

Effect of airborne *Alternaria* conidia, ozone exposure, PM₁₀ and weather on emergency visits for asthma in school-age children in Kolkata city, India

Pampa Chakraborty · Arindom Chakraborty ·
Debajyoti Ghosh · Jyotshna Mandal · Shilpi Biswas ·
Ujjal Kumar Mukhopadhyay · Swati Gupta Bhattacharya

Received: 4 February 2013 / Accepted: 25 July 2013 / Published online: 4 August 2013
© Springer Science+Business Media Dordrecht 2013

Abstract *Alternaria* is one of the important allergenic fungal spores having special role in childhood asthma. Aerodynamic particle of <10 µm diameter (PM₁₀) and ozone exacerbate respiratory conditions including asthma. There is no documentation determining the role of airborne concentration of *Alternaria* conidia, pollutants like PM₁₀ and ozone and weather on school-age (5–18 years) children causing life-threatening episodes requiring emergency hospitalization for asthma in the megacity of Kolkata, Eastern India. We examined the relationship of daily hospitalization for asthma in school-age children and daily concentration

of outdoor *Alternaria* conidia, ozone, PM₁₀ and weather parameters in the atmosphere of Kolkata during 2010 using nonparametric generalized additive model (GAM). After a baseline survey (2008–2009), asthma hospitalization data for school-age children (based on case history and symptom-medication) were collected from two important city hospitals near study area in 2010. Daily *Alternaria* concentration was recorded using Burkard volumetric sampler. Its allergenic potential was confirmed by skin reactivity. Ozone and PM₁₀ concentration data were collected from West Bengal Pollution Control Board. The daily time series analyses of data were performed using GAM. There are two peak seasons of asthma hospitalization, one in

Pampa Chakraborty and Arindom Chakraborty have contributed equally to this work.

P. Chakraborty
Department of Botany, Sree Chaitanya College, North 24
Parganas, Habra, West Bengal 743268, India
e-mail: pampa_c@rediffmail.com

A. Chakraborty
Department of Statistics, Visva-Bharati, Santiniketan,
West Bengal 731235, India

D. Ghosh · J. Mandal · S. Gupta Bhattacharya (✉)
Division of Plant Biology, Bose Institute, 93/1 Acharya
P.C. Road, Kolkata 700009, West Bengal, India
e-mail: swati@bosemain.boseinst.ac.in

Present Address:
D. Ghosh
Division of Immunology, Allergy and Rheumatology, UC
College of Medicine, 3255 Eden Avenue # 350, ML 0563,
Cincinnati, OH 45267, USA

Present Address:

J. Mandal
Institute of Evolutionary Biology and Environmental
Studies, University of Zurich, Winterthurerstrasse 190,
8057 Zurich, Switzerland

S. Biswas · U. K. Mukhopadhyay
West Bengal Pollution Control Board, 10A, LA Block,
Sector III, Salt Lake, Kolkata 700098, West Bengal, India

spring-early summer (end of March to mid April) and other in autumn (mid September–October). In GAM analyses, *Alternaria* conidia, ozone and PM₁₀ were found to be significant both in spline and LOcal regrESSion smoothing method. Airborne *Alternaria* conidia, ozone and PM₁₀ in the city of Kolkata are found to have effect on school-age children with asthma and appear responsible for severe attacks leading to hospitalization.

Keywords Airborne *Alternaria* conidia · Asthma hospitalization · Kolkata · Generalized additive model (GAM) · Ozone · PM₁₀ · School-age children

1 Introduction

Airborne allergen exposure, climatic changes and airborne pollutants influence the symptoms of asthma patients (Reid and Gamble 2009; D'Amato et al. 2005). Allergic sensitization is an important risk factor for asthma. On the other hand, several studies have been documented the role of pollutants and weather in asthma exacerbation and subsequent hospitalization (Lewis et al. 2000; Wilson et al. 2007). Among environmental allergens, fungal spores are important in both indoor and outdoor. More than 80 fungal genera are reported to induce type 1 allergy in susceptible individuals (Simon-Nobbe et al. 2008). Among the most important allergenic fungal genera, *Alternaria* is important (Knutsen et al. 2012) with a special role in childhood asthma (Downs et al. 2001; Halonen et al. 1997; Perzanowski et al. 1998). In addition to fungal allergen exposure, the level of atmospheric pollutants like ozone and respirable particulate matter with less than 10-μm diameter (PM₁₀) have also found to be associated with daily asthma severity (Delfino et al. 1996; Adhikari et al. 2006) even in childhood (Lierl and Hornung 2003). The prevalence of asthma in children is continued to increase in developing countries undergoing rapid industrialization (Eder et al. 2009) with distinct seasonal variation. One of the possible reasons is the increase in industrialized lifestyle, which has resulted in declining air quality. Reports show that in several cases, the peak of asthma epidemic occurs first in school-age children followed by pre-school children

and then adults (Johnston et al. 2006). However, for children below 18 years of age, the asthma hospitalization rates show a seasonal pattern with autumn being the common season, followed by spring and summer, which has the lowest admission rate (Kao et al. 2001; Yeh et al. 2008). Aeroallergens, specially mold spores, air pollutants and their interaction with climate are important factors in the genesis of asthma exacerbations (Dales et al. 2004). It has also been reported that mold spore allergens may be more prevalent at higher levels in schools than in homes (Taskinen et al. 1999).

According to a multi-centric Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis (INSEARCH), the Indian population aged ≥ 15 years (845 millions) reported 2.04 % people having asthma, which corresponds nearly 17.25 million (Jindal et al. 2012). The total burden for the entire population will actually be higher considering its higher prevalence among children (Pal et al. 2009). The prevalence of asthma in Mumbai megacity in western India was found to be more (3.5 % by physicians' diagnosis) in comparison (Chowgule et al. 1998). Kolkata (previously Calcutta) is the largest and fastest growing metropolis in Eastern India (22°34'N, 88°24'E), in lower Gangetic plain, undergoing a process of rapid urbanization. The urban agglomeration had a population 14,112,536 in 2011 (Census of India 2011). The occurrence of a number of airborne allergenic pollen and spores are reported to be statistically associated with hospitalization for asthma in city population (Ghosh et al. 2010). Till date, there is no detail statistical analysis relating environmental allergen or pollutants to childhood asthma hospitalization over the time in this megacity.

Since all data of environmental exposure and health effects were recorded over different time point, it is essentially a time series data with wave-like seasonal fluctuations. To get a valid inference from such huge discrete data, statistical regression analyses using generalized additive models (GAMs) were found to be most widely applied. GAM allows for nonparametric adjustments for nonlinear confounding effects of seasonality, trends and weather variables (Hastie and Tibshirani 1986; Schwartz 2000; Dominici et al. 2002). GAMs are useful where the relationship between the variables is expected to be of a complex

form, not easily fitted by standard linear or nonlinear models, and there is no a priori reason for using a particular model. GAM extends traditional generalized linear models (GLM) by replacing linear predictors of the form $\eta = \sum_j \beta_j x_j$ with $\eta = \sum_j f_j(x_j)$, where $f_j(x_j)$ are unspecified nonparametric functions.

Methods for estimating f_j include smoothing by spline regression or LOESS (stands for LOcal regrESSion) smoothers to remove irregularities of data. It helps to provide a clear view of the true underlying behavior of the series. GLMs with regression splines (to which we refer here as the fully parametric alternative of the GAM with nonparametric smoothers) commonly define f_j to be regression splines, such as natural cubic splines or B-splines with a pre-specified number of knots at known locations. A nonparametric smoothed function (LOESS) of time is used to remove low frequency variability (Cleveland and Devlin 1988). This procedure removes smooth long-term variability in the time series and has been successfully used to remove seasonal variability from similar datasets in many previous studies (Stieb et al. 2000; Lin et al. 2002; Wong et al. 2002). Alternatively, one can smooth using GLM with parametric natural cubic splines (GLM NS) instead of the nonparametric smoothers LOESS. This smoother again requires the specification of degrees of freedom (number of knots +2) so that the autocorrelation in the residuals is minimized. In this study, we want to compare both LOESS and NS. The smoothing parameter identified for each diagnosis was also used to smooth the atmospheric variables in the models to ensure comparison between identically smoothed data and reduce the possibility of introducing spurious associations. The main result of interest, of course, is how the predictors are related to the dependent variable.

In addition to reducing the possibility of residual confounding between the predictors and the response, a significant benefit of pre-adjustment is the removal of potentially spurious associations between predictor variables.

Hence, the goal of the present study was to examine the relationship of daily asthma hospital admission of school-age children and daily concentration of outdoor *Alternaria* conidia, ozone, PM₁₀ and weather parameters in the atmosphere of Kolkata, using nonparametric generalized additive model.

2 Methods

2.1 *Alternaria* exposure data

The daily data (2008–2010) for atmospheric *Alternaria* conidial spore concentration (number/m³ of air) were recorded (The British Aerobiology Federation 1995) by Burkard 7-day volumetric sampler (Burkard Manufacturing Co., UK), which was located within 2 km of distance from two hospitals. The suction flow rate (10 l/min) of the sampler was constant throughout the study period. For the confirmed identification of the *Alternaria* and its species, petriplates containing potato dextrose agar (PDA) and Sabouraud's dextrose agar (SDA) media were exposed at the sampling site for a period of 15 min. After exposure, the petriplates were incubated at 25 °C. The fungal colonies developed in the petriplates were isolated by subculturing and studied by both light and scanning electron microscopy. The daily concentration data of 2010 were used for statistical analyses.

2.2 Skin prick tests (SPT)

To check the allergenic potential of *Alternaria* conidial spore, skin tests were performed with *Alternaria* conidial spore extracts on 398 respiratory allergic school-age patients (age range: 5–18 years), attending the Allergy Department of The Institute of Child Health, Calcutta, during 2008–2009. They all had demonstrated clinical manifestations of rhinoconjunctivitis and/or bronchial asthma. The exclusion criteria were perennial or severe asthma and other severe systemic diseases during skin testing. To avoid masking of possible symptoms, corticosteroids and antihistamines were prohibited. The detailed history including age, sex, family history, onset and duration of symptoms were recorded.

Tests were performed with 20 µl of allergenic extract placed on the ventral side of the forearm with a 26 G. disposable hypodermic needle. Extraction was performed using the conidial spore at 95 % purity with 0.1 M PBS (1:50 w/v). Histamine phosphate (1 mg/ml) and PBS (1 mg/ml) were used as positive and negative controls, respectively. The reaction measurement was performed after 20 min. According to International guidelines, positivity was defined as mean wheal diameter ≥ 3 mm compared with negative control (Dreborg and Frew 1993).

2.3 Study population

The data of daily emergency hospital admission due to asthma attack for school-age children were recorded from Sir Nilratan Sarkar Hospital and National Medical College Hospital, the two important government hospital of Central Kolkata during 2008–2010. After the baseline study (2008–2009) to confirm the trend of hospitalization, the data of the year 2010 were used for statistical analyses, which included the patients ($n = 2,708$) between 5 and 18 [mean age (9 ± 1.2)] years of age with principal diagnosis of asthma. Hospitalized patients were selected on the basis of International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM) code (WHO 1975) for asthma (493.xx) ICD-10 (WHO 1993) code J45/4d listed as the first discharge diagnosis (symptom, medication and case history) for children and adolescents from birth to 19 years (the age group mostly covered in present study). The symptoms included were wheeze, cough, chest tightness, chest pain, cold, shortness of breath and associated complications. The exacerbations were acute that does not respond to inhaled bronchodilators, corticosteroids, etc., and in most of the cases, oxygen was provided. In some cases ($n = 467$), pulmonary function tests were performed. In these cases, the percentage of forced expiratory volume in 1 s (FEV_1) of predicted value was $<60\%$ (data not shown).

2.4 Ozone and PM_{10} exposure data

The daily data of Ozone (O_3 , ppb) and PM_{10} ($\mu g/m^3$ of air) exposure were collected from Victoria Memorial (Central Kolkata) for the year 2010. The measurement was conducted by West Bengal Pollution Control Board, India. The weather parameters [average daily temperature ($^{\circ}C$), relative humidity (%), rainfall (mm) and wind speed (km/h)] data were collected from Alipore Meteorological station, Kolkata.

2.5 Statistical analyses

Whenever we are interested in a study concerning the regression of a variable (here hospitalization) over a few predictors, we usually use a statistical model. Once such a model is proposed, the next job is to test for its validation. This is usually done by testing the hypothesis regarding the effect of covariates on

response. GAM is nothing but a regression model, which includes different covariates. The null hypothesis (assuming that all predictors are insignificant for predicting the number of hospitalization) of non-significant effects are studied using appropriate test statistics. Predictors with lower p value (<0.05) are treated as statistically significant. The nonparametric smooth curve fitted to daily asthma hospital visits over study months indicated a time trend characterized by a restricted cubic splines with three knots.

Hence, the overall model was summarized as:

$$\text{Visit}_t \sim \text{Poisson}(\eta_t)$$

$$\begin{aligned} \log \eta_t = & \alpha + \beta_1 \text{Spore count} + \beta_2 \text{PM}_{10} + \beta_3 (\text{Ozone}) \\ & + ns(\text{Average humidity}) \\ & + ns(\text{Average temperature}) \\ & + ns(\text{Average wind speed}) \end{aligned}$$

where $(\text{Visit})_t$ is the expected number of hospital asthma visits on a particular day t ; α is the intercept term; β_1 and β_2 are exposure parameters, which are equal to the coefficient of the *Alternaria* conidial spore count and PM_{10} , respectively. We then repeat the analysis replacing the smoothing splines with LOESS smoothers. We fitted the following GAM with LOESS smoothers:

$$\begin{aligned} \log \eta_t = & \alpha + \beta_1 \text{Spore count} + \beta_2 \text{PM}_{10} + \beta_3 (\text{Ozone}) \\ & + lo(\text{Average humidity}) \\ & + lo(\text{Average temperature}) \\ & + lo(\text{Average wind speed}) \end{aligned}$$

3 Results

The survey on airborne *Alternaria* conidial spore count showed its presence throughout the year 2010 (entire 365 days period) with variable concentrations. When the viable conidial spores are considered in term of colony forming units in petriplate exposure, among the species of *Alternaria*, we found *A. alternata* to be the most prevalent (61.35 %), followed by *A. tenuissima* (23.71 %) and *A. padwickii* (11.05 %). In skin reaction results, ($n = 398$) on respiratory allergic school-age children, with allergenic extract of the conidial spore of these three species, *A. tenuissima* showed highest positive response (40.70 %) followed by *A. alternata* (21.35 %) and *A. padwickii* (4.02 %).

The survey on asthma-related hospitalization in school-age children (5–18 years of age) of Kolkata

city showed the admission rate of 2,708/year in 2010. There is a significant seasonal variation in daily asthma hospitalization of this age group, which ranges within 2–24/day (Fig. 1a). There were two peak seasons, the first one (up to 24/day) was in spring-

early summer (last week of March to mid April) and the second one (up to 23/day) was in autumn (mid September–October).

In 2010 (Fig. 1b), the concentration of airborne *Alternaria* conidia was high up to 191.5 conidia/day/

Fig. 1 a–h Data on daily hospital admission due to asthma attack for school-age children at Kolkata along with all the possible factors. Broken lines correspond to smoothing by spline. The labels on x-axis refer to days of the year (**a** School children hospitalized for asthma/day, **b** *Alternaria* conidial spore count/day/m³ of air, **c** Ozone concentration in ppb, **d** PM₁₀ in µg/m³ of air, **e** average daily temperature in °C, **f** average daily humidity in %, **g** Average daily rainfall in mm, **h** average daily wind speed in km/h.)

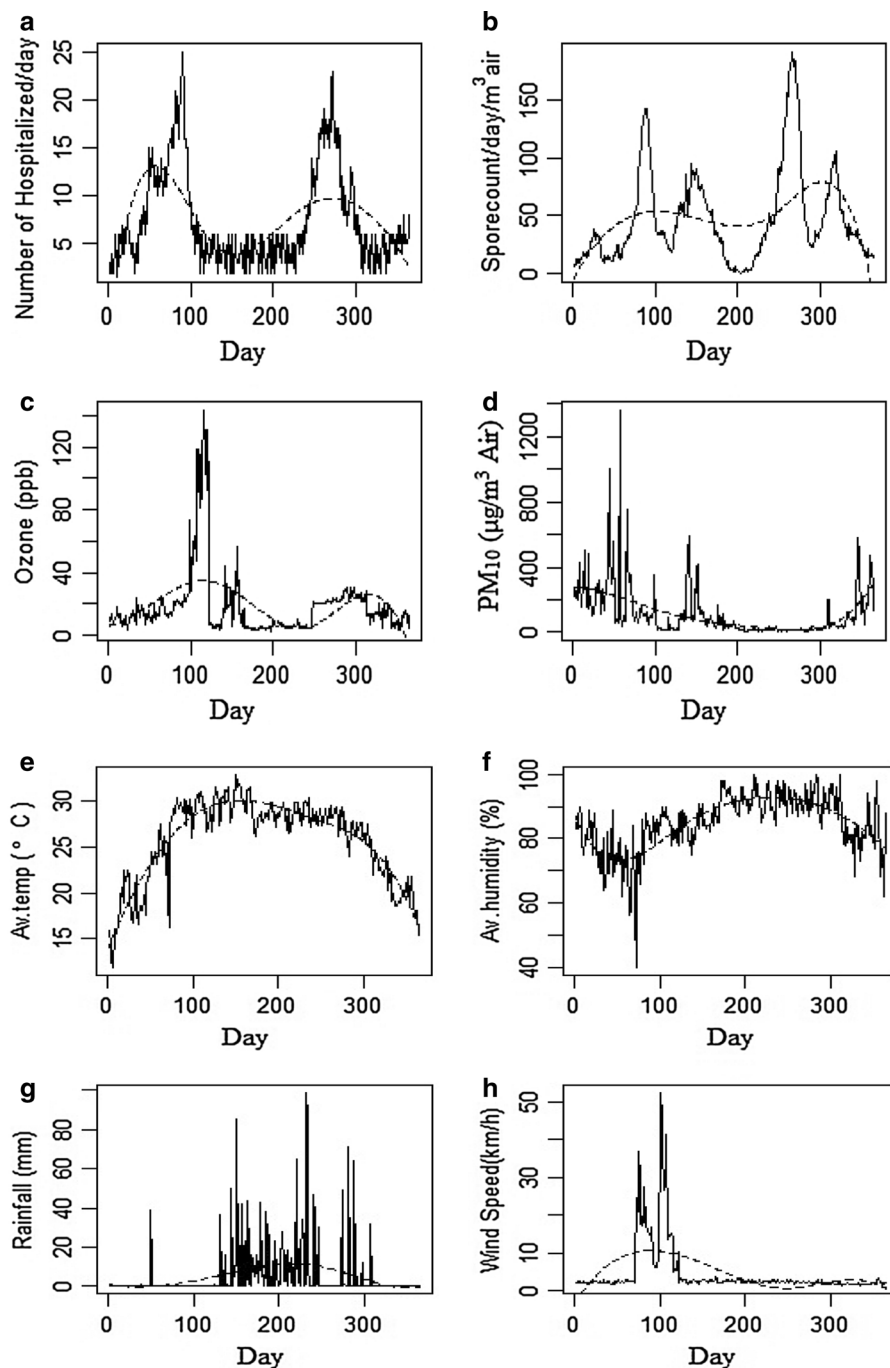


Table 1 Quarterly mean, median and SD values for asthma admission data, *Alternaria* conidial spore count, Ozone and PM₁₀ and weather variables

Quarter	Number of asthma hospital admissions	Conidial spore count (no./m ³ of air)	Ozone (ppb)	Average humidity (%)	Average temperature (°C)	PM ₁₀ (µg/m ³ of air)	Wind speed (km/h)	Rainfall (mm)
1	9.71 ± 5.72	35.02 ± 35.62	13.844 ± 4.93	75.76 ± 9.00	21.92 ± 4.86	235.20 ± 196.91	6.18 ± 7.73	0.56 ± 4.24
	8.50	21.20	13.00	76.00	21.85	207.00	2.50	0.00
2	5.39 ± 3.54	54.21 ± 24.68	32.48 ± 37.68	85.77 ± 5.1385.00	29.50 ± 1.60	99.25 ± 114.30	7.86 ± 10.89	5.97 ± 13.53
	4.00	52.45	18.00		29.70	72.50	3.00	0.00
3	8.355 ± 5.73	54.481 ± 62.40	10.366 ± 7.06	92.02 ± 3.51	28.51 ± 0.90	19.86 ± 15.83	2.55 ± 0.29	9.67 ± 18.08
	6.0	26.2	6.0	92.0	28.5	15.0	2.6	0.30
4	6.53 ± 3.43	44.50 ± 24.92	17.378 ± 7.78	85.511 ± 8.04	22.87 ± 3.44	98.88 ± 121.52	2.033 ± 0.38	2.23 ± 10.65
	6.0	36.00	16.50	84.50	22.45	43.00	1.95	0.00

First row in each cell corresponds to mean ± SD and second row corresponds to median values

m³ of air in March and September, moderate in October–December and low (≤ 1 conidia/day/m³) in July, in the midst of the rainy season.

The concentration of ozone in the atmosphere varied from 2 to 145 ppb around the year with lower level in January, June–July period and higher level during April, September–November period (Fig. 1c).

The quantum of PM₁₀ (Fig. 1d) showed its higher level in the atmosphere of study area in May–June and January–April (up to 2,422 µg/m³ of air). Its concentration was lower during end of July–September (minimum up to 2 µg/cubic meter of air).

Regarding the weather parameters, the average daily temperature of the city of Kolkata (Fig. 1e) ranged from 11.9 °C in winter (December–January) to 32.9 °C in summer (May–June). The average relative humidity (Fig. 1f) was found to be high from the end of June–October and lower during December–March with a range of 70–100 %. In case of rainfall (Fig. 1g), there was almost no rainfall in winter (December–January and spring (March–April). It was sporadic or low during autumn (September–November) but high in rainy season (July–August). The average daily rainfall over the year ranged from 0 to 98.5 mm. The wind speed showed the range from 1.6 km/h in December to 52.3 km/h in April with great variation (Fig. 1h).

Number of asthma-related hospitalization in school-age children varied from one season to other. Seasonal variation is present in different covariates used here, which is evident from Fig. 1. It can also be observed that whenever the *Alternaria* conidial spore count is high (in the first and third quarters of the year), number of hospitalization is also high. It gives an indication of some positive correlation between *Alternaria* conidial spore count and number of hospital admission.

Table 1 describes the summary of the data including the mean, medians and standard deviations for the data of all predictor test variables and asthma hospitalization by combining the quarterly data for the whole year.

A 30-day smoothing parameter was chosen for all diagnostic classes to remove temporal cycles, minimize autocorrelation and optimize goodness-of-fit. In Fig. 2, we have smoothed out the fluctuations. It can be seen that the smoothing technique performs well in almost all the cases except rainfall (Fig. 1g), which is obvious. Spline method is also used for smoothing.

Both methods result in similar results. To make our study more focused, we have not included the Spline-based results here.

Table 2 shows product–moment correlations between the predictors. This relationship can be computed using Pearson’s product–moment formula, which is necessary to have an idea regarding the relationship between variables. This formula is useful for most of the cases, when variables are of some numeric value. These correlations should be used with much attention to remove spurious correlation among the predictors. A significant benefit of smoothing is the removal of potentially spurious correlations between predictor variables (Table 3). For example, the correlation between ozone and average temperature is positive ($r = 0.196$) for raw data, but highly negative ($r = -0.756$) for smoothed data. Similar phenomenon may be observed in case of *Alternaria* conidial spore

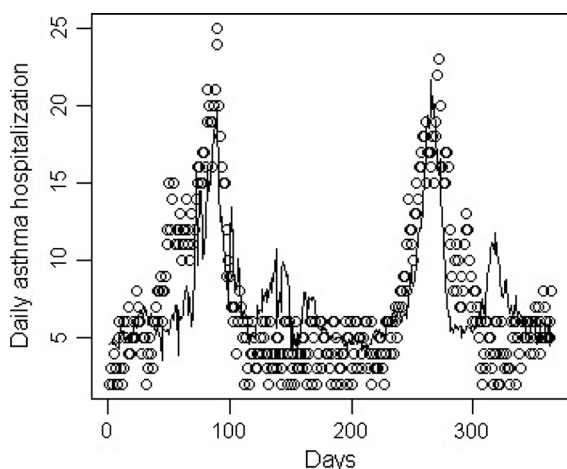


Fig. 2 Fitting of discrete numeric data of asthma hospitalization by standard Poisson regression

count and wind speed. In some cases (e.g., wind speed and rainfall), magnitudes of the correlations also vary except for ozone and PM_{10} .

To get an idea about the significant factors, a generalized linear model (GLM) with log link is fitted. Though this model completely ignores the correlation structure present in a time series data, it may be used for preliminary analysis. From Table 3, first model obtained using GLM is as follows:

$$\begin{aligned} \text{Log}(Y) = & 2.2384 + 0.0083 * \text{Spore count} - 0.0023 \\ & * \text{Ozone} - 0.0089 * \text{Average humidity} \\ & + 0.0173 * \text{Wind speed} \end{aligned}$$

where, Y is the counts of daily hospital admission due to asthma. Surprisingly, PM_{10} is not included in the above model since it is not significant statistically. This shows inefficiency of the proposed GLM where the correlation structure of the data is completely ignored. The fitted values, using this GLM, are plotted in Fig. 2. The Akaike information criteria (AIC) value is found to be 1,981.1. However, the fitting of this GLM is not so good for obvious reasons. First, the spurious correlation present in the data is not removed, and secondly, the time series correlation is not considered. These two problems can be solved by using general additive model which is a less restrictive model, with moderately flexible nonparametric terms for each of the predictors.

General additive model (GAM) is fitted using R-2.10.1. Since the response (number of hospital visit) is assumed to follow a Poisson distribution (Fig. 2), the link function for the GAM is taken to be log. We first model the response using all the factors. The result of the analysis is given in Table 4.

It is found that smooth term corresponding to average temperature does not have much impact on the

Table 2 Correlation coefficients (by product–moment correlation analysis, specific for numeric variables) for the environmental predictors of hospitalization in case of raw data

	Conidia	Ozone	Average humidity	Average temperature	PM_{10}	Wind speed	Rainfall
Conidia	1	0.070	0.138	0.343	−0.019	0.086	−0.063
Ozone		1	−0.650	0.196	−0.114	0.406	−0.118
Average humidity			1	0.388	−0.437	−0.111	0.202
Average temperature				1	−0.440	0.238	0.229
PM_{10}					1	−0.0947	−0.111
Wind speed						1	−0.105
Rainfall							1

Table 3 Correlation coefficients (by product–moment correlation analysis, specific for numeric variables) for the environmental predictors of hospitalization in case of smoothed (to reduce spurious correlation) data

	Conidia	Ozone	Average humidity	Average temperature	PM ₁₀	Wind speed	Rainfall
Conidia	1	0.136	0.623	0.471	−0.819	−0.191	0.324
Ozone		1	0.321	−0.756	−0.094	−0.925	−0.788
Average humidity			1	0.742	−0.950	−0.123	0.804
Average temperature				1	−0.636	0.710	0.956
PM ₁₀					1	0.085	−0.638
Wind speed						1	0.681
Rainfall							1

Table 4 Analysis of parameter estimates of *Alternaria* conidial spore, PM₁₀, ozone, average humidity, average temperature and wind speed for standard multiple Poisson regression with standard error and *p* value) for level of significance)

Parameter	Estimate	SE	<i>p</i> value
Intercept	2.2384	0.2288	<0.05*
Conidial spore count	0.0083	0.0004	<0.05*
PM ₁₀	0.0001	0.0001	0.3446
Ozone	−0.0023	0.0011	0.0388*
Average humidity	−0.0089	0.0025	<0.05*
Average temperature	0.0003	0.0055	0.9446
Wind speed	0.0173	0.0025	<0.05*

response. So, we exclude this covariate from our final model. To get an idea about the model fit, Un-Biased Risk Estimator (UBRE) score is computed. From the above, it is found that there exists hardly any difference between these two methods except the inclusion of ozone as a predictor (Table 5). In LOESS smoothing, the *p* value is very high for the ozone implying the insignificance of ozone as a predictor. But in case of spline smoothing, ozone is found to be significant at 5 % level. Both the methods indicate the significance of the *Alternaria* conidial spore count and PM₁₀ in the air. From the unbiased risk estimates (UBRE) score as well as from the value of adjusted R^2 (a statistical term explaining the goodness of a predictor with other), it is observed that Spline smoothing perhaps is an edge over the Spline regression in terms of model fitting. More deviance is explained by the Spline regression compared to the LOESS method (Table 6).

In Fig. 3a, b, the dotted line represents the fitted value, whereas the points are the observed ones.

From here, it is clear that the GAM is well fitted irrespective of LOESS or spline.

GAM with nonparametric smoothers provides a more flexible approach for adjusting for nonlinear confounders compared with fully parametric alternatives in time series studies of air pollution and health. A significant advantage of nonparametric regression is that we need not rely on difficult-to-verify assumptions about the functional form of the dependence of the outcome on individual risk factors. We have considered *Alternaria* conidial spore count, Ozone and PM₁₀ as the possible cofactors smoothing the other cofactors. However, it will be of our interest to deal with some more models by incorporating the cofactors in regression without smoothing. In this work, two popular smoothing techniques were applied; some other smoothing techniques can also be used so that the best model can be fitted.

4 Discussion

An average of one out of every ten school-age children now has asthma in USA. It is a leading cause of school absenteeism due to chronic condition accounting for nearly 13 million missed school days per year (United States Environmental Protection Agency 2012). The level of outdoor air pollutants are also increasing sharply in India, including the rise of asthmatics. In the present study on school-age children of Calcutta (Kolkata) City, India, we recorded two peak seasons of asthma hospitalization, one during spring-early summer period and another in autumn. The most intense peak of epidemic of asthma exacerbation requiring hospital admission of children during autumn have been reported from many countries like

Table 5 Estimates of *Alternaria* conidial spore count, PM₁₀ and ozone with their SE (in parentheses) in case of spline regression and LOESS smoothing of data to reduce irregularities

	Spline		LOESS	
	Estimate	<i>p</i> value	Estimate	<i>p</i> value
<i>Alternaria</i> conidial spore count	0.0081 (0.0004)	<0.01*	0.0083 (0.004)	<0.01*
PM ₁₀	0.0037 (0.0007)	<0.01*	0.0010 (0.0008)	<0.01*
Ozone	0.0022 (0.0011)	0.0024*	0.0024 (0.0018)	0.8244

* Significant effects

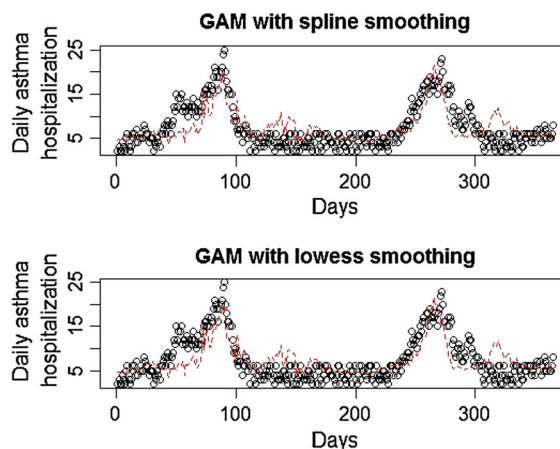
Table 6 Deviances for spline regression and LOESS smoothing of data to check the goodness-of-fit of prediction model

	Spline	LOESS
Adjusted R^2 value	0.534	0.527
Unbiased risk estimates (UBRE) score for GAM	0.546	0.6093
Deviance explained	62.7 %	54 %

Canada (Crichton et al. 2001), United States (Kimes et al. 2004), Israel (Garty et al. 1998), etc.

To correlate different factors with childhood asthma hospitalization, statistical tools are necessary for summarizing the data, predicting the response and finding the causal inference. To analyze huge data on environmental health studies, complications may arise due to the nature of study, e.g., time series data, measurement error, high dimensional data, etc. Since the data is a time series data, it contains cyclical fluctuations (i.e., wave-like ups and downs) and autocorrelations giving irregularities. In case of such time-dependent variables, the actual correlations may be confounded. To remove these confusions, we smooth the data first (by spline and LOESS smoother tool) to get rid of such dependency and then we compute the correlation between two predictors. This feature has been used in modeling the response using regression analysis by GAM to view the actual underlying correlation with cofactors like airborne *Alternaria* conidia, PM₁₀, ozone, etc.

The tropical climate and agricultural practice may release significantly higher level of fungal spores (Chakraborty et al. 2003) including *Alternaria* in the city atmospheric environments. In outdoor environment, *Alternaria* is one of the most commonly reported fungal genera, causing respiratory hypersensitivity (Feo Brito et al. 2012), which sometimes shows seasonal two-peak pattern as in present study

**Fig. 3** Fitted values for daily hospitalization of school children for asthma under generalized additive models (GAM) with smoothing Splines (above) and LOWESS smoothers (below). Circles are the observed counts. Time is given in days

(Maya-Manzano et al. 2012). We found a high level of allergenic sensitivity against *Alternaria* in school-age children. Showing similarity to the present study, results from the middle-school children population survey showed that sensitization to this mold is strongly associated with asthma ($p < 0.001$) by univariate analysis (Guo et al. 1999).

Supporting the strong positive correlation of hospitalization and coarse aerodynamic particles in our study, there are several reports indicating PM₁₀ as an aggravation factor for asthma in children (Iskandar et al. 2012; Weinmayr et al. 2010). A recent study on respiratory health and PM₁₀ exposure data on 45,000 children from comparable cross-sectional studies in 12 countries showed that it is associated with seasonal respiratory symptoms (Hoek et al. 2012). Association of PM₁₀ concentration with higher percentage of nasal irritation and wheeze is also common in asthmatic children (Preuttipan et al. 2004; Mann et al. 2010).

The ambient pollutant most strongly associated with asthma morbidity is the ozone, which can enhance airway inflammation. A strong association between ozone and asthma emergency visits has been established in school children (Villeneuve et al. 2007). In the present study, although there is significant positive correlation of ozone concentration and asthma hospitalization in LOESS smoothing of data, it is not highly significant in spline smoothing method. In this context, it can be mentioned that data from the APHEA (Air Pollution and Health: A European Approach) study for the period of 1986–1992 from Barcelona, Spain; Helsinki, Finland; Paris, France and London, UK, failed to find any association between emergency asthma hospitalization and ambient ozone level in children <15 years of age (Sunyer et al. 1997). The reason behind such variation of association is a subject of further detail analyses.

5 Conclusion

In conclusion, the result of this study provides a better understanding of the correlation of airborne *Alternaria* conidial spore concentration, ozone exposure, particulate matter (PM₁₀) and weather on emergency visits for asthma in school-age children of Kolkata city using generalized additive model. The findings demonstrate that airborne *Alternaria* conidial spore, PM₁₀ and to some extent ozone might have a positive significant impact on emergency asthma hospitalization in school-age children of the city. Future research is required to provide more specific model and trends to help for the awareness about the pollutants and allergens as well.

Acknowledgments Thanks are due to University Grants Commission, New Delhi, for providing fund to first author; Mr. Chanchal Chakraborty, Division of Plant Biology, Bose Institute for his kind assistance during collection of data. We are also thankful to the Director, Institute of Child Health (ICH), Kolkata; Dr. Indrani Roy and all the staff members of Allergy Department, ICH, Kolkata, for their kind help during skin reaction study on school-age children.

References

- Adhikari, A., Reponen, T., Grinshpun, S. A., Martuzevicius, D., & LeMasters, G. (2006). Correlation of ambient inhalant bioaerosols with particulate matter and ozone: A two-year study. *Environmental Pollution*, 140, 16–28.
- Census of India. (2011). Provisional population totals. Retrieved 26 January 2012. Registrar General and Census Commissioner, India. http://censusindia.gov.in/2011-prov-results/paper2/data_files. Accessed on 19.07.2012.
- Chakraborty, P., Gupta-Bhattacharya, S., & Chanda, S. (2003). Aeromycoflora of an agricultural farm in West Bengal, India: A five-year study (1994–1999). *Grana*, 42(4), 248–254.
- Chowgule, R. V., Shetye, V. M., Parmar, J. R., et al. (1998). Prevalence of respiratory symptoms, bronchial hyper reactivity and asthma in a megacity. Results of the European Community Respiratory health Survey in Mumbai (Bombay). *American Journal of Respiratory and Critical Care Medicine*, 158, 547–554.
- Cleveland, W. S., & Devlin, S. (1988). Locally weighted regression: An approach to regression analysis by local fitting. *Journal of American Statistical Association*, 83, 596–610.
- Crighton, E. J., Mamdani, M. M., & Upshur, R. E. G. (2001). A population based time series analysis of asthma hospitalizations in Ontario, Canada: 1988 to 2000. *BMC Health Services Research*, 1, 7.
- D'Amato, G., Liccardi, G., D'Amato, M., & Holgate, S. (2005). Environmental risk factors and allergic bronchial asthma. *Clinical and Experimental Allergy*, 35(9), 1113–1124.
- Dales, R. E., Cakmak, S., Judek, S., Dann, T., Coates, F., Brook, J. R., et al. (2004). Influence of outdoor aeroallergens on hospitalization for asthma in Canada. *Journal of Allergy and Clinical Immunology*, 113, 303–306.
- Delfino, R. J., Coate, B. D., Zeiger, R. S., Selter, J. M., Street, D. H., & Koutrakis, P. (1996). Daily asthma severity in relation to personal ozone exposure and outdoor fungal spores. *American Journal of Respiratory and Critical Care Medicine*, 154(3), 633–641.
- Dominici, F., McDermott, A., Zeger, S. L., & Samet, J. M. (2002). On the use of generalized additive models in time-series studies of air pollution and health. *American Journal of Epidemiology*, 156(3), 193–203.
- Downs, S. H., Mitakakis, T. Z., Marks, G. B., Car, N. G., Belousova, E. G., Leuppi, J. D., et al. (2001). Clinical importance of *Alternaria* exposure in children. *American Journal of Respiratory and Critical Care Medicine*, 164, 455–459.
- Dreborg, S., & Frew, A. J. (1993). Allergen standardization and skin tests. *Allergy*, 48(14), 49–82.
- Eder, W., Ege, M. J., & von Mutius, E. (2009). The asthma epidemic. *New England Journal of Medicine*, 355, 2226–2272.
- Feo Brito, F., Alonso, A. M., Carnes, J., Martin-Martin, R., Fernandez-Caldas, E., Galindo, P. A., et al. (2012). Correlation between *Alt a 1* allergen and symptom levels in *Alternaria alternata* monosensitized patients. *Journal of Investigational Allergology and Clinical Immunology*, 22(3), 154–159.
- Garty, B. Z., Kosman, E., Ganor, E., Berger, V., Garty, L., Wietzen, T., et al. (1998). Emergency room visits of asthmatic children, relation to air pollution, weather and airborne allergens. *Annals of Allergy, Asthma & Immunology*, 81, 563–570.
- Ghosh, D., Chakraborty, P., Gupta, J., Biswas, A., & Gupta-Bhattacharya, S. (2010). Asthma related hospital

- admissions in an Indian megacity: Role of ambient aeroallergen and inorganic pollutants. *Allergy*, 65, 795–796.
- Guo, Y. L., Lin, Y. C., Sung, F. C., Huang, S. L., Ko, Y. C., Lai, J. S., et al. (1999). Climate, traffic-related air pollutants and asthma prevalence in middle school children in Taiwan. *Environmental Health Perspectives*, 107(12), 1001–1006.
- Halonen, M., Stern, D. A., Wright, A. L., Taussig, L. M., & Martinez, F. D. (1997). *Alternaria* as a major allergen for asthma for children raised in a desert environment. *American Journal of Respiratory and Critical Care Medicine*, 155, 1356–1361.
- Hastie, T., & Tibshirani, R. (1986). Generalized additive models. *Statistical Science*, 1, 297–318.
- Hoek, G., Pattenden, S., Willers, S., Antova, T., Fabianova, E., Braun-Fahrlander, C., et al. (2012). PM₁₀, and children's respiratory symptoms and lung function in the PATY study. *European Respiratory Journal*, 40(3), 538–547.
- Iskandar, A., Andersen, Z. J., Bonnelykke, K., Ellermann, T., Andersen, K. K., & Bisgaard, H. (2012). Coarse and fine particles but not ultrafine particles in urban air trigger hospital admission for asthma in children. *Thorax*, 67(3), 252–257.
- Jindal, S. K., Aggarwal, A. N., Gupta, D., Agarwal, R., Kumar, R., Kaur, T., et al. (2012). Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH). *International Journal of Tuberculosis and Lung Disease*, 16(9), 1270–1277.
- Johnston, N. W., Johnston, S. L., Norman, G. R., Dai, J., & Sears, M. R. (2006). The September epidemic of asthma hospitalization: School children as disease vectors. *Journal of Allergy and Clinical Immunology*, 117, 557–562.
- Kao, C. C., See, L. C., Yan, D. C., Ou, L. S., & Huang, J. L. (2001). Time trends and seasonal variations in hospital admission for childhood asthma in Taiwan from 1990 to 1998. *Asian Pacific Journal of Allergy and Immunology*, 19, 63–68.
- Kimes, D., Levine, E., Timmins, S., Weiss, S. R., Bollinger, M. E., & Blaisdell, C. (2004). Temporal dynamics of emergency department and hospital admission of pediatric asthmatics. *Environmental Research*, 94, 7–17.
- Knutsen, A. P., Bush, R. K., Demain, J. G., Denning, D. W., Dixit, A., Fairs, A., et al. (2012). Fungi and allergic lower respiratory tract diseases. *Journal of Allergy and Clinical Immunology*, 129, 280–291.
- Lewis, S. A., Corden, J. M., Forster, G. E., & Newlands, M. (2000). Combined effect of aerobiological pollutants, chemical pollutants and meteorological conditions on asthma admissions and A & E attendances in Derbyshire, UK, 1993–96. *Clinical and Experimental Allergy*, 30, 1724–1732.
- Lierl, M. B., & Hornung, R. W. (2003). Relation of outdoor air quality to pediatric asthma exacerbation. *Annals of Allergy, Asthma & Immunology*, 90, 28–33.
- Lin, M., Chen, Y., Burnett, R. T., Villeneuve, P. J., & Krewski, D. (2002). The influence of ambient coarse particulate matter on asthma hospitalization in children, case-cross-over and time-series analyses. *Environmental Health Perspectives*, 110(6), 575–581.
- Mann, J. K., Balmes, J. R., Bruckner, T. A., Mortimer, K. M., Margolis, H. G., Pratt, B., et al. (2010). Short-term effects of air pollution on wheeze in asthmatic children in Fresno, California. *Environmental Health Perspectives*, 118(10), 1497–1502.
- Maya-Manzano, J. M., Fernandez-Rodriguez, S., Hernandez-Trejo, F., Diaz-Perez, G., Gonzalo-Garjito, A., Silva-Palacios, I., et al. (2012). Seasonal Mediterranean pattern for airborne spores of *Alternaria*. *Aerobiologia*, 28, 515–525.
- Pal, R., Dahal, S., & Pal, S. (2009). Prevalence of bronchial asthma in Indian children. *Indian J Community Med*, 34(4), 310–316.
- Perzanowski, M. S., Sporik, R., Squillace, S. P., Gelber, L. E., Call, R., Carter, M., et al. (1998). Association of sensitisation to *Alternaria* allergens with asthma among school-age children. *Journal of Allergy and Clinical Immunology*, 101, 626–632.
- Preutthipan, A., Udomsubpayakul, U., Chaisupamongkollarp, T., & Pentamwa, P. (2004). Effect of PM₁₀ in Bangkok on children with and without asthma. *Pediatric Pulmonology*, 37, 187–192.
- Reid, C. E., & Gamble, J. L. (2009). Aeroallergens, allergic disease and climatic change: Impacts and adaptation. *EcoHealth*, 6, 458–470.
- Schwartz, J. (2000). Assessing confounding, effect modification and thresholds in the association between ambient particles and daily healths. *Environmental Health Perspectives*, 108, 563–568.
- Simon-Nobbe, B., Denk, U., Pöhl, V., Rid, R., & Breitenbach, M. (2008). The spectrum of fungal allergy. *International Archives of Allergy and Immunology*, 145, 58–86.
- Stieb, D. M., Beveridge, R. C., Brook, J. R., Smith-Doiron, M., Burnett, R. T., Dales, R. E., et al. (2000). Air pollution, aeroallergens and cardio-respiratory emergency department visits in Saint John, Canada. *Journal of Exposure Analysis and Environmental Epidemiology*, 10(5), 461–477.
- Sunyer, J., Spix, C., Quenel, P., Ponce-de-Leon, A., Ponka, A., Barumandzadeh, T., et al. (1997). Urban air pollution and emergency admissions for asthma in four European cities. The APHEA project. *Thorax*, 52, 760–765.
- Taskinen, T., Hyvärinen, A., Meklin, T., Husnan, T., Nevalainen, A., & Korppi, M. (1999). Asthma and respiratory infections in school children with special reference to moisture and mold problems in the school. *Acta Paediatrica*, 88, 1373–1379.
- The British Aerobiology Federation. (1995). Airborne pollen and spores: A guide to trapping and counting. Larkfield, Aylesford, Kent, UK, Kimberley Clark Ltd.
- United States Environmental Protection Agency. Managing Asthma. (2012). Available on: <http://www.epa.gov/iaq/schools/managingasthma>. Accessed on July, 2012.
- Villeneuve, P., Chen, L., Rowe, B., & Coates, F. (2007). Outdoor air pollution and emergency department visits for asthma among children and adults: A case crossover study in northern Alberta, Canada. *Environmental Health*, 6, 40–54.
- Weinmayr, G., Romeo, E., De Sario, M., Weiland, S. K., & Forastiere, F. (2010). Short-term effects of PM₁₀ and NO₂ on respiratory health among children with asthma or asthma-like symptoms: A systematic review and meta-analysis. *Environmental Health Perspectives*, 118(4), 449–457.
- Wilson, A. M., Wake, C. P., Kelly, T., & Salloway, J. C. (2007). Air pollution, weather and respiratory emergency room

- visits in two Northern New England cities: An ecological time series study. *Environmental Research*, 97, 312–321.
- Wong, C. M., Atkinson, R. W., Anderson, H. R., Hedley, A. J., Chau, P. Y., & Cam, T. H. (2002). A tale of two cities, effects of air pollution on hospital admissions in Hong Kong and London compared. *Environmental Health Perspectives*, 110(1), 67–77.
- World Health Organization. (1975). International classification of diseases, 9th revision. Geneva: World Health Organization. Available: <http://icd9cm.chrisendres.com/>. Accessed July 8th, 2008.
- World Health Organization. (1993). International classification of diseases, 10th revision. Geneva: World Health Organization; Available: <http://www.who.int/classifications/apps/icd/icd10online>. Accessed on July 8th, 2008.
- Yeh, K. W., Fang, W., & Huang, J. L. (2008). Increasing the hospitalization of asthma in children not in adults—Form a national survey in Taiwan 1996–2002. *Pediatric Allergy and Immunology*, 19, 13–19.