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Air pollution in Calcutta elicits adverse pulmonary reaction in children

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Background & objective: **Pulmonary responses of children chronically exposed to ambient air pollution in Calcutta have been investigated.**

Methods: **A total number of 153 children from Calcutta and 116 from rural West Bengal in the age group of 6-17 yr were included in this study. Respiratory symptom complex, sputum cytology and micronucleus (MN) count of buccal epithelial cells were evaluated. Blood smears were examined for WBC differential count and RBC morphology.**

Results: **Marked rise in respiratory symptoms (43% in urban vs 14% in rural) and sputum alveolar macrophage (AM) number was observed in urban children compared to their rural counterparts (14.2±1.4 AM/hpf vs 6.7±1.4 AM/hpf, mean±SE, $P<0.001$). The urban group also demonstrated increased numbers of neutrophils, eosinophils and iron-laden AM in their sputum. Besides, buccal epithelial cells of urban children exhibited higher MN frequency than their rural counterparts (0.22 vs 0.17%, $P<0.05$). While sputum neutrophilia and eosinophilia suggest inflammatory and allergic lung reactions, elevated MN count is indicative of greater genotoxic effect on the exposed tissues of urban children. Hypochromic red cells in peripheral blood smear was a common finding in both urban and rural groups, but eosinophils and monocytes were present in elevated frequencies in the rural children.**

Interpretation & conclusion: **The study demonstrated that children inhaling grossly polluted air of Calcutta suffer from adverse lung reactions and genetic abnormality in the exposed tissues.**

Key words Air pollution - children - genotoxicity - lung response - respiratory symptoms

The potential health hazards associated with inhalation of airborne pollutants are now well recognized. In this context, children are reported to be more susceptible to environmental exposures than adults[1,2]. An estimated 4 million children in developing countries die each year from respiratory diseases which are potentially preventable and/or treatable[3]. In addition, some of the adverse effects of air pollution may be obvious in the adult owing to the prolonged latent period[4].

It is therefore important to investigate the health effects of chronic exposure to ambient air pollution among children. We have undertaken a comprehensive study on the lung response to ambient air pollution among the school-going children in and around Calcutta using a panel of sensitive cytological and cytochemical parameters and haematological indices.

Material & Methods

This study was conducted between November 1997 and May 1999. The sample size was decided on the basis of Simple Random Sampling Without Replacement (SRSWOR) method[5] where the

expected mean alveolar macrophage (AM) count was based on published reports[6,7]. The study group consisted of 153 students (81 boys and 72 girls) from five schools of north and south Calcutta. The students were in the age group of 6-17 yr. The control group comprised 116 students (61 boys and 55 girls aged 6-17 yr) from rural schools in South 24-Parganas and Burdwan districts within 100 km from Calcutta where the level of air pollution was much less than in Calcutta due to infrequent vehicular traffic and the absence of factories. The students of the selected schools came from the middle to lower middle income groups.

Sample collection: Each student was asked to answer a questionnaire, either by themselves or assisted by the mother, for information about age, state of health specially respiratory problems like bronchitis, asthma, tonsillitis and allergy to dust, fumes and other environmental contaminants following the procedure of Dockery *et al*[8]. Habits such as smoking, drinking and/or chewing of tobacco were also considered. Biological samples were collected from the children after informed consent of the parents. The children were given a container to collect the deep sputum after vigorous coughing. The non-transparent high viscosity parts of the sputum sample were selected and smears were made on clean glass slides. Buccal mucosa was scraped by a sterile spatula and smeared on the slides. Blood samples were collected from finger tips by pricking with a sterile 24-gauge needle.

Fixation : Smears of sputum samples were immediately fixed in (*i*) ether alcohol (1:1, v/v) for 30 min for Papanicolaou staining; (*ii*) buffered formalin (0.2*M* phosphate buffer, *p*H 7.6 and formalin in 3:1 ratio) for 10 min for non-specific esterase staining; and (*iii*) methanol for 10 min for Perl's prussian blue reaction. Buccal mucosal cells were fixed in absolute methanol for 15 min for staining in Wright-Giemsa and in Carnoy's fixative for 10 min for Feulgen reaction. Blood smears were fixed in absolute methanol for 10 min for staining with Wright-Giemsa.

Staining: Papanicolaou staining was done following the method of Hughes and Dodds[9]. Parallel slides were stained for non-specific esterase, the marker enzyme for macrophages, following the Fast Blue RR method[10]. Deposition of ferric iron in macrophages was evaluated by Perl's prussian blue reaction[11]. Iron deposition in AM was graded subjectively 0-4+ following established criteria[12]. Blood smears were stained with Wright-Giemsa (Qualigens, India) following standard procedure[13].

Cell count: All the slides were coded and scored blindly by three independent observers. Alveolar macrophages in sputum were identified as non-specific esterase-positive cells. Mean AM count was expressed as mean number of cells in 10 high power fields (hpf, 40X objective and 10X eyepiece) of a light microscope (Leitz, Germany).

Statistical analysis: The results were statistically analyzed by Student's 't' test and Chi-square test and $P<0.05$ was considered as significant.

Results

Respiratory symptoms: About 43 per cent (66/153) of the urban children had respiratory symptoms compared to 14 per cent (16/116) among the rural children. Cough, either productive or dry, was observed to be the most common (19%, 29/153) symptom in urban children followed by allergic rhinitis (10%, 15/153). In contrast, these two symptoms were present in only 3 and 2 per cent children in rural areas respectively. Other problems in children of Calcutta were headache (2%, 3/153), wheezing (8%, 13/153), tonsillitis (3%, 4/153) and bronchitis (1%, 2/153). Except for headache (3%; 4/116), the other problems were much less among the rural children.

Sputum AM count of urban and rural children : Thirteen children (9 urban and 4 rural) were unable to produce adequate sputum samples at the time of investigation and produced saliva only. These children were excluded from cytological studies. Children in Calcutta had many more AM in their sputum than their age and sex matched rural counterparts. For example, compared to 6.7±1.4AM/hpf (mean ±SE) in rural children, 14.2±1.4 AM/hpf was recorded in children of Calcutta, thereby showing an increase of about 112 per cent ($P<0.001$, Table I). In Calcutta, boys had slightly higher AM counts than the girls, while in the rural areas girls had slightly higher AM number than the boys. In

Table I. Alveolar macrophage number in sputum of Calcutta school children compared to their rural counterparts

Group	Number studied	AM/hpf, mean±SE
Rural:		
Boys	59	6.3±1.4
Girls	53	7.1±1.3
Total	112	6.7±1.4
Urban:		
Boys	76	15.0±2.1*
Girls	68	13.3±1.9*
Total	144	14.2±1.4*

AM, alveolar macrophage; hpf, high power field (40X objective and 10X eye piece); *$P<0.001$ as compared to the rural group

both the situations the differences between boys and girls were not statistically significant ($P>0.05$). However, the differences in AM counts between rural and urban girls as well as between rural and urban boys were highly significant ($P<0.001$).

Sputum AM number of urban children were found to be much higher in winter (16.4±1.6/hpf) than in the monsoon and summer (9.3±2.0/hpf; $P<0.001$). Similar seasonal variation in AM count was also evident in rural children (7.6±0.8/hpf in winter vs 4.8±1.1/hpf in the rest of the year, $P<0.05$).

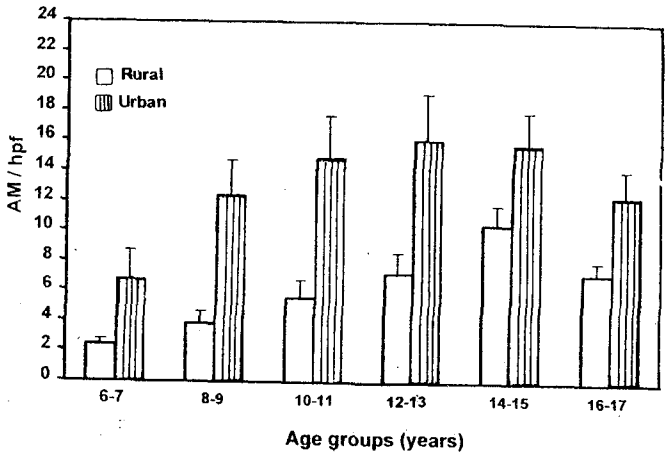

Fig. Alveolar macrophage count (mean ± SE) in sputum of rural and urban children in different age groups. Group A, 6-7 yr (11 rural, 9 urban); B, 8-9 yr (13 R, 16 U); C, 10-11 yr (14 R, 29 U); D, 12-13 yr (20 R, 32 U); E, 14-15 yr (25 R, 44 U); F, 16-17 yr (29 R, 14 U). The differences in AM count between rural and urban children in all age groups are statistically significant ($P<0.05$).

In rural children, highest AM count (10.5±1.3/hpf) in sputum was found in the age group of 14-15 yr, while it was highest in the age group of 12-13 yr (16.0±3.1/hpf) among urban children (Fig.). The differences in AM count between rural and urban children in all the age groups were statistically significant ($P<0.05$).

Differential cell count of sputum: Papanicolaou-stained smears of sputum samples from urban children showed a significantly increased number of neutrophils and eosinophils and markedly reduced number of lymphocytes when compared to rural children (Table II; $P<0.05$). But the relative distributon (%) of epithelial cells and macrophages were more or less unchanged in these two groups of children. However, sputum samples of urban children contained significantly higher number of cells than that of the rural group (63.5±3.1/hpf vs 17.2±2.2/hpf, $P<0.01$). As a result, the total number of sputum macrophages and epithelial cells along with

Table II. Differential cell count of sputum in Papanicolaou-stained smears

Group	Epithelial	Neutrophil	Eosinophil	Lymphocyte	Macrophage
Rural (n=112)	47.2±2.2	29.2±1.9	2.5±0.5	11.5±1.5	9.6±1.9
Urban (n=144)	45.9±1.8	38.7±1.8*	4.7±0.6*	2.5±0.3*	8.3±1.2

Results are expressed in %± SEM. *$P<0.05$ compared to the rural group

Table III. Iron deposition in alveolar macrophages (AM) of rural and urban children

Area	n	Numer of children with different grades of iron in AM		
		Negligible (±)	Mild to moderate 1+ to 2+	High 3+ to 4+
Rural	112	15	89	8
Urban	144	15	90	39*

*$P<0.05$ compared to rural group

Table IV. WBC differential count (%) of rural and urban children

(Data are mean ±SE)

Group	Basophil	Eosino-phil	Neutro-phil	Lymphocyte	Monocyte
Rural (n=112)	0.02±0.01	7.8±0.4*	59.6±1.9	29.5±0.8	3.0±0.2*
Urban (n=144)	0.02±0.01	4.7±0.3	63.1±1.8	31.3±1.0	0.7±0.1

*$P<0.05$ when compared with the urban group

neutrophils and eosinophils in urban children were markedly elevated compared to their rural counterparts.

Iron deposition in AM: Mild (1+) to moderate (2+) iron deposition in AM was recorded in 90 (62.8%) urban children, while sputum macrophages of 39 (26.7%) children had high iron content (3+ and 4+). Only 15 (10.5%) urban children showed negligible amount of iron in AM (Table III). In contrast, iron deposition in AM was mild to moderate in a large majority (89, *i.e.*, 79.7%) of rural children. Sputum AM of only 8 (6.8%) students from the rural areas contained heavy iron deposits.

Micronucleus assay in buccal epithelium: Children in Calcutta had a mean value of 0.22±0.04 per cent micronucleated buccal epithelial cells against 0.17±0.03 per cent in their rural counterparts ($P<0.05$).

Haematological studies: A common finding in both rural and urban children was the presence of hypochromic red cells in the peripheral blood smear. But the severity of the problem appeared to be more acute among the rural students. Differential count of WBCs demonstrated raised eosinophil and monocyte counts in the rural students compared to their urban counterparts (Table IV).

Discussion

Of the 153 school children studied in Calcutta, 144 produced adequate sputum while 112 of the 116 rural children produced satisfactory sputum. Thus, about 3-6 per cent children in our study groups failed to expectorate material from the deeper regions of their respiratory tracts and produced only saliva. The high frequency of sputum producing children in our study is quite unusual because children in comparable age groups are not likely to produce sputum samples unless they are habitual smokers[14]. Only 5.5 per cent of children in our study were occasional smokers. Therefore, the observation cannot be attributed to the habit of smoking. Presumably, high level of ambient air pollution was responsible for this difference. The evidence in support of this assumption comes from the study of Plamenac *et al*[15] showing marked increase in the number of deep sputum producing students in highly polluted urban areas of Yugoslavia compared to that of the less polluted countryside. Our results, however, did not show any appreciable difference between the rural and urban students in this regard. It is possible that the rural environment in this study had pollution levels sufficient enough to act as a threshold for producing deep sputum.

The level of respirable particulate matter (RSPM or PM_{10}, size <10 μm) of Calcutta during the period of study ranged between 133 μg/m^3 and 232 μg/m^3 (annual average)[16] which is far above the National Ambient Air Quality Standard of 60 μg/m^3 (annual average in residential areas)[17]. The harmful effects of RSPM on respiratory diseases and mortality of children have been reported in developed countries[18-20]. The deleterious effect of air pollution on the respiratory symptom complex in pre-school children has also been reported in a study from Lucknow[21]. In conformity with these findings, the present study demonstrated an alarmingly high percentage of respiratory symptoms in the school children of Calcutta.

The elevated number of alveolar macrophages in sputum of children in Calcutta compared to their rural counterparts is consistent with the relatively high level of air pollution in the city. The findings also corroborate those of Mylius and Gullvag[6] who showed a positive correlation between the number of AM and the level of air pollution. Higher AM count during winter months both in the urban and rural groups reaffirms this association. In both the groups, the highest AM count was observed in adolescent children *i.e.*, between 12-15 yr and this

is consistent with the fact that adolescent children are more susceptible to the adverse effect of air pollution[22].

A significant finding of this study is the prevalence of iron-laden macrophages in the sputum of children of Calcutta. Since prussian blue-positive ferric iron in AM generally comes from the disintegration of circulating erythrocytes, high iron content may suggest chronic pulmonary irritation leading to microscopic haemorrhage[23]. Since the pulmonary tract of growing children is more susceptible to insult by air pollutants[24], haemorrhage in lung capillaries is not unlikely.

The presence of cells like neutrophils, eosinophils and lymphocytes in the sputum is indicative of underlying inflammatory and allergic reactions[25]. Hence, the increased number of neutrophils in the sputum of the urban children indicates inflammatory response to air pollution. Similarly, sputum eosinophilia in a large number of children in Calcutta is suggestive of allergic reactions to airborne pollutants. Compared to the rural children, the urban children show adverse lung reaction at the cellular level to increased level of ambient air pollution. Our results are at variance with the reported absence of neutrophils and eosinophils in bronchoalveolar lavage of nonsmokers[26]. This could be explained by the difference in air quality standards between the developed and developing countries as well as differential lung response between children and adults. Haematological studies revealed large numbers of hypochromic red cells in both rural and urban groups suggesting iron deficiency anaemia. But its severity was more pronounced in rural children. Relatively poor hygienic conditions and frequent helminth infestation in rural areas rather than air pollution may account for this.

Our results demonstrating an appreciable rise in micronucleus count among the urban children suggest a higher degree of genetic damage caused by air pollution in this group. Thus, air pollution in Calcutta not only affects the respiratory system of the city's children, but also the genome of their exposed tissues. Since mutation may lead to an array of health problems including the genesis of malignant tumours after a long latent period, the finding has far-reaching consequences.

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