4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 19, 20, 21 Figure S1, TableS1-S3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 11, 12, 13
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	13, 12-25
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13, 29-37
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study	13, 47-51

and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Short-term associations between ambient air pollution and stroke hospitalizations: A time-series study in Shenzhen, China

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Short-term associations between ambient air pollution and stroke hospitalizations: A time-series study in Shenzhen, China.

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Abstract

Objective: To investigate the association between ambient air pollution and stroke morbidity in different subgroups and seasons.

Methods: We performed a time-series analysis based on generalized linear models to study the short-term exposure-response relationships between air pollution and stroke hospitalizations, and conducted subgroup analyses to identify possible sensitive populations.

Results: For every 10 μg/m^3 increase in the concentration of air pollutant, across lag 0-3 days, the relative risk (RR) of stroke hospitalization was 1.029 (95% CI: 1.013-1.045) for $PM_{2.5}$, 1.054 (95% CI: 1.031-1.077) for NO_2, and 1.012 (1.002-1.022) for O_3. Subgroup analyses showed that statistically significant associations were found in both men and women, middle-aged and older populations, and both cerebral infarction and intracerebral hemorrhage. The seasonal analyses showed that statistically significant associations were only found in the winter.

Conclusions: Our study indicates that short-term exposure to $PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, and the government should take actions to mitigate air pollution and protect sensitive populations.

Keywords: Air pollution; Stroke morbidity; Time-series analysis; Season

Article Summary

Strengths and limitations of this study

► This study investigated the association between air pollution and subtypes of stroke morbidity.
► This study included more than 67,000 cases, ensuring high statistical power.
► The modification effects of different demographic characteristics and seasons were explored.
► Single-city study limited the generalization of the findings.
► Associations between air pollution and stroke hospitalizations did not prove causality.

Introduction

Stroke is one of the leading causes of disability-adjusted life years (DALYs) and mortality, accounting for approximately 132 million DALYs and 6.2 million deaths globally in 2017.[1 2] It is also the leading cause of long-term adult disability and leading cause of death in China; 20.19% of total deaths were caused by stroke in 2017,[3 4] and the age-standardized incidence and mortality rates were 246.8 and 114.8 per 100,000 person-years, respectively.[5] Well-known risk factors of stroke include history of hypertension, current smoking status, and alcohol intake.[6]

With the deterioration of global air quality, concerns have been raised about the relationship between ambient air pollution and stroke. According to the Global Burden of Disease, approximately 12.75% of stroke cases in China in 2017 were linked to air pollution.[3] Possible mechanisms linking ambient air pollution to stroke include inflammation, oxidative stress, atherosclerosis and autonomic dysregulation.[7] To date, most of the relevant epidemiological studies have focused on stroke mortality instead of morbidity,[8 9] but it should be noted that morbidity is more indicative of the early stages of stroke; early prevention of stroke has an important public health significance. Studies that examined the relationship between ambient air pollution and stroke morbidity obtained inconsistent results, and most of them studied the association over a full year but failed to consider the seasonal differences in pollutant concentration and their health effects.[10-12]

In this study, a time-series analysis was conducted to investigate the association between ambient air pollution and stroke morbidity in different seasons; we also researched possible sensitive populations by conducting subgroup analyses by sex, age group, education level and stroke subtypes.

Materials and Methods

Study area

Shenzhen is located within the Pearl River Delta, China, is one degree south of the Tropic of Cancer, and has a permanent population of approximately 12.5 million people.[13] Because of the Siberian anticyclone, it has a warm and humid subtropical climate. Six kinds of air pollutants, including ozone (O_3), fine particulate matter ($PM_{2.5}$), nitrogen dioxide (NO_2), inhalable particulate matter (PM_{10}), sulfur dioxide (SO_2) and carbon monoxide (CO), are monitored in Shenzhen, and the main ambient air pollutants are O_3, $PM_{2.5}$ and NO_2.

Data collection

Stroke data, containing 67,078 cases from Jan 1st 2014 to Dec 31st 2016, were collected from the Shenzhen Center for Chronic Disease Control. Sixty-nine hospitals from 10 administrative districts reported their stroke case information (including patient age and sex, clinical diagnosis and classification, onset date, diagnosis date, and major risk factors) to the Shenzhen Center for Chronic Disease Control. All the cases were coded according to the World Health Organization's International Classification of Diseases, 10th revision (ICD-10), ranging from I60-I64: (1) Subarachnoid hemorrhage (SAH), which was coded as I60; (2) intracerebral hemorrhage (ICH), which was coded as I61; (3) other nontraumatic intracranial hemorrhage (ONIH), which was coded as I62; (4) cerebral infarction (CBI), which was coded as I63; (5) stroke not specified as hemorrhage or infarction (SNSHI), which was coded as I64. All cases were diagnosed in the hospital with computed tomography, magnetic resonance imaging or neurological examination. In the subgroup analysis for stroke subtypes, we focused on ICH and CBI, which accounted for 94.8% of all stroke cases.

Hourly concentrations of O_3, $PM_{2.5}$ and NO_2 were obtained from the National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035/). The daily mean concentrations for each air pollutant were averaged from all the 11 fixed-site air pollution monitoring stations in Shenzhen.[14] Daily meteorological data were obtained from the National Meteorological Data Sharing Platform (http://data.cma.cn/), including the daily mean, maximum and minimum temperatures, the mean relative humidity, the mean wind speed, and the mean atmospheric pressure.

Statistical modeling

This time-series analysis adopted generalized linear Poisson models (GLMs) to estimate the association between air pollutants and stroke hospitalizations.[15] The daily mean concentrations of air pollutant ($PM_{2.5}$, NO_2, or O_3) were the independent variables, and the number of daily hospital admissions for stroke was the dependent variable. Meteorological variables (i.e. temperature, relative humidity and air pressure), long-term trends and seasonal patterns, as well as day of the week (DOW) were used as adjustment variables. The influence of other pollutants was examined in two-pollutant models.

Considering the possible delayed and/or cumulative effects of air pollution on stroke morbidity, we created cross-basis matrix for air pollutants, within the framework of distributed lag linear model (DLM).[16] The selection of lag days was justified by the polynomial lag models.

The model to represent the relationship between the daily mean concentration of air pollutants and hospital admissions for stroke is as follows:

$$\log\left[\mathrm{E}(Y_t)\right] = \alpha + \beta POL_{t,l} + ns(TEM_t, df = 3) + ns(RH_t, df = 3) + ns(PRE_t, df = 3) + ns(TIME_t, df = 7/\text{year} * No.\ of\ years) + \gamma DOW_t$$

Here, $\mathrm{E}(Y_t)$ indicates the expected number of hospital admissions for stroke on day t; $POL_{t,l}$ is the cross-basis matrix of the air pollutants ($PM_{2.5}$, NO_2, or O_3), with β as the corresponding vector of coefficients; and $ns()$ is the natural cubic spline function. DOW_t is a dummy variable for the day of week. We used 7 degrees of freedom (df) per year for the time trend ($TIME_t$), 3 df for the previous 14 days' moving average temperature (TEM_t),[17] and 3 df for both the relative humidity (RH_t) and the air pressure (PRE_t) on day t; the choice of df for each variable was based on the Akaike Information Criterion for quasi-Poisson models.

Subgroup analyses were conducted for different sexes, age groups (<40, 40-64, ≥65), education levels (primary school or below, junior high school, high school or above) and stroke subtypes (CBI, ICH). When comparing seasonal differences, we defined spring as the period from Mar 1st to May 31st, summer as the period from Jun 1st to Aug 31st, autumn as the period from Sept 1st to Nov 30th, and winter as the period from Dec 1st to Feb 28th of the next year. The differences among the effect estimates between different subgroups were tested via the Z-test.[18] Sensitivity analyses were performed by adjusting the df values of $TIME_t$ and TEM_t, as well as the number of lags considered for the air pollutant. All results were reported as relative risks (RRs) and 95% confidence intervals (CIs) of hospital admissions for stroke for every 10 μg/m^3 increase in air pollutants exposure, and two-tailed $p < 0.05$ were considered statistically significant. Main analyses were performed using the "dlnm" and "splines" packages in R version 3.5.1.

Patient and Public Involvement

This study belongs to ecological research and does not reveal any personal information; patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research. Therefore, patients did not have to be asked for informed consent.

Results

Data description

In total, during 2014-2016, 67,078 hospital admissions for stroke were recorded, of which 77.8% were first-time cases, and 61.9% were men. Patients aged 40-64 years accounted for 49.3% of the total, and 44.7% were older (age≥65 years old). In total, 27.7% of the cases had an education level of primary school or below, 39.8% completed junior high school, and 32.5% had a high school education or higher. Among the subtypes of stroke, CBI and ICH accounted for 52,168 (77.8%) and 11,415 (17.0%), respectively. The other three subtypes accounted for approximately 5% of the total (Table 1).

On average, there were approximately 61 hospital admissions for stroke per day during our study period, and the 24-hour mean concentrations of $PM_{2.5}$, NO_2 and O_3 were 30.1 μg/m^3, 33.2 μg/m^3 and 56.9 μg/m^3, respectively. The daily mean temperature was 23.5 °C, the mean relative humidity was 74.7%, and the daily mean air pressure was 1005.7 hPa (Table 2). In general, the pollutant concentrations of $PM_{2.5}$ and NO_2 were higher in winter than in other seasons (Table S1). Correlations between air pollutants and meteorological factors are in Table 3.

Table 1. Summary statistics of stroke hospitalizations in Shenzhen

Variables	Number	Percentage (%)
Stroke cases	67,078	100.0
Sex		
Male	41,546	61.9
Female	25,526	38.1
Age (years)		
<40	4,051	6.0
40-64	33,053	49.3
≥65	29,968	44.7
Education		
Primary school or below	18,558	27.7
Junior high school	26,686	39.8
High school or above	21,792	32.5
Stroke subtypes		
CBI	52,168	77.8
ICH	11,415	17.0
SAH	2,369	3.5
SNSHI	219	0.3
ONIH	783	1.2

Table 2. Distribution of daily air pollution concentrations, meteorological factors and stroke hospitalizations in Shenzhen

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Air Pollutants							
$PM_{2.5}$, μg/m^3	30.1	16	6.3	16.8	27	39.9	96.1
NO_2, μg/m^3	33.2	11.1	11.3	25	31	39.5	84.8
O_3, μg/m^3	56.9	22.1	16.9	38.8	52.9	71.9	147.8
Meteorological Factors							
Temperature, ℃	23.5	5.7	3.5	19.0	25.1	28.2	33.0
Relative humidity, %	74.7	12.2	19.0	69.0	76.0	83.0	99.0
Air pressure, hPa	1005.6	6.6	986.8	1000.7	1005.3	1010.6	1027.3
Stroke cases							
All strokes	61.2	15.7	23.0	51.0	59.0	70.0	182.0
Type							
ICH	10.4	4.2	1.0	7.0	10.0	13.0	40.0
CBI	47.6	13.1	17.0	39.0	46.0	54.0	143.0
Sex							
Male	37.9	10.2	15.0	31.0	37.0	43.0	113.0
Female	23.3	7.4	7.0	18.0	22.0	27.0	77.0
Age (years old)							
<40	3.7	2.1	0.0	2.0	4.0	5.0	12.0
40-64	30.2	8.4	9.0	24.0	30.0	35.0	90.0
≥65	27.3	8.7	9.0	21.0	26.0	32.0	101.0
Education							
Primary school or below	16.9	5.4	3.0	13.0	16.0	20.0	51.0
Junior high school	24.4	7.9	7.0	19.0	23.0	29.0	85.0
High school or above	19.9	7.6	5.0	14.0	19.0	24.0	67.0

Table 3. Spearman's rank coefficients between daily mean air pollutants and meteorological variables

Variables	$PM_{2.5}$	NO_2	O_3	Temperature	Relative humidity	Air pressure
$PM_{2.5}$	1.00					
NO_2	0.55*	1.00				
O_3	0.56*	0.02	1.00			
Temperature	-0.43*	-0.27*	-0.09*	1.00		
Relative humidity	-0.53*	-0.1*	-0.51*	0.27*	1.00	
Air pressure	0.50*	0.26*	0.21*	-0.85*	-0.45*	1.00

*p<0.05.

Regression analysis

When the lag length was set as 7 days, the results showed that the lag effect mainly lasted for 3 days (Figure S1). Therefore, we used 3 days as the lag length in later analyses. Positive associations and lag effects were observed between stroke morbidity and air pollution. The cumulative effect of a 10 μg/m^3 increase in $PM_{2.5}$ concentrations across lag 0-3 days corresponded to a 2.9% (95% CI: 1.3%-4.5%) increase in daily stroke hospitalizations; it was a 5.3% (3.1%-7.7%) increase in the case of NO_2, and a 1.2% (0.2%–2.2%) increase for O_3.

Table 4 showed that, for $PM_{2.5}$, an association was found in both men and women, populations older than 40 years old, and people with education levels of junior high school and above, and the CBI stroke subtype. For NO_2, the association was consistently significant in both men and women, populations in all age groups and all education levels, and subtypes CBI and ICH. For O_3, the association was significant in women, the middle-aged population, populations with an education level of junior high school and the CBI stroke subtype. The associations mainly occurred in the winter. Comparing the effect estimates between different subgroups, we found none of the differences to be statistically significant.

For the two-pollutant model (Figure 1), the results did not vary substantially compared to those in the single-pollutant models, especially for NO_2. The results were stable after adjusting the df values of $TIME_t$ and TEM_t, as well as the number of lags considered for the air pollutant in the model, indicating the stability of the estimated effects (Table S2-S3).

Table 4. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2, and O_3 in the single-pollutant model, across lag 0-3 days.

Subgroups	RR(95% CI)		
	$PM_{2.5}$	NO_2	O_3
All strokes	1.029 (1.013-1.045)	1.054 (1.031-1.077)	1.012 (1.002-1.022)
Sex			
Male	1.029 (1.011-1.046)	1.056 (1.032-1.080)	1.011 (0.999-1.022)
Female	1.030 (1.009-1.052)	1.050 (1.020-1.080)	1.013 (1.000-1.027)
Age			
<40	1.030 (0.990-1.070)	1.084 (1.027-1.145)	0.998 (0.974-1.023)
40-64	1.025 (1.008-1.044)	1.058 (1.033-1.084)	1.013 (1.002-1.024)
>65	1.033 (1.012-1.055)	1.046 (1.017-1.076)	1.012 (0.999-1.025)
Education			
Primary and below	1.013 (0.991-1.036)	1.050 (1.019-1.081)	1.007 (0.993-1.021)
Junior high school	1.043 (1.021-1.065)	1.072 (1.041-1.104)	1.023 (1.009-1.037)
Senior high school and above	1.028 (1.006-1.049)	1.036 (1.007-1.066)	1.003 (0.990-1.016)
Stroke subtype			
ICH	1.021 (0.995-1.048)	1.058 (1.021-1.096)	1.008 (0.991-1.025)
CBI	1.029 (1.011-1.047)	1.050 (1.026-1.075)	1.012 (1.001-1.023)
Season			
Spring	1.031 (0.989-1.076)	1.034 (0.989-1.081)	1.012 (0.989-1.035)
Summer	1.031 (0.989-1.075)	0.992 (0.944-1.044)	1.015 (0.990-1.041)
Autumn	1.021 (0.993-1.050)	1.040 (0.994-1.089)	1.009 (0.992-1.025)
Winter	1.073 (1.022-1.126)	1.095 (1.024-1.170)	1.033 (0.983-1.086)

Discussion

In this study, a positive association was found between the daily stroke hospitalizations and the concentration of ambient $PM_{2.5}$. Previous studies showed inconsistent results when exploring the short-term associations between $PM_{2.5}$ and stroke morbidity. Some studies observed a nonsignificant association, while other studies found a significant positive association.[10 19] For example, a 2014 meta-analysis of nonfatal strokes found an RR of 0.7-0.8% for short-term effects per 10 μg/m^3 $PM_{2.5}$.[20] Exposure to particulate matter increases inflammation, antioxidant activity and circulating blood platelet activation, and decreases vascular endothelial functions and enzyme activity, the latter of which may increase peripheral thrombosis and blood clotting.[21-23] Furthermore, compared with PM_{10}, $PM_{2.5}$ has a smaller size and larger surface area and can carry more harmful substances and penetrate deep into the human body,[24] finally induce a stroke.

We found that daily stroke hospitalizations increased by 5.4% for every 10 μg/m^3 increase of NO_2. These results are similar to those of previous studies, but some of the previous studies obtained different effect estimates.[25-27] For example, a study in Wuhan, China showed a 2.9% increase in stroke hospitalizations per 10 μg/m^3 increase in NO_2 in the cold season, which was smaller than the result (9.5%) from our study.[28] Evidence has shown that NO_2 is related to plasma fibrinogen[29] and is also correlated with fine particulate matter, which leads to increased coagulability through the release of cytokines.[30] Gaseous NO_2 can also be mixed with water to form acid aerosols after inhalation, which damages health through a series of oxidation mechanisms.[31]

The study found that the relative risk of stroke associated with an increase in the level of air pollution from the first to the third quartile was slightly higher for NO_2 than for $PM_{2.5}$. Previous studies showed similar results;[14 32] gaseous pollutants appeared to have a stronger effect on stroke mortality,[33 34] and some studies indicated that, after adjusting for other pollutants, only NO_2 showed consistent results with a single-pollutant model.[35 36] Concern has been raised that the observed health effects associated with NO_2 might be a result of exposure to traffic-related emissions or fine particles.[37]

A weak positive association between O_3 and hospital admissions for stroke was found in our study. Previous studies exploring this relationship showed different results. For example, a 10-year study conducted in France showed a significant positive relationship between O_3 exposure and ischemic stroke in men over 40 years of age, and the result was observed for a 1-day lag (OR 1.133, 95% CI 1.052 to 1.220).[38] In the United States, where air pollution

levels are low, the incidence of CBI/TIA was also associated with O_3 exposure.[39] However, there was no statistically significant association found between O_3 pollution and the incidence of stroke in South Carolina and Nueces County, Texas.[40 41] Some studies even showed a significant or nonsignificant negative association between O_3 and CBI.[42 43] The mechanisms linking O_3 and stroke need to be further studied.

The associations were found in both men and women; no clear trends were found across age groups and education levels. We found that the associations between air pollution and stroke morbidity had no statistically significant differences between different age groups. Similar results have been found in some previous studies.[14 44] Elderly people may spend more time indoors, and they are likely to wear masks when air pollution is severe, thus their individual exposure may be less.[45] On the other hand, Tian et al. found that the associations between air pollution and stroke morbidity were stronger in the elderly.[32] Therefore, the modification effect of age on the associations between air pollution and stroke morbidity needs further study. In addition, vascular risk factors, such as hypertension and hyperlipidemia, may also modify these associations, which needs to be addressed in future research.

The seasonal analyses showed that statistically significant associations between ambient air pollutant concentrations and hospital admissions for stroke were only found in the winter; Dong et al. also found that the harmful effects of NO_2 and SO_2 were more robust in the cold season[14]. One possible reason for this result is that the air pollution concentrations in Shenzhen are higher in winter than in summer (Table S1). The substances carried by particles may vary between seasons, which would influence the effect.

Our study found that $PM_{2.5}$, NO_2 and O_3 were related to hospitalizations for CBI but found different results for ICH. Similarly, previous studies showed consistent associations between CBI and air pollution, while the association between ICH was more variable and had a higher imprecision.[46] One reason is that the prevalence of ICH was much lower than CBI. In our study, 77.8% of stroke hospitalizations were for CBI, and ICH only accounted for 17.0% of stroke hospitalizations. Therefore, the risk estimates for ICH were more imprecise, and the confidence interval of the RR for ICH was wider. Another reason may be the differential mechanisms linking air pollution exposure to CBI and ICH. $PM_{2.5}$ exposure is conducive to atherosclerosis and the oxidative stress of the heart. These conditions may upregulate visfatin, reduce heart rate variability and induce CD36-dependent 7-ketocholesterol, which accumulate in macrophages[47-50]. All of these factors are more likely to induce CBI than ICH.

There are several limitations in this study. First, the air pollution data from fixed-site monitoring stations may not be representative of average population exposure. For example, the air pollution concentrations near the main roads with heavy traffic were often higher than those near the parks with few vehicles, and the air pollution monitoring stations were usually located far away from the main roads with heavy traffic. Second, it is difficult to completely eliminate the possibility of ecological fallacy in time-series studies, and we cannot provide absolute risks in this study. Moreover, the associations observed in the present study cannot prove causality.

Conclusions

$PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, especially in the winter. All three air pollutants were associated with higher risks of hospital admission due to cerebral infarction, the most prevalent stroke subtype in the study location. The government should take actions to address air pollution issues and develop warning systems for sensitive populations during high-risk periods.

Contributors: The study was conceived and designed by Bao J, Peng J and Guo Y; Xie X and Guo Y conducted statistical analysis and drafted the manuscript; Lei L, Zhou H, and Deng S contributed to data collection and processing; Xu Y and Liu Z helped in study management and the interpretation of the results; Bao J, Peng J and Huang C reviewed the manuscript for important intellectual content; All authors read and approved the final version.

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Patient consent: Not required.

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Data sharing statement: No additional data are available.

Conflicts of Interest: The authors declare no conflict of interest.

Ethics approval： The institutional review board at the School of Public Health, Sun Yat-sen University, approved the study protocol (No. 2019-029) with a waiver of informed consent. Data were analyzed at the aggregate level. All patients were anonymous, and no patient privacy were revealed.

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Figure legend/caption：

Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2 and O_3 in the two-pollutant model, across lag 0-3 days

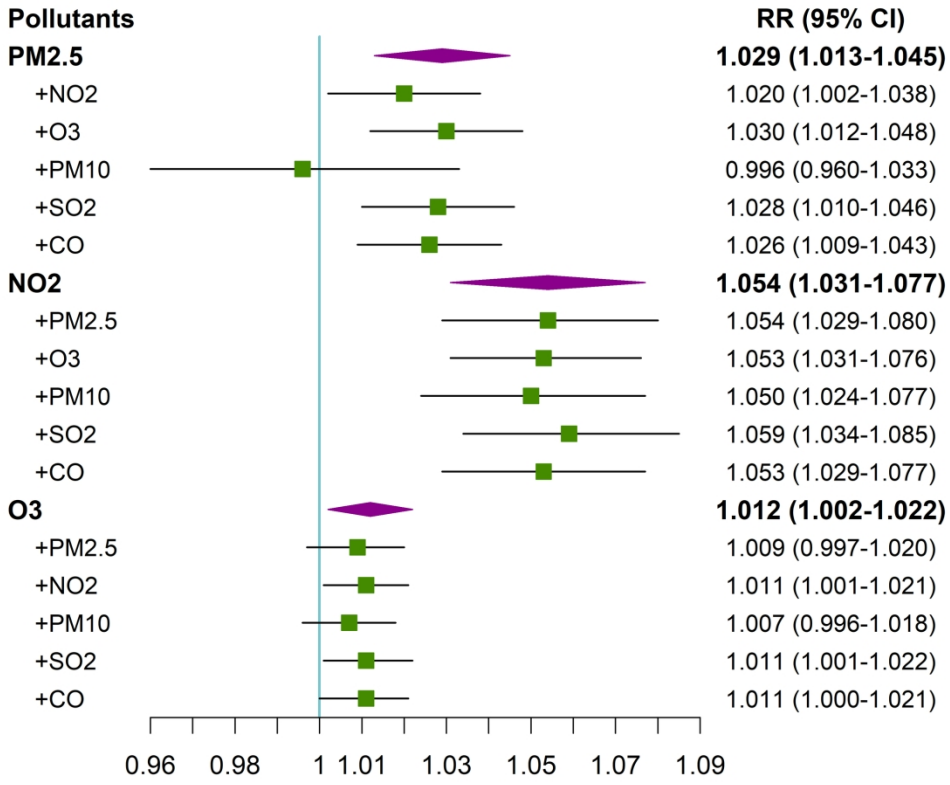

Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m3 increase in ambient PM2.5, NO2 and O3 in the two-pollutant model, across lag 0-3 days

193x233mm (300 x 300 DPI)

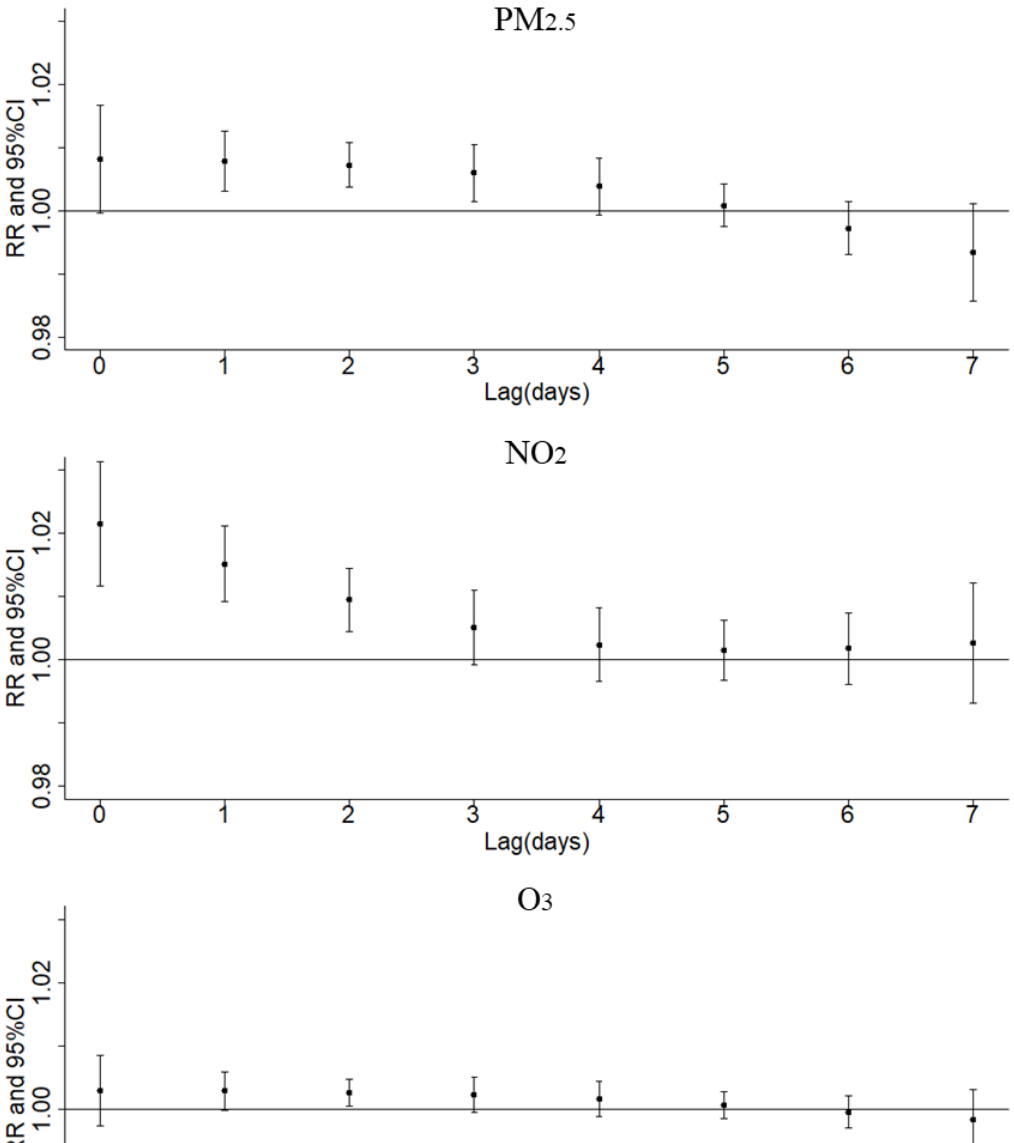

Figure S1. The distribution of lagged associations (RR, 95% CI) between air pollutants ($PM_{2.5}$, NO_2 and O_3) and stroke hospitalizations

Table S1. The distribution of daily air pollutant concentrations and temperature in four seasons of Shenzhen.

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Spring							
$PM_{2.5}$, μg/m^3	27.3	11.8	6.3	17.9	25.1	33.8	72.5
NO_2, μg/m^3	33.9	11.0	17.6	25.0	31.0	40.1	81.0
O_3, μg/m^3	53.7	22.8	17.0	37.0	47.2	68.9	130.7
Temperature	22.9	4.2	9.6	20.2	23.5	26.2	30.3
Summer							
$PM_{2.5}$, μg/m^3	18.4	10.3	7.1	11.4	14.7	18.4	59.5
NO_2, μg/m^3	28.7	8.4	12.0	22.4	27.5	33.4	61.2
O_3, μg/m^3	47.6	18.7	19.9	33.4	42.7	56.6	115.4
Temperature	29.1	1.3	25.0	28.17	29.4	30.0	33.0
Autumn							
$PM_{2.5}$, μg/m^3	33.2	14.8	8.5	22.5	31.2	43.0	84.0
NO_2, μg/m^3	32.3	9.1	11.3	25.3	30.9	38.4	60.0
O_3, μg/m^3	67.8	23.8	23.8	49.5	66.0	83.2	147.8
Temperature	25.5	3.2	14.5	24.2	26.1	27.9	30.7
Winter							
$PM_{2.5}$, μg/m^3	40.4	16.7	7.3	27.7	39.1	51.8	84.1
NO_2, μg/m^3	38.9	13.0	12.8	30.1	37.7	46.9	84.8
O_3, μg/m^3	58.3	17.1	26.6	44.6	56.4	70.2	113.0
Temperature	16.2	3.5	3.5	14.2	16.3	18.8	23.5

Table S2. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adjusting the df values of $TIME_t$ and TEM_t.

df of $TIME_t$	df of TEM_t	RR (95% CI)		
		$PM_{2.5}$	NO_2	O_3
5x3	2	1.023(1.007-1.039)	1.054(1.032-1.076)	1.004(0.994-1.014)
	3	1.022(1.006-1.038)	1.052(1.030-1.075)	1.005(0.995-1.015)
	4	1.022(1.006-1.039)	1.053(1.031-1.076)	1.004(0.995-1.015)
6x3	2	1.028(1.012-1.044)	1.053(1.031-1.075)	1.010(0.999-1.020)
	3	1.027(1.011-1.044)	1.054(1.031-1.076)	1.010(1.000-1.020)
	4	1.028(1.012-1.044)	1.054(1.032-1.077)	1.010(0.999-1.020)
7x3	2	1.029(1.013-1.046)	1.054(1.032-1.077)	1.011(1.001-1.021)
	3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
	4	1.029(1.013-1.046)	1.056(1.033-1.080)	1.011(1.001-1.022)

Table S3. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adopting different lag days.

Lag days	RR (95% CI)		
	$PM_{2.5}$	NO_2	O_3
1	1.023(1.009-1.037)	1.039(1.022-1.056)	1.009(0.999-1.017)
2	1.029(1.013-1.044)	1.052(1.032-1.072)	1.010(1.000-1.020)
3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
4	1.028(1.012-1.046)	1.050(1.025-1.075)	1.012(1.001-1.023)
5	1.028(1.010-1.046)	1.054(1.027-1.081)	1.010(0.999-1.021)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page/line
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3, 6-16
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3, 18-29
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6-36
Objectives	3	State specific objectives, including any prespecified hypotheses	5, 38-43
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5, 51-60 6, 6-39
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	NA
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 43-55 6, 13-24
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 6-60 7, 3-45
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	6, 6-27
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7, 3-44
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6, 43-60 7, 3-44
		(*b*) Describe any methods used to examine subgroups and interactions	7, 25-36
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7, 36-43

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8, 3-14
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 3-26 Table 1, Table2
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 30-60 Table 4 Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	11, 30-60 Table 4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 24, 25, 26 Figure S1, TableS1-S3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 13, 14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15, 3-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15, 21-28
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15, 39-42

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Short-term associations between ambient air pollution and stroke hospitalizations: A time-series study in Shenzhen, China

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Short-term associations between ambient air pollution and stroke hospitalizations: A time-series study in Shenzhen, China.

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Abstract

Objective: To investigate the association between ambient air pollution and stroke morbidity in different subgroups and seasons.

Methods: We performed a time-series analysis based on generalized linear models to study the short-term exposure-response relationships between air pollution and stroke hospitalizations, and conducted subgroup analyses to identify possible sensitive populations.

Results: For every 10 μg/m^3 increase in the concentration of air pollutant, across lag 0-3 days, the relative risk (RR) of stroke hospitalization was 1.029 (95% CI: 1.013-1.045) for $PM_{2.5}$, 1.054 (95% CI: 1.031-1.077) for NO_2, and 1.012 (1.002-1.022) for O_3. Subgroup analyses showed that statistically significant associations were found in both men and women, middle-aged and older populations, and both cerebral infarction and intracerebral hemorrhage. The seasonal analyses showed that statistically significant associations were only found in the winter.

Conclusions: Our study indicates that short-term exposure to $PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, and the government should take actions to mitigate air pollution and protect sensitive populations.

Keywords: Air pollution; Stroke morbidity; Time-series analysis; Season

Article Summary

Strengths and limitations of this study

► This study investigated the association between air pollution and subtypes of stroke morbidity.
► This study included more than 67,000 cases, ensuring high statistical power.
► The modification effects of different demographic characteristics and seasons were explored.
► Single-city study limited the generalization of the findings.
► Associations between air pollution and stroke hospitalizations did not prove causality.

Introduction

Stroke is one of the leading causes of disability-adjusted life years (DALYs) and mortality, accounting for approximately 132 million DALYs and 6.2 million deaths globally in 2017.[1 2] It is also the leading cause of long-term adult disability and leading cause of death in China; 20.19% of total deaths were caused by stroke in 2017,[3 4] and the age-standardized incidence and mortality rates were 246.8 and 114.8 per 100,000 person-years, respectively.[5] Well-known risk factors of stroke include history of hypertension, current smoking status, and alcohol intake.[6]

With the deterioration of global air quality, concerns have been raised about the relationship between ambient air pollution and stroke. According to the Global Burden of Disease, approximately 12.75% of stroke cases in China in 2017 were linked to air pollution.[3] Possible mechanisms linking ambient air pollution to stroke include inflammation, oxidative stress, atherosclerosis and autonomic dysregulation.[7] To date, most of the relevant epidemiological studies have focused on stroke mortality instead of morbidity,[8 9] but it should be noted that morbidity is more indicative of the early stages of stroke; early prevention of stroke has an important public health significance. Studies that examined the relationship between ambient air pollution and stroke morbidity obtained inconsistent results, and most of them studied the association over a full year but failed to consider the seasonal differences in pollutant concentration and their health effects.[10-12]

In this study, a time-series analysis was conducted to investigate the association between ambient air pollution and stroke morbidity in different seasons; we also researched possible sensitive populations by conducting subgroup analyses by sex, age group, education level and stroke subtypes.

Materials and Methods

Study area

Shenzhen is located within the Pearl River Delta, China, is one degree south of the Tropic of Cancer, and has a permanent population of approximately 12.5 million people.[13] Because of the Siberian anticyclone, it has a warm and humid subtropical climate. Six kinds of air pollutants, including ozone (O_3), fine particulate matter ($PM_{2.5}$), nitrogen dioxide (NO_2), inhalable particulate matter (PM_{10}), sulfur dioxide (SO_2) and carbon monoxide (CO), are monitored in Shenzhen, and the main ambient air pollutants are O_3, $PM_{2.5}$ and NO_2.

Data collection

Stroke data, containing 67,078 cases from Jan 1st 2014 to Dec 31st 2016, were collected from the Shenzhen Center for Chronic Disease Control. Sixty-nine hospitals from 10 administrative districts reported their stroke case information (including patient age and sex, clinical diagnosis and classification, onset date, diagnosis date, and major risk factors) to the Shenzhen Center for Chronic Disease Control. All the cases were coded according to the World Health Organization's International Classification of Diseases, 10th revision (ICD-10), ranging from I60-I64: (1) Subarachnoid hemorrhage (SAH), which was coded as I60; (2) intracerebral hemorrhage (ICH), which was coded as I61; (3) other nontraumatic intracranial hemorrhage (ONIH), which was coded as I62; (4) cerebral infarction (CBI), which was coded as I63; (5) stroke not specified as hemorrhage or infarction (SNSHI), which was coded as I64. All cases were diagnosed in the hospital with computed tomography, magnetic resonance imaging or neurological examination. In the subgroup analysis for stroke subtypes, we focused on ICH and CBI, which accounted for 94.8% of all stroke cases.

Hourly concentrations of O_3, $PM_{2.5}$ and NO_2 were obtained from the National Urban Air Quality Real-time Publishing Platform (http://106.37.208.233:20035/). The daily mean concentrations for each air pollutant were averaged from all the 11 fixed-site air pollution monitoring stations in Shenzhen.[14] Daily meteorological data were obtained from the National Meteorological Data Sharing Platform (http://data.cma.cn/), including the daily mean, maximum and minimum temperatures, the mean relative humidity, the mean wind speed, and the mean atmospheric pressure.

Statistical modeling

This time-series analysis adopted generalized linear Poisson models (GLMs) to estimate the association between air pollutants and stroke hospitalizations.[15] The daily mean concentrations of air pollutant ($PM_{2.5}$, NO_2, or O_3) were the independent variables, and the number of daily hospital admissions for stroke was the dependent variable. Meteorological variables (i.e. temperature, relative humidity and air pressure), long-term trends and seasonal patterns, as well as day of the week (DOW) were used as adjustment variables. The influence of other pollutants was examined in two-pollutant models.

Considering the possible delayed and/or cumulative effects of air pollution on stroke morbidity, we created cross-basis matrix for air pollutants, within the framework of distributed lag linear model (DLM).[16] The selection of lag days was justified by the polynomial lag models.

The model to represent the relationship between the daily mean concentration of air pollutants and hospital admissions for stroke is as follows:

$$\log\left[\mathrm{E}(Y_t)\right] = \alpha + \beta POL_{t,l} + ns(TEM_t, df = 3) + ns(RH_t, df = 3) + ns(PRE_t, df = 3) + ns(TIME_t, df = 7/\text{year} * No.\ of\ years) + \gamma DOW_t$$

Here, $\mathrm{E}(Y_t)$ indicates the expected number of hospital admissions for stroke on day t; $POL_{t,l}$ is the cross-basis matrix of the air pollutants ($PM_{2.5}$, NO_2, or O_3), with β as the corresponding vector of coefficients; and $ns()$ is the natural cubic spline function. DOW_t is a dummy variable for the day of week. We used 7 degrees of freedom (df) per year for the time trend ($TIME_t$), 3 df for the previous 14 days' moving average temperature (TEM_t),[17 18] and 3 df for both the relative humidity (RH_t) and the air pressure (PRE_t) on day t; the choice of df for each variable was based on the Akaike Information Criterion for quasi-Poisson models.

Subgroup analyses were conducted for different sexes, age groups (<40, 40-64, ≥65), education levels (primary school or below, junior high school, high school or above) and stroke subtypes (CBI, ICH). When comparing seasonal differences, we defined spring as the period from Mar 1st to May 31st, summer as the period from Jun 1st to Aug 31st, autumn as the period from Sept 1st to Nov 30th, and winter as the period from Dec 1st to Feb 28th of the next year. The differences among the effect estimates between different subgroups were tested via the Z-test.[19] Sensitivity analyses were performed by adjusting the df values of $TIME_t$ and TEM_t, as well as the number of lags considered for the air pollutant. All results were reported as relative risks (RRs) and 95% confidence intervals (CIs) of hospital admissions for stroke for every 10 μg/m^3 increase in air pollutants exposure, and two-tailed $p < 0.05$ were considered statistically significant. Main analyses were performed using the "dlnm" and "splines" packages in R version 3.5.1.

Patient and Public Involvement

This study belongs to ecological research and does not reveal any personal information; patients or the public were not involved in the design, or conduct, or reporting, or dissemination of our research. Therefore, patients did not have to be asked for informed consent.

Results

Data description

In total, during 2014-2016, 67,078 hospital admissions for stroke were recorded, of which 77.8% were first-time cases, and 61.9% were men. Patients aged 40-64 years accounted for 49.3% of the total, and 44.7% were older (age≥65 years old). In total, 27.7% of the cases had an education level of primary school or below, 39.8% completed junior high school, and 32.5% had a high school education or higher. Among the subtypes of stroke, CBI and ICH accounted for 52,168 (77.8%) and 11,415 (17.0%), respectively. The other three subtypes accounted for approximately 5% of the total (Table 1).

On average, there were approximately 61 hospital admissions for stroke per day during our study period, and the 24-hour mean concentrations of $PM_{2.5}$, NO_2 and O_3 were 30.1 μg/m^3, 33.2 μg/m^3 and 56.9 μg/m^3, respectively. The daily mean temperature was 23.5 °C, the mean relative humidity was 74.7%, and the daily mean air pressure was 1005.7 hPa (Table 2). In general, the pollutant concentrations of $PM_{2.5}$ and NO_2 were higher in winter than in other seasons (Table S1). Correlations between air pollutants and meteorological factors are in Table 3.

Table 1. Summary statistics of stroke hospitalizations in Shenzhen

Variables	Number	Percentage (%)
Stroke cases	67,078	100.0
Sex		
Male	41,546	61.9
Female	25,526	38.1
Age (years)		
<40	4,051	6.0
40-64	33,053	49.3
≥65	29,968	44.7
Education		
Primary school or below	18,558	27.7
Junior high school	26,686	39.8
High school or above	21,792	32.5
Stroke subtypes		
CBI	52,168	77.8
ICH	11,415	17.0
SAH	2,369	3.5
SNSHI	219	0.3
ONIH	783	1.2

Table 2. Distribution of daily air pollution concentrations, meteorological factors and stroke hospitalizations in Shenzhen

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Air Pollutants							
$PM_{2.5}$, μg/m^3	30.1	16	6.3	16.8	27	39.9	96.1
NO_2, μg/m^3	33.2	11.1	11.3	25	31	39.5	84.8
O_3, μg/m^3	56.9	22.1	16.9	38.8	52.9	71.9	147.8
Meteorological Factors							
Temperature, ℃	23.5	5.7	3.5	19.0	25.1	28.2	33.0
Relative humidity, %	74.7	12.2	19.0	69.0	76.0	83.0	99.0
Air pressure, hPa	1005.6	6.6	986.8	1000.7	1005.3	1010.6	1027.3
Stroke cases							
All strokes	61.2	15.7	23.0	51.0	59.0	70.0	182.0
Type							
ICH	10.4	4.2	1.0	7.0	10.0	13.0	40.0
CBI	47.6	13.1	17.0	39.0	46.0	54.0	143.0
Sex							
Male	37.9	10.2	15.0	31.0	37.0	43.0	113.0
Female	23.3	7.4	7.0	18.0	22.0	27.0	77.0
Age (years old)							
<40	3.7	2.1	0.0	2.0	4.0	5.0	12.0
40-64	30.2	8.4	9.0	24.0	30.0	35.0	90.0
≥65	27.3	8.7	9.0	21.0	26.0	32.0	101.0
Education							
Primary school or below	16.9	5.4	3.0	13.0	16.0	20.0	51.0
Junior high school	24.4	7.9	7.0	19.0	23.0	29.0	85.0
High school or above	19.9	7.6	5.0	14.0	19.0	24.0	67.0

Table 3. Spearman's rank coefficients between daily mean air pollutants and meteorological variables

Variables	$PM_{2.5}$	NO_2	O_3	Temperature	Relative humidity	Air pressure
$PM_{2.5}$	1.00					
NO_2	0.55*	1.00				
O_3	0.56*	0.02	1.00			
Temperature	-0.43*	-0.27*	-0.09*	1.00		
Relative humidity	-0.53*	-0.1*	-0.51*	0.27*	1.00	
Air pressure	0.50*	0.26*	0.21*	-0.85*	-0.45*	1.00

*p<0.05.

Regression analysis

When the lag length was set as 7 days, the results showed that the lag effect mainly lasted for 3 days (Figure S1). Therefore, we used 3 days as the lag length in later analyses. Positive associations and lag effects were observed between stroke morbidity and air pollution. The cumulative effect of a 10 μg/m^3 increase in $PM_{2.5}$ concentrations across lag 0-3 days corresponded to a 2.9% (95% CI: 1.3%-4.5%) increase in daily stroke hospitalizations; it was a 5.3% (3.1%-7.7%) increase in the case of NO_2, and a 1.2% (0.2%–2.2%) increase for O_3.

Table 4 showed that, for $PM_{2.5}$, an association was found in both men and women, populations older than 40 years old, and people with education levels of junior high school and above, and the CBI stroke subtype. For NO_2, the association was consistently significant in both men and women, populations in all age groups and all education levels, and subtypes CBI and ICH. For O_3, the association was significant in women, the middle-aged population, populations with an education level of junior high school and the CBI stroke subtype. The associations mainly occurred in the winter. Comparing the effect estimates between different subgroups, we found none of the differences to be statistically significant.

For the two-pollutant model (Figure 1), the results did not vary substantially compared to those in the single-pollutant models, especially for NO_2. The results were stable after adjusting the df values of $TIME_t$ and TEM_t, as well as the number of lags considered for the air pollutant in the model, indicating the stability of the estimated effects (Table S2-S3).

Table 4. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2, and O_3 in the single-pollutant model, across lag 0-3 days.

Subgroups	RR(95% CI)		
	$PM_{2.5}$	NO_2	O_3
All strokes	1.029 (1.013-1.045)	1.054 (1.031-1.077)	1.012 (1.002-1.022)
Sex			
Male	1.029 (1.011-1.046)	1.056 (1.032-1.080)	1.011 (0.999-1.022)
Female	1.030 (1.009-1.052)	1.050 (1.020-1.080)	1.013 (1.000-1.027)
Age			
<40	1.030 (0.990-1.070)	1.084 (1.027-1.145)	0.998 (0.974-1.023)
40-64	1.025 (1.008-1.044)	1.058 (1.033-1.084)	1.013 (1.002-1.024)
>65	1.033 (1.012-1.055)	1.046 (1.017-1.076)	1.012 (0.999-1.025)
Education			
Primary and below	1.013 (0.991-1.036)	1.050 (1.019-1.081)	1.007 (0.993-1.021)
Junior high school	1.043 (1.021-1.065)	1.072 (1.041-1.104)	1.023 (1.009-1.037)
Senior high school and above	1.028 (1.006-1.049)	1.036 (1.007-1.066)	1.003 (0.990-1.016)
Stroke subtype			
ICH	1.021 (0.995-1.048)	1.058 (1.021-1.096)	1.008 (0.991-1.025)
CBI	1.029 (1.011-1.047)	1.050 (1.026-1.075)	1.012 (1.001-1.023)
Season			
Spring	1.031 (0.989-1.076)	1.034 (0.989-1.081)	1.012 (0.989-1.035)
Summer	1.031 (0.989-1.075)	0.992 (0.944-1.044)	1.015 (0.990-1.041)
Autumn	1.021 (0.993-1.050)	1.040 (0.994-1.089)	1.009 (0.992-1.025)
Winter	1.073 (1.022-1.126)	1.095 (1.024-1.170)	1.033 (0.983-1.086)

Discussion

In this study, a positive association was found between the daily stroke hospitalizations and the concentration of ambient $PM_{2.5}$. Previous studies showed inconsistent results when exploring the short-term associations between $PM_{2.5}$ and stroke morbidity. Some studies observed a nonsignificant association, while other studies found a significant positive association.[10 20] For example, a 2014 meta-analysis of nonfatal strokes found an RR of 0.7-0.8% for short-term effects per 10 μg/m^3 $PM_{2.5}$.[21] Exposure to particulate matter increases inflammation, antioxidant activity and circulating blood platelet activation, and decreases vascular endothelial functions and enzyme activity, the latter of which may increase peripheral thrombosis and blood clotting.[22-24] Furthermore, compared with PM_{10}, $PM_{2.5}$ has a smaller size and larger surface area and can carry more harmful substances and penetrate deep into the human body,[25] finally induce a stroke.

We found that daily stroke hospitalizations increased by 5.4% for every 10 μg/m^3 increase of NO_2. These results are similar to those of previous studies, but some of the previous studies obtained different effect estimates.[26-28] For example, a study in Wuhan, China showed a 2.9% increase in stroke hospitalizations per 10 μg/m^3 increase in NO_2 in the cold season, which was smaller than the result (9.5%) from our study.[29] Evidence has shown that NO_2 is related to plasma fibrinogen[30] and is also correlated with fine particulate matter, which leads to increased coagulability through the release of cytokines.[31] Gaseous NO_2 can also be mixed with water to form acid aerosols after inhalation, which damages health through a series of oxidation mechanisms.[32]

The study found that the relative risk of stroke associated with an increase in the level of air pollution from the first to the third quartile was slightly higher for NO_2 than for $PM_{2.5}$. Previous studies showed similar results;[14 33] gaseous pollutants appeared to have a stronger effect on stroke mortality,[34 35] and some studies indicated that, after adjusting for other pollutants, only NO_2 showed consistent results with a single-pollutant model.[36 37] Concern has been raised that the observed health effects associated with NO_2 might be a result of exposure to traffic-related emissions or fine particles.[38]

A weak positive association between O_3 and hospital admissions for stroke was found in our study. Previous studies exploring this relationship showed different results. For example, a 10-year study conducted in France showed a significant positive relationship between O_3 exposure and ischemic stroke in men over 40 years of age, and the result was observed for a 1-day lag (OR 1.133, 95% CI 1.052 to 1.220).[39] In the United States, where air pollution

levels are low, the incidence of CBI/TIA was also associated with O_3 exposure.[40] However, there was no statistically significant association found between O_3 pollution and the incidence of stroke in South Carolina and Nueces County, Texas.[41 42] Some studies even showed a significant or nonsignificant negative association between O_3 and CBI.[43 44] The mechanisms linking O_3 and stroke need to be further studied.

The associations were found in both men and women; no clear trends were found across age groups and education levels. We found that the associations between air pollution and stroke morbidity had no statistically significant differences between different age groups. Similar results have been found in some previous studies.[14 45] Elderly people may spend more time indoors, and they are likely to wear masks when air pollution is severe, thus their individual exposure may be less.[46] On the other hand, Tian et al. found that the associations between air pollution and stroke morbidity were stronger in the elderly.[33] Therefore, the modification effect of age on the associations between air pollution and stroke morbidity needs further study. In addition, vascular risk factors, such as hypertension and hyperlipidemia, may also modify these associations, which needs to be addressed in future research.

The seasonal analyses showed that statistically significant associations between ambient air pollutant concentrations and hospital admissions for stroke were only found in the winter; Dong et al. also found that the harmful effects of NO_2 and SO_2 were more robust in the cold season[14]. One possible reason for this result is that the air pollution concentrations in Shenzhen are higher in winter than in summer (Table S1). The substances carried by particles may vary between seasons, which would influence the effect.

Our study found that $PM_{2.5}$, NO_2 and O_3 were related to hospitalizations for CBI but found different results for ICH. Similarly, previous studies showed consistent associations between CBI and air pollution, while the association between ICH was more variable and had a higher imprecision.[47] One reason is that the prevalence of ICH was much lower than CBI. In our study, 77.8% of stroke hospitalizations were for CBI, and ICH only accounted for 17.0% of stroke hospitalizations. Therefore, the risk estimates for ICH were more imprecise, and the confidence interval of the RR for ICH was wider. Another reason may be the differential mechanisms linking air pollution exposure to CBI and ICH. $PM_{2.5}$ exposure is conducive to atherosclerosis and the oxidative stress of the heart. These conditions may upregulate visfatin, reduce heart rate variability and induce CD36-dependent 7-ketocholesterol, which accumulate in macrophages[48-51]. All of these factors are more likely to induce CBI than ICH.

There are several limitations in this study. First, the air pollution data from fixed-site monitoring stations may not be representative of average population exposure. For example, the air pollution concentrations near the main roads with heavy traffic were often higher than those near the parks with few vehicles, and the air pollution monitoring stations were usually located far away from the main roads with heavy traffic. Second, it is difficult to completely eliminate the possibility of ecological fallacy in time-series studies, and we cannot provide absolute risks in this study. Moreover, the associations observed in the present study cannot prove causality.

Conclusions

$PM_{2.5}$, NO_2 and O_3 may induce stroke morbidity, especially in the winter. All three air pollutants were associated with higher risks of hospital admission due to cerebral infarction, the most prevalent stroke subtype in the study location. The government should take actions to address air pollution issues and develop warning systems for sensitive populations during high-risk periods.

Contributors: The study was conceived and designed by Bao J, Peng J and Guo Y; Xie X and Guo Y conducted statistical analysis and drafted the manuscript; Lei L, Zhou H, and Deng S contributed to data collection and processing; Xu Y and Liu Z helped in study management and the interpretation of the results; Bao J, Peng J and Huang C reviewed the manuscript for important intellectual content; All authors read and approved the final version.

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Ethics approval：The institutional review board at the School of Public Health, Sun Yat-sen University, approved the study protocol (No. 2019-029) with a waiver of informed consent. Data were analyzed at the aggregate level. All patients were anonymous, and no patient privacy were revealed.

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Figure legend/caption:

Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m^3 increase in ambient $PM_{2.5}$, NO_2 and O_3 in the two-pollutant model, across lag 0-3 days

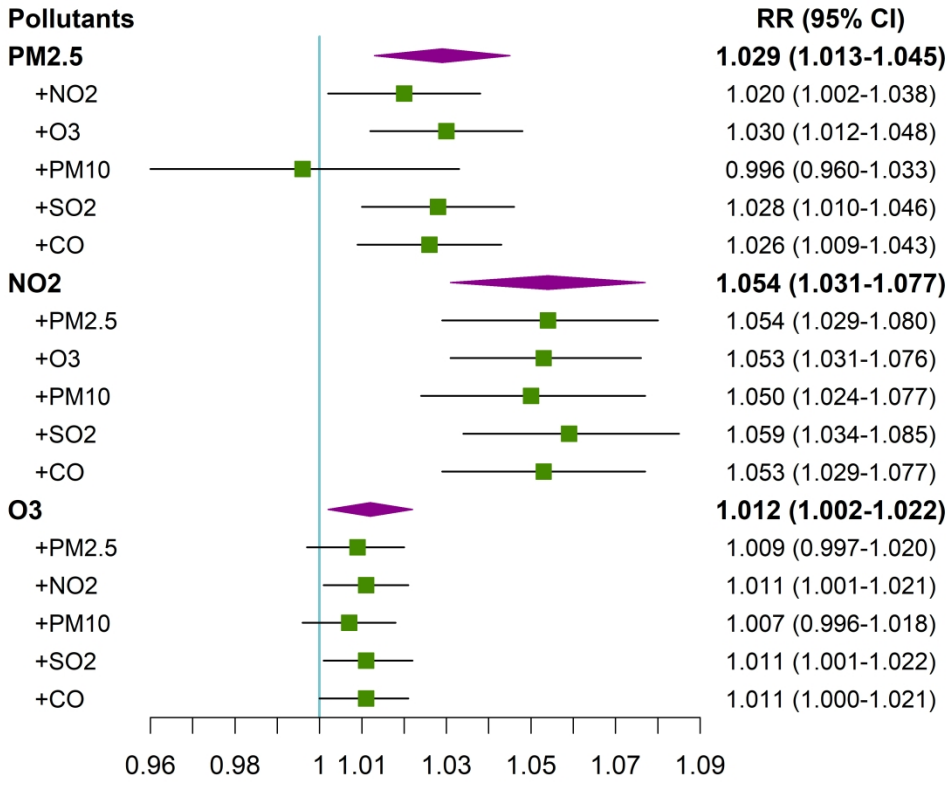

Figure 1. Relative risks (95% CI) of stroke hospitalizations for every 10 μg/m3 increase in ambient PM2.5, NO2 and O3 in the two-pollutant model, across lag 0-3 days

193x233mm (500 x 500 DPI)

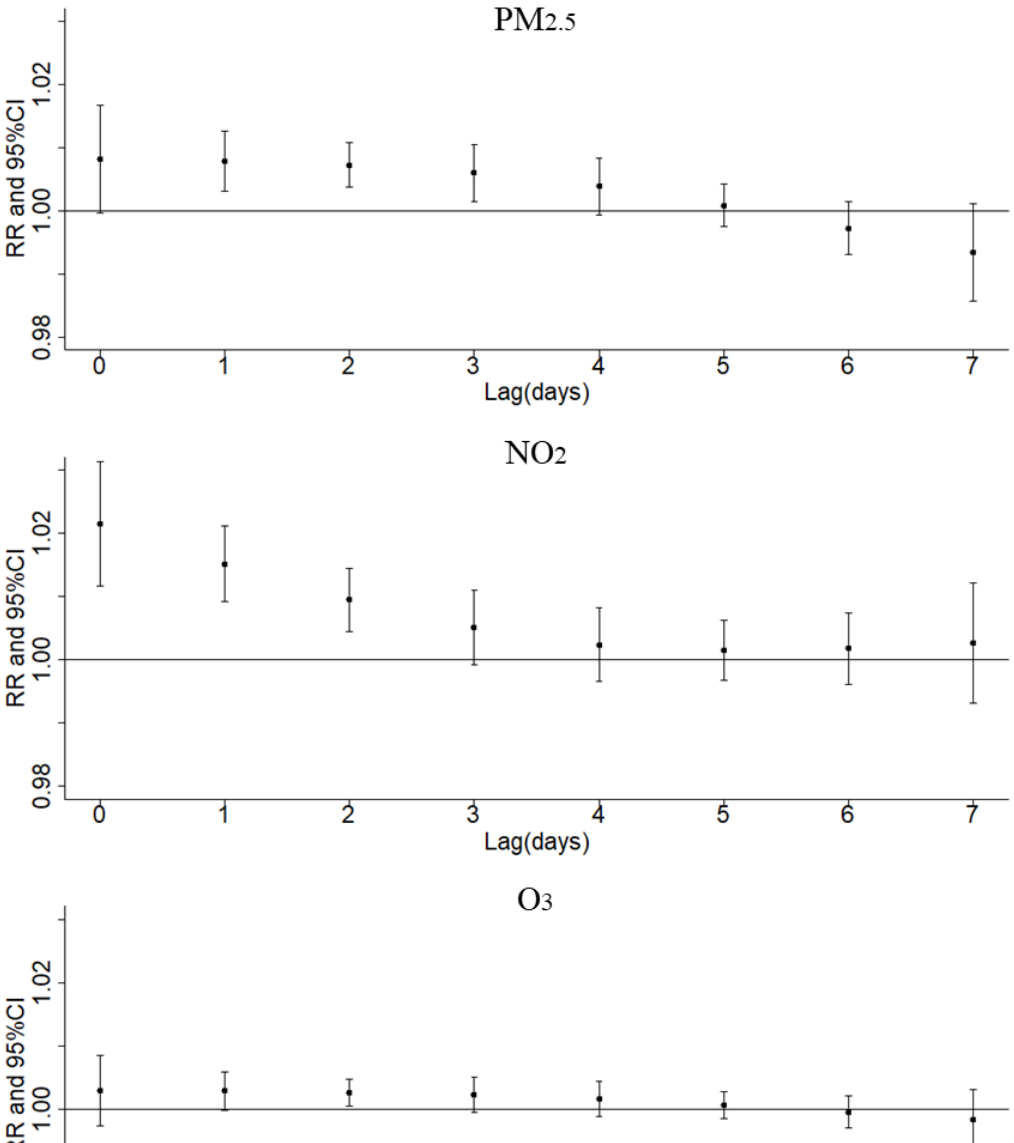

Figure S1. The distribution of lagged associations (RR, 95% CI) between air pollutants ($PM_{2.5}$, NO_2 and O_3) and stroke hospitalizations

Table S1. The distribution of daily air pollutant concentrations and temperature in four seasons of Shenzhen.

Variables	Mean	SD	Min	P_{25}	P_{50}	P_{75}	Max
Spring							
$PM_{2.5}$, μg/m^3	27.3	11.8	6.3	17.9	25.1	33.8	72.5
NO_2, μg/m^3	33.9	11.0	17.6	25.0	31.0	40.1	81.0
O_3, μg/m^3	53.7	22.8	17.0	37.0	47.2	68.9	130.7
Temperature	22.9	4.2	9.6	20.2	23.5	26.2	30.3
Summer							
$PM_{2.5}$, μg/m^3	18.4	10.3	7.1	11.4	14.7	18.4	59.5
NO_2, μg/m^3	28.7	8.4	12.0	22.4	27.5	33.4	61.2
O_3, μg/m^3	47.6	18.7	19.9	33.4	42.7	56.6	115.4
Temperature	29.1	1.3	25.0	28.17	29.4	30.0	33.0
Autumn							
$PM_{2.5}$, μg/m^3	33.2	14.8	8.5	22.5	31.2	43.0	84.0
NO_2, μg/m^3	32.3	9.1	11.3	25.3	30.9	38.4	60.0
O_3, μg/m^3	67.8	23.8	23.8	49.5	66.0	83.2	147.8
Temperature	25.5	3.2	14.5	24.2	26.1	27.9	30.7
Winter							
$PM_{2.5}$, μg/m^3	40.4	16.7	7.3	27.7	39.1	51.8	84.1
NO_2, μg/m^3	38.9	13.0	12.8	30.1	37.7	46.9	84.8
O_3, μg/m^3	58.3	17.1	26.6	44.6	56.4	70.2	113.0
Temperature	16.2	3.5	3.5	14.2	16.3	18.8	23.5

Table S2. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adjusting the df values of $TIME_t$ and TEM_t.

df of $TIME_t$	df of TEM_t	RR (95% CI)		
		$PM_{2.5}$	NO_2	O_3
5x3	2	1.023(1.007-1.039)	1.054(1.032-1.076)	1.004(0.994-1.014)
	3	1.022(1.006-1.038)	1.052(1.030-1.075)	1.005(0.995-1.015)
	4	1.022(1.006-1.039)	1.053(1.031-1.076)	1.004(0.995-1.015)
6x3	2	1.028(1.012-1.044)	1.053(1.031-1.075)	1.010(0.999-1.020)
	3	1.027(1.011-1.044)	1.054(1.031-1.076)	1.010(1.000-1.020)
	4	1.028(1.012-1.044)	1.054(1.032-1.077)	1.010(0.999-1.020)
7x3	2	1.029(1.013-1.046)	1.054(1.032-1.077)	1.011(1.001-1.021)
	3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
	4	1.029(1.013-1.046)	1.056(1.033-1.080)	1.011(1.001-1.022)

Table S3. The distribution of RR (95% CI) for stroke hospitalizations for every 10 μg/m^3 increase in air pollutants concentrations, adopting different lag days.

Lag days	RR (95% CI)		
	$PM_{2.5}$	NO_2	O_3
1	1.023(1.009-1.037)	1.039(1.022-1.056)	1.009(0.999-1.017)
2	1.029(1.013-1.044)	1.052(1.032-1.072)	1.010(1.000-1.020)
3	1.029(1.013-1.045)	1.054(1.031-1.077)	1.012(1.002-1.022)
4	1.028(1.012-1.046)	1.050(1.025-1.075)	1.012(1.001-1.023)
5	1.028(1.010-1.046)	1.054(1.027-1.081)	1.010(0.999-1.021)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page/line
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3, 6-16
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3, 18-29
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5, 6-36
Objectives	3	State specific objectives, including any prespecified hypotheses	5, 38-43
Methods			
Study design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5, 51-60 6, 6-39
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	NA
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6, 43-55 6, 13-24
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6, 6-60 7, 3-45
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	6, 6-27
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7, 3-44
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6, 43-60 7, 3-44
		(*b*) Describe any methods used to examine subgroups and interactions	7, 25-36
		(*c*) Explain how missing data were addressed	NA
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	NA

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	7, 36-43

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8, 3-14
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8, 3-26 Table 1, Table2
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	NA
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	NA
		Cross-sectional study—Report numbers of outcome events or summary measures	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 30-60 Table 4 Figure 1
		(*b*) Report category boundaries when continuous variables were categorized	11, 30-60 Table 4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 24, 25, 26 Figure S1, TableS1-S3
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 13, 14
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	15, 3-16
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	15, 21-28
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15, 39-42

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.