Marked increase in sputum alveolar macrophages in residents of Calcutta: Possible exposure effect of severe air pollution

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Air pollution is a major problem in Calcutta. In this study, exfoliated sputum cytology of the exposed population was done in order to get an insight into the response of the lungs to air pollutants at the individual level. 468 residents from Calcutta and 60 from Sunderban islands, where ambient air pollution is negligible, were studied. Results showed remarkable increase in alveolar macrophage (AM) number in the sputum of the urban group (22.7/hpf) than that of rural controls (2.8/hpf, P < 0.001). Inflammatory cells like neutrophils and eosinophils were also found in increased numbers in the sputum of city dwellers. In the urban group, AM count was highest among residents of north Calcutta where the pollution level was maximum and lowest in the relatively less polluted south Calcutta. Similarly, pollution level and AM count were maximum during the winter, minimum during the monsoon and intermediate in summer. Thus, a close parallelism was observed between the magnitude of air pollution and the AM count in sputum of the exposed persons. Since AM count is simple, non-intrusive and relatively inexpensive the results envisage usefulness of sputum AM count as an indicator of exposure to ambient air pollution especially in large population-based studies in developing countries.

Air pollution is a serious problem in Calcutta. The city air is reported to have high concentrations of total suspended particulate matter, NOx, CO, aromatic hydrocarbons, namely benzene, toluene and xylene, polycyclic aromatic hydrocarbons including benz(a)pyrene, benz(a)anthracene and dibenzanthracene, and heavy metals such as iron, lead and chromium. Besides other toxic effects, many of these polyaromatic hydrocarbons are carcinogenic. The major causes of air pollution in Calcutta are the exponential increase in the number of petrol and diesel-fuelled vehicles coupled with narrow road space (6% of total city area), innumerable small factories in and around the city producing various toxic compounds and burning of coal and wood for domestic cooking in many households.

Pollution of ambient air is generally associated with several health problems. The respiratory tract is the main entry point of air pollutants and the lung is the ultimate target organ for their adverse effects. Thus increase in chronic pulmonary diseases like bronchitis, pneumonia and exacerbation of pre-existing heart and lung diseases is well known in pollution-exposed population. Considering the air pollution scenario in Calcutta, it seems important to study the lung responses among the residents to ambient air pollution. To our knowledge, no such attempt has been made till date. Therefore, we have undertaken this study on the basis of sputum cytology at the individual level.

Alveolar macrophage (AM) are among the first cells to encounter inhaled pollutants in the lower respiratory tract. Their phagocytic capacity provides an efficient nonspecific defence against inhaled particles. In addition, they play a role in the mediation of acute and chronic inflammatory processes in the lung by producing an array of cytokines. An increase in macrophages and leukocytes in the lung has been reported to be the primary lung response to air pollutants in animals and also in humans. Marked increase in the number of AM in the sputum is regarded as the reflection of the reaction of the lung to air pollutants, and the change appeared to be highly sensitive and reproducible. In fact, cytological changes in the sputum are recognized as the most sensitive indicators of the effects of exposure to polluted air. In the light of these reports, we have adopted the exfoliated sputum cytology procedure in the present study for evaluating the lung responses to urban air pollution among the residents of Calcutta.

Materials and methods

This cross-sectional study was carried out during July 1996 and June 1997 in the following six areas of Calcutta with variable severity of air pollution: Bhowanipur, Ballygunge in the south, Behala–Thakurpukur in
the south-west; Moula Ali and B.B.D. Bag in the central
part of the city, Beliaghata in the east, Kidderpore in the
west and Shyambazar in the north. Excluding B.B.D.
Bag, which is the main office-cum-commercial area of
the city, all other places are residential areas with plenty
of small shops and busy traffic.

The study was conducted on 468 Calcutta residents,
352 males and 116 females. As controls, we have exam-
ined 60 individuals who are permanent residents of Sun-
derban islands in the Gangetic delta about 100 km south
of Calcutta where the level of air pollution was negli-
gible.

Sample collection

Each subject was asked to answer a questionnaire for
information about the place of work, duration of outdoor
duties, state of health and smoking habits. Persons with
asthma, chest and heart diseases and/or those who took
medicines daily were excluded. Each remaining person
was given a container to collect the deep cough after
vigorous coughing. The non-transparent high viscosity
parts of the sputum sample were selected and smears
were made on clean glass slides.

Fixation, staining and cell count

Smears of sputum samples were immediately fixed in
ether alcohol (1:1, v/v) for 30 min or in buffered for-
malin (0.2 M phosphate buffer, pH 7.6 and formalin in 3:1
ratio) for 10 min for Papanicolaou and non-specific es-
terase staining, respectively.

Papanicolaou staining was done following the method
of Hughes and Dodd. The fixed slides were brought
to water through graded alcohol and stained with Harris’
haematoxylin for 2 min, washed in running tap water
and dehydrated in 95% alcohol. Thereafter, the slides
were stained in orange-G for 2 min, washed in 95% alcohol
followed by staining in EA-50 mixture for 2 min and
dehydration in absolute ethanol. The slides were cleared
in xylene and mounted in DPX.

Parallel slides were stained for non-specific esterase,
the marker enzyme for macrophages, following the Fast
Blue RR method. The substrate solution was prepared
by dissolving 10 mg of o-naphthyl acetate (Sigma
Chemical, USA) in 0.25 ml of acetone and 20 ml of
0.1 M phosphate buffer (pH 7.4). Thereafter, 100 mg of
Fast Blue RR (Gurr, Germany) was added. The mixture
was filtered directly onto the slides. After incubation for
30 min at room temperature, the slides were washed in
running water for 2 min, dehydrated in ethanol and
mounted in DPX.

Pap and non-specific esterase-stained slides were
coded and scored blindly by three independent observ-
cers. Each observer screened at least 10 high power fields
(40 x objective, 10 x eye piece) in each slide. The aver-
age of the three mean values obtained was then calcu-
lated. The smears were considered as being
representative of the lower part of the airway when ei-
ther cylindrical epithelial cells or alveolar macrophages
or both were found. AM, bronchial epithelial cells and
sputum neutrophils, eosinophils and lymphocytes were
identified by standard criteria.

Statistical analysis

The results were statistically analysed by Student’s t test
and P < 0.05 was considered as significant.

Results

AM count in sputum

The number of AM in the sputum was expressed as the
mean number of cells in 10 high power fields (hpf) of
non-specific esterase-stained slides. It is evident from
Table 1 that the control group had 2.8 AM/hpf in their
sputum samples (Figure 1a). In contrast, residents of
Calcutta had as high as 22.7 AM/hpf in their sputum, i.e.
an increase of about 10-fold over the controls; the dif-
fERENCE being highly significant (P < 0.001, Figure 1b,
c). Among the residents of Calcutta, females had a rela-
tively higher number of AM than males (Figure 1d).

AM population in the sputum in different age groups
of residents of Calcutta is presented in Table 2. Highest
AM number was recorded in the age group of 31–40
years. Interestingly, teenagers (10–20 years) also had
substantially high number of macrophages in the sputum
which was greater than that of the mean value of 40+
adults.

Sputum macrophage numbers of city dwellers were
found to be highest in winter, lowest in monsoon and
intermediate in summer (Table 3). The differences in
AM numbers between winter and summer (P < 0.01),
winter and monsoon (P < 0.01) and monsoon and sum-
mer (P < 0.01) are all statistically significant.

The urban study group of 468 persons were residents
of different parts of the city within the Calcutta Munici-
pal Corporation. Appreciable differences in AM num-
bers were observed among them (Table 4). Highest AM
count of 30.5/hpf was found in residents of Calcutta
whereas residents of south Calcutta had the lowest num-
ber (18.8/hpf).

Tobacco smoking seems to have an enhancing effect
on the sputum AM count. For instance, both in the rural
(control) and urban groups, smokers had a higher num-
ber of AM in the sputum compared to their non-smoking
counterparts (Table 5). However, there was little
Table 1. Alveolar macrophage number in sputum of residents of Calcutta compared with their rural counterparts

<table>
<thead>
<tr>
<th>Control group</th>
<th>n</th>
<th>AM per hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>37</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Female</td>
<td>23</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>2.8 ± 0.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urban group</th>
<th>n</th>
<th>AM per hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>352</td>
<td>20.7 ± 1.2</td>
</tr>
<tr>
<td>Female</td>
<td>116</td>
<td>29.0 ± 2.8*</td>
</tr>
<tr>
<td>Total</td>
<td>468</td>
<td>22.7 ± 1.6**</td>
</tr>
</tbody>
</table>

Results are mean ± SE; n, number of subjects studied; AM, alveolar macrophage; hpf, high power field (40x objective and 10x eye piece); *P < 0.05 between male and female in urban group; **P < 0.001 between control and urban groups.

Table 2. Sputum alveolar macrophage count of residents of Calcutta in different age groups

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>n</th>
<th>Macrophage count/hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–20</td>
<td>95</td>
<td>17.3 ± 1.6</td>
</tr>
<tr>
<td>21–30</td>
<td>199</td>
<td>26.3 ± 1.8*</td>
</tr>
<tr>
<td>31–40</td>
<td>101</td>
<td>29.8 ± 3.0*</td>
</tr>
<tr>
<td>41–50</td>
<td>47</td>
<td>15.3 ± 2.2</td>
</tr>
<tr>
<td>51–60</td>
<td>20</td>
<td>13.9 ± 2.9</td>
</tr>
<tr>
<td>Above 60</td>
<td>6</td>
<td>12.8 ± 3.2</td>
</tr>
</tbody>
</table>

Results are mean ± SE; n, number of subjects; hpf, high power field (400x); *P < 0.05 when compared with groups 1 and 4–6.

Table 3. Seasonal variation in the number of alveolar macrophages (mean ± SE) in sputum of residents of Calcutta

<table>
<thead>
<tr>
<th></th>
<th>Monsoon (July–September)</th>
<th>Winter (October–February)</th>
<th>Summer (March–June)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>106</td>
<td>292</td>
<td>70</td>
</tr>
<tr>
<td>Macrophage/hpf</td>
<td>11.4 ± 1.2</td>
<td>27.2 ± 2.0</td>
<td>18.3 ± 2.4</td>
</tr>
</tbody>
</table>

The differences in AM count during the three seasons are all statistically significant (P < 0.05).

Table 4. Alveolar macrophage count in sputum of persons residing in different areas of Calcutta

<table>
<thead>
<tr>
<th>Area</th>
<th>n</th>
<th>AM/hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>North</td>
<td>64</td>
<td>30.5 ± 3.8</td>
</tr>
<tr>
<td>South</td>
<td>212</td>
<td>18.8 ± 1.7</td>
</tr>
<tr>
<td>East</td>
<td>25</td>
<td>25.9 ± 5.0</td>
</tr>
<tr>
<td>West</td>
<td>30</td>
<td>23.9 ± 5.4</td>
</tr>
<tr>
<td>Central</td>
<td>61</td>
<td>24.4 ± 3.6</td>
</tr>
<tr>
<td>South–west</td>
<td>76</td>
<td>25.3 ± 3.4</td>
</tr>
</tbody>
</table>

Results are mean ± SE; n, number of subjects; P < 0.05 between north and south.

Table 5. Comparison of sputum alveolar macrophage number between tobacco users and non-users

<table>
<thead>
<tr>
<th>Habits</th>
<th>n</th>
<th>No. of AM per hpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>13</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Chewing</td>
<td>3</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>Smoking + chewing</td>
<td>5</td>
<td>3.5 ± 0.6</td>
</tr>
<tr>
<td>None</td>
<td>39</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Urban group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>87</td>
<td>33.7 ± 3.5</td>
</tr>
<tr>
<td>Chewing</td>
<td>92</td>
<td>19.2 ± 1.6</td>
</tr>
<tr>
<td>Smoking + chewing</td>
<td>76</td>
<td>30.6 ± 3.2</td>
</tr>
<tr>
<td>None</td>
<td>213</td>
<td>16.9 ± 1.2</td>
</tr>
</tbody>
</table>

Results are mean ± SE; n, number of subjects studied; hpf, high power field (40x objective, 10x eye piece). In the urban group, P < 0.05 in (i) smokers vs chewers; (ii) smokers vs none; (iii) smokers + chewers vs chewers; and (iv) smokers + chewers vs none.

among residents of Calcutta (Figures 2a and 3) but not in the control group.

Discussion

This study was undertaken to evaluate the pulmonary responses of residents of Calcutta to ambient air pollution. Individual lung reaction was assessed in terms of the number of AM in deep cough of the exposed persons. One of the prime objectives of this work was to ascertain whether the AM count of the sputum could be used as a sensitive indicator of exposure to ambient air pollution. The present results showing markedly elevated AM count in the sputum of the residents of Calcutta compared to their rural counterparts seem to be compatible with the general air pollution scenario of the city. In agreement with the present findings, sharp increase in the number of AM in the sputum has been reported in persons occupationally exposed to severe air pollution8–11.

Our results are also in good agreement with the estimate of seasonal variation in the air pollutants in the

_difference in AM count between tobacco chewers and those who never smoked or chewed tobacco.

Sputum cytology

Residents of Calcutta had more nucleated cells in their sputum than their control counterparts. Examination of Pap-stained smears in these two groups revealed that while the urban group had an average of 63 cells/hpf, the rural group had only 16 cells/hpf (Figure 2a, b). A comparative study of the leukocytes in the sputum between the rural and urban groups revealed a sharp increase in the number of neutrophils, eosinophils and lymphocytes in the urban population (Table 6). Sputum neutrophilia with sheets of neutrophils were often found
city's ambient air. The total suspended particulate matter (SPM) was found to be highest in the city during winter. In a recent study, Samanta et al. reported that the average SPM concentration in Calcutta during winter is as high as 1181 μg/m³. In contrast, the lowest SPM value was reported during the rainy season when Calcutta's air is comparatively clean due to heavy rainfall. SPM value during the summer months was also low as winds with high velocity blow away the pollutants and the air becomes relatively less polluted. An exactly similar picture emerged from our study: highest AM count in winter, lowest during rainy season and

![Figure 1](image.png)

**Figure 1.** Alveolar macrophages in sputum of pollution-exposed individuals of Calcutta. a, cab driver; c, student; d, housewife. Note the high AM count in these persons when compared with that of a rural control (b). Non-specific esterase-stained, 400x.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Calcutta group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrophage</td>
<td>23.2 ± 4.4</td>
<td>18.8 ± 2.5</td>
</tr>
<tr>
<td>Epithelial</td>
<td>20.3 ± 2.7</td>
<td>17.0 ± 2.2</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>47.1 ± 4.6</td>
<td>47.9 ± 3.6</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>9.3 ± 3.1</td>
<td>16.2 ± 3.4</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.01 ± 0.01</td>
<td>0.1 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>No./hpf</td>
<td>No./hpf</td>
</tr>
<tr>
<td>Macrophage</td>
<td>3.7 ± 0.7</td>
<td>14.8 ± 1.6*</td>
</tr>
<tr>
<td>Epithelial</td>
<td>3.2 ± 0.4</td>
<td>10.7 ± 1.4*</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>7.3 ± 0.7</td>
<td>30.2 ± 2.3*</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>1.5 ± 0.5</td>
<td>10.2 ± 2.1*</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>0.001 ± 0.001</td>
<td>0.06 ± 0.02*</td>
</tr>
</tbody>
</table>

Results are mean ± SE; hpf, high power field (40x objective, 10x eye piece); *P < 0.05 when compared with controls; Total no. of cells per hpf was 16 and 63 in control and residents of Calcutta respectively.
intermediate during summer. A close parallelism also exists between the present findings with respect to variability in AM count among the residents in different parts of the city and the reported air quality in terms of SPM content in these regions. The highest SPM concentration was found in the northern parts of the city, viz. Shyambazar and adjoining areas and minimum in the south. A strikingly similar trend was recorded in our study: the highest AM count among the residents of north Calcutta and the lowest in residents of the southern parts. Taken together, our observations underscore a positive relationship between the level of pollution in the city as reported by several investigators and the number of AM in the sputum of the exposed persons.

Besides macrophages, marked difference was also observed in the number of leukocytes in the sputum between the urban and rural (control) groups. Subjects of Calcutta inhaling polluted air had 30 or more neutrophils per hpf (400x). Even a lower number of 10 neutrophils per field is indicative of pathological change in the lung. It is reasonable to assume, therefore, that the pollution-exposed individuals in our study group are probably in the danger of developing pulmonary diseases. Presence of eosinophils and neutrophils in the sputum in increased numbers has been reported to be associated with respiratory obstructions. Sputum eosinophilia has also been reported among smokers with bronchial allergy and consequent respiratory problems. Based on these reports our finding of nearly seven-fold rise in sputum eosinophil number in residents of Calcutta assumes great significance.

Interestingly it has been reported that bronchial asthma is rapidly increasing in the residents of Calcutta, which is compatible with our observation of sputum eosinophilia. On the other hand, Lacost and Maestrelli et al. demonstrated a close association between sputum neutrophilia and airway obstruction. Accordingly, the presence of inflammatory cells in ele-
vated numbers in the sputum of residents of Calcutta indicates underlying respiratory problems. The quality of the city's air is likely to account for this. Indeed, diseases of the respiratory system excluding pulmonary tuberculosis are responsible for a high number of deaths (11.4%) in this city\textsuperscript{18}. As for the mechanism of sputum neutrophilia, the change could be mediated by the macrophages since human alveolar macrophages have been shown to prevent apoptosis of polymorphonuclear (PMN) cells\textsuperscript{19}. It was also suggested that the AM suppress PMN apoptosis as long as PMN are needed to eliminate microbes and other offending agents from the pulmonary parenchyma. Since the exposed persons in this study are constantly inhaling air-borne pollutants, PMN seem to be needed for an extended duration. Hence, prevention of PMN apoptosis by AM appears to be a likely phenomenon in our subjects.

High AM count can occur due to several reasons. Increased turnover of monocytes from the blood or proliferation of the resident pulmonary macrophages could elevate the AM count. The first possibility, i.e. egress of mononuclear cells from the circulation seems unlikely because we have not recorded any drop in the number of monocytes in the peripheral blood. Alternatively, proliferation of resident macrophages as observed in some studies\textsuperscript{20} is a distinct possibility to combat the assault of air-borne pollutants on the pulmonary tissues. This hypothesis is supported by the fact that increase in inflammatory cells and macrophages in the lungs is the primary response to challenge by most air pollutants in laboratory animals\textsuperscript{7}.

Another interesting finding which emerged from this study is that highest AM count was found in relatively younger age groups of 21–40 years rather than in older people who are exposed to the city's polluted air for a longer duration. The reason for this is as yet unexplained but could be attributed to the habit of smoking which is more prevalent in the younger age group. Also more outdoor activities in this group may have some bearing on their sputum AM count.

Increased number of AM in the sputum did not appear to be specific for a particular pollution source, rather it seems to be in response to pollution in general. Elevated AM count among the urban subjects in variable age groups and the parallelism between AM number and the seasonal variation in air quality support this contention. This makes sputum AM count a good bio-indicator.

In conclusion, we found the AM test to be highly sensitive for evaluating the lung response to ambient air pollution. AM test appears to be rapid, sensitive, simple and relatively inexpensive for the assessment of lung response to air pollution in humans. Hence, the test is ideally suited for biomonitoring of air pollution particularly in the developing countries.


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