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Dusty Air Pollution is Associated with an Increased Risk of Allergic Diseases in Southwestern Part of Iran

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ABSTRACT

Concerns have been raised about the adverse impact of dusty air pollution (DAP) on human health. The aim of this study was to find the association between dusty air pollution based on air quality index (AQI) and the risk of allergic diseases in southwestern provinces of Iran, with assessing cytokine profiles and lymphocyte immunophenotypes.

In this case control study 148 individuals participated. The sampling was done in hazardous condition (AQI >300) as the case and clean air (AQI <50) as the control. We measured cytokine production by using ELISA method and phenotypes of T-lymphocytes (CD4+ and CD8+), CD19+ B-lymphocytes, CD25+, CD4+ CD25+ cells by FACSort flow cytometer.

The mean serum level of IL-4 (33.4±2.9 *vs* 0.85± 0.65 pg/dl) and IL-13 (15.1±4.4 *vs.* 0.12±0.7 pg/dl) in the subjects exposed to ambient DAP was increased significantly compared to the individuals in the clean air condition. Also, CD19+ B-lymphocytes (12.6± 4.9 vs 8.9±3.2%) and CD4+ CD25+ cell count (13.6± 4.6 vs 7.7± 3.8%) in peripheral blood were increased significantly in subjects exposed to ambient DAP compared with the controls.

The result of our study suggested that ambient DAP affected immune system in a way that might lead to allergic diseases in the population.

Keywords: Allergy; Dusty air pollution; Immune system

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INTRODUCION

Dusty air events have been occurring in southwestern cities of Iran, one of which is Bushehr. Dusty air pollution (DAP) in Iran originates from the deserts of neighboring countries including Saudi Arabia, Iraq, Syria and is the main cause of meteorological phenomenon in Iran.[1] Public concerns about the possible adverse effects of the phenomenon have increased, because the occurrence of dusty air events have become more frequent during recent years.[2] The DAP events lead to increased levels of particulate matter (PM_{10}). Epidemiological studies have shown that the high levels of ambient PM are associated with increased respiratory and cardiac morbidity and mortality.[3-5] A detailed study has been carried out to determine components of DAP in Iran, and some studies have reported that the size of dust particles are mostly less than 1 μm in diameter.[1] The main source of DAP composition are clay, minerals (especially Ca^{2+}, Na^{+}), quartz as well as microorganisms and pollens (fungi as Cladosporium, Alternaria and Aspergillus). Also there are various other plant-derived materials that induce allergic inflammation.[1,6]

With respect to DAP composition, exposure to these compounds and elements could have impact on immune system towards allergic diseases. Recent studies reported an association between PM and absolute number of B-cells, CD4+, CD8+ T-cells, and natural killer (NK) cells in cord blood and school children.[7,8] They reported that air pollution caused a decrease in the percentage of T-lymphocyte, and an increase in NK cell count among the residents. Moreover ambient PM pollution causes oxidative stress and may influence allergic, immunologic and systemic inflammatory responses.[7,9]

Descriptive epidemiological studies in Iran show that allergic diseases are more common among children and adolescents.[10-12] The goal of the present study was to determine the effects of DAP on the immune system lymphocytes including CD4+, CD8+ as T-lymphocytes, CD19+ as B-lymphocytes, CD4+ CD25+ cells as regulatory T lymphocytes with especial regard to helper T lymphocyte 2 (Th2) responses that lead to allergic diseases in the population living in Bushehr, Iran.

MATERIALS AND METHODS

DAP Events Data Based on Meteorological Reports

We obtained DAP data on daily ambient air pollution level of PMs including PM_{10} from Bushehr National Meteorological Research Center (BNMRC). The concentration of fine PM_{10} calculated by BNMRC was extracted daily. With respect to BNMRC daily reports on air quality index (AQI), as a measure of the condition of air relative to the requirements of one or more biotic species or to any human need or purpose, we considered AQI <50 (as clean air) and AQI >300 (as hazardous condition). Blood samples were collected by venipuncture from those exposed to hazardous condition (as the case group) and those with clean air (as the control) for a period of one year. The study was approved by the ethics committee of Medical University of Bushehr and written informed consent was obtained from the parents of all the participants.

Demographic Data and Sampling

In this case control study, 148 subjects (age, 20-59 years) were chosen and divided into two groups; 97 participants (65.5%) were exposed to hazardous condition as the case and 51 persons (34.5%) were in the clean air as the control. All the subjects (cases and the controls) were exposed to DAP several times before sampling because of living in Bushehr. The blood sampling was done for cases in hazardous condition days while it was done for the controls in clean air days. The blood samples were collected from blood donors at Bushehr Blood Transfusion Organization (BBTO). Demographic data including age, gender, atopic diseases, place of living and other clinical conditions of the participants were assessed. We excluded atopic subjects and those who did not refer for the next sampling.

Serum Cytokines Measurement

The serum samples were collected and stored at -70°C until the analysis. IFN-γ (the main Th1 cytokine with antiallergic effects), IL-4 and IL-13 (the main Th2 cytokine which induces allergic inflammation) and IL-10 (an Immunoregulatory cytokine) were measured by ELISA method (U-CyTech biosciences, Utrecht, The Netherlands). The sensitivity of the method was 2 pg/ml.

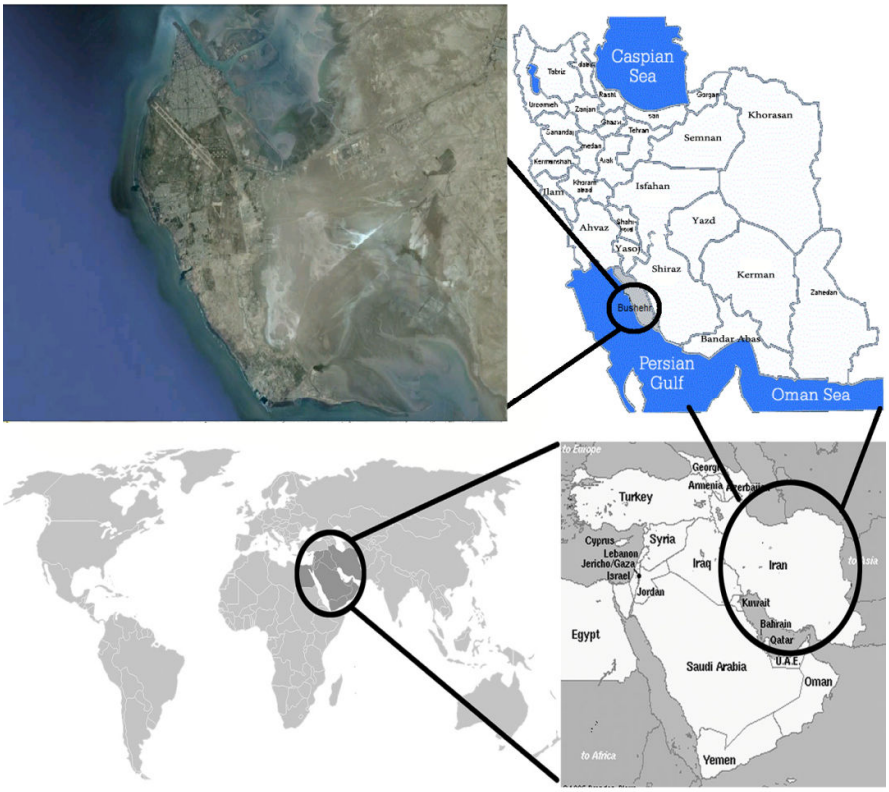


Figure 1. Geographic location of study area

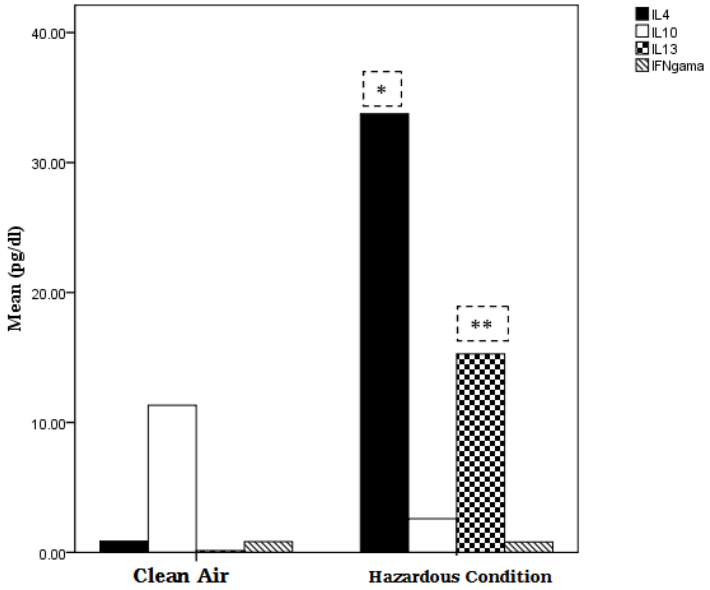
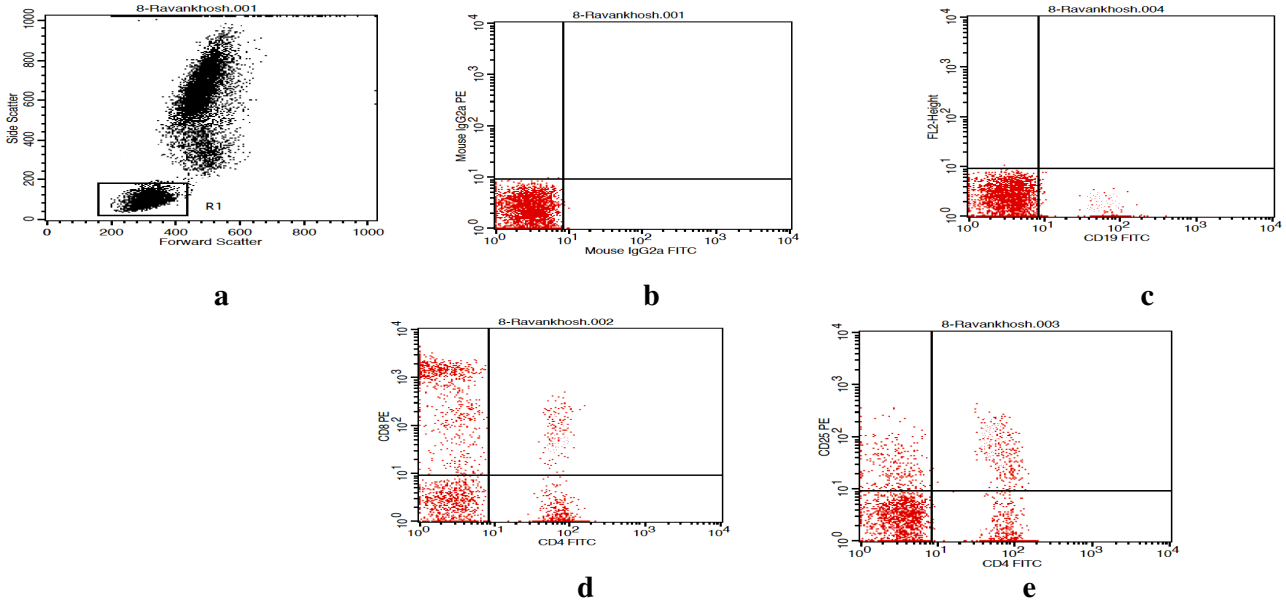


Figure 2. Serum cytokine levels (IL-4, IL-10, IL-13 and IFN-γ) of general population after exposure to ambient DAP compared with controls. *P*<0.05 for both IL-4 and IL-13

A. Hazardous Condition



B. Clean Air

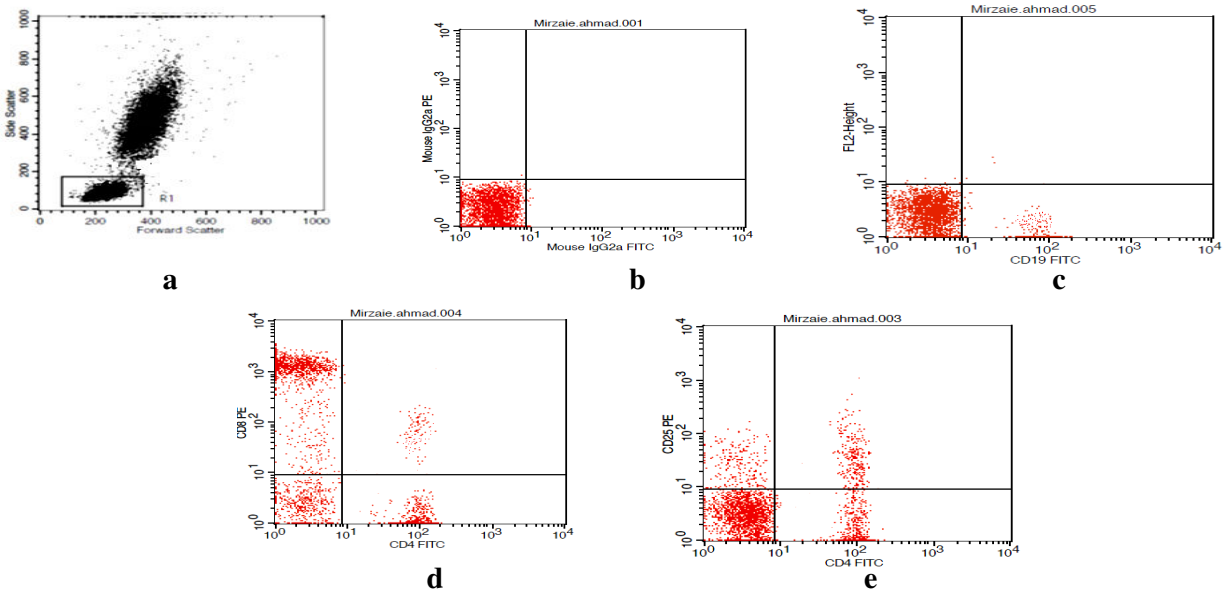


Figure 3. Immunophenotyping analysis of subjects by flow cytometry after exposure to ambient DAP in comparison to the controls. A) in hazardous condition: a. selected PBMC, b. double negative control, c. CD19+ lymphocytes, d. CD4+ and CD8+ lymphocytes, e. CD4+ and CD25+ lymphocyte. B) in clean air: a. selected PBMC, b. double negative control, c. CD19+ lymphocytes, d. CD4+ and CD8+ lymphocytes, e. CD4+ and CD25+ lymphocytes.

Immunophenotyping Assay by Flow Cytometry

Venous blood samples were collected into heparinized Vacutainers (5-mL Vacuette; Greiner, Kremsmuenster, Austria). Lymphocytes obtained pfrom whole blood were immunophenotyped using a FACSort flow cytometer and monoclonal antibodies (Becton Dickinson Immunocytometry Systems, San Jose, California, USA), then lymphocyte subsets including CD4+, CD8+ T-lymphocytes, CD19+ B-lymphocytes, CD25+ and CD4+ CD25+ cells were measured (Figure 3).

Statistics

The effect of DAP ambient exposure on alteration of serum cytokine profiles and on lymphocyte subsets were compared using Mann-Whitney U test and independent T tests. Comparisons of the other results in both, hazardous air and clean air were also done using Chi-Square Test. *P* values of <0.05 were considered statistically significant. Statistical analysis was performed using SPSS 16 software package (San Diego, CA).

RESULTS

Demographic data of the subjects is shown in Table 1. Regarding age, no difference was found between the two groups. Statistical_test for age difference was not done, because almost all subjects were male who referred to BBTO for blood donating.

Effect of Ambient DAP Exposure on Cytokines Production

The serum levels of IFN-γ, IL-4, IL-10 and IL-13 of the two groups are shown in Table 2. There was no difference in the mean serum concentration of IFN-γ in individuals who were exposed to ambient DAP as compared with the controls (p= 0.8). The mean serum level of IL-4 and IL-13 in the case group were significantly higher than the control (p= 0.001). There was no difference in the mean serum concentration of IL-10 between the two groups (p=0.1) (Table 2).

Effect of Ambient DAP Exposure on Lymphocyte Phenotype Subsets

The data from flow cytometric analysis showed significant rise in CD19+ cells (B lymphocytes) in the population upon exposure to ambient DAP compared to the controls (p= 0.035). Also it was found that the percentage of CD4+CD25+ cells were significantly increased in the case group compared to the control (p=0.004). On the contrary, the number of CD4+, CD8+ and CD25+ lymphocytes in the case group were not changed after exposure to ambient DAP as compared to the control (p= 0.1, 0.2 and 0.8, respectively) (Table 2).

In addition, our results indicated that subjects with higher weight, exposed to ambient DAP several times (more than 10 in the past), smoking and being undergraduate, secreted significantly higher serum levels of IL-4 and IL-13 (p=0.03). Meanwhile, the above factors did not influence the serum levels of IFN-γ and IL-10.

Table 1. Demographic data of subjects exposed to ambient DAP as case group in comparison with control group in clean air

	Hazardous Condition (Case Group)	Clean Air (Control Group)
Age (y)	35.7±9.1	34.2±9.3
Sex		
Male	96 (99%)	48 (94%)
Female	1 (1%)	3 (5.9%)
Weight (Kg)	81.2±10.3	85.2±12.6
Smoking	20 (20.6%)	19 (37.3%)
Graduation		
Undergraduate (≤ Diploma)	65 (67%)	39 (76.4%)
Postgraduate (> Diploma)	31 (33%)	12 (23.6%)
PM2.5 (µg/m3)	801.5±12.6	58.3±8.2

Y= Year; Kg= Kilogram

Table 2. Serum cytokine profiles and lymphocyte counts of individuals exposed to ambient DAP in comparison with control group

	Hazardous Condition (Case Group)	Clean Air (Control Group)	*P* value
IFN-γ (pg/dl)	0.79±0.6	0.82±0.6	0.8
IL-4 (pg/dl)	33.4±2.9	0.85± 0.65	0.001
IL-10 (pg/dl)	2.5±0.9	11.3±6.2	0.1
IL-13 (pg/dl)	15.1±4.4	0.12±0.7	0.001
CD4+ Cells (%)	40.4±6.7	35.8± 7.4	0.1
CD8+ Cells (%)	35.1±5.7	38± 5.7	0.2
CD19+ Cells (%)	12.6±4.9	8.9± 3.2	0.035
CD25+ Cells (%)	10.2±1.6	10.8± 7.2	0.8
CD4+CD25+ Cells (%)	13.6±4.6	7.7± 3.8	0.004

Pg/dl= Picogram/ Deciliter

DISCUSSION

To the best of our knowledge, this is the first study that shows the effect of DAP exposure on the immune system. The results of the present study indicated that subjects exposed to ambient DAP secrete higher levels of serum IL-4 and IL-13 cytokines compared to the controls. It is well known that IL-4 and IL-13 cytokines are factors involved in the development of allergic responses through the induction of Th2 lymphocytes.[13,14] In an experimental study, it was shown that bronchitis and alveolitis occurred after mice were exposed to Asian sand-dust (ASD).[15] Also it has been reported that ASD increases neutrophils by chemokines and cytokines including IL-12, tumor necrosis factor- α (TNF-α), IL-1β, IL-6, IL-12 and IL-17A in bronchoalveolar lavage fluid (BALF).[15] In the same experimental setting it was observed that the mice exposed to ovalbumin (OVA) and SAD, enhanced eosinophil and neutrophil infiltration in their airway [15]. Meanwhile, the cellular profile of BALF was changed by the production of OVA-specific IgE, IgG1 and the expression of Th2- associated molecules.[15] In another experimental study it was found that ASD containing mineral particles and/or the microbiological materials in the air, caused neutrophilic lung inflammation, while subjects co-exposed to ASD and OVA, enhanced eosinophilic lung inflammation related to IL-5.[16]

Recent epidemiological studies have shown that ASD can deteriorate allergic diseases, such as asthma, allergic rhinitis and atopic dermatitis. [17-20] The mechanism of action of ASD in worsening the skin and asthma symptoms may be related to allergic reaction to metals and mineral elements such as SiO2 which is the main component of Asian dust particles. It may also be as a result of secretion of Th2-related cytokines (IL-4, IL-5 and IL-13) [20]. The impact of some pollens in the form of suspended particulate matter have been reported in the deterioration of skin symptoms [21]. The results of our study suggested that increase in the serum level of IL-4 and IL-13 following ambient exposure to DAP may cause the stimulation of B cells which leads to the production of antigen specific antibodies and also promotes allergic inflammation. DAP present in southwestern provinces of Iran contains microorganisms, pollens and molds.[1] With reference to the results from our and the other studies, it seems that increase in the serum level of IL-4 and IL-13 as the result of ambient exposure to DAP affects the immune system shifting towards Th2 lymphocytes.[15, 16]

In addition, our results showed that CD19+ and CD4+CD25+ lymphocyte counts of the subjects exposed to ambient DAP increased significantly. This was in line with a European study which reported that air pollution was significantly associated with absolute number of B-cells, CD4+, CD8+ T-cells and NK cells in school children.[8] Moreover, the result of another study showed that ambient exposure to 12 polycyclic aromatic hydrocarbons (PAHs) and PM2.5 during the last 2 weeks of gestation was associated with an obvious increase in the B-lymphocyte (CD19+) fraction and a decrease in the percentage of T-lymphocytes in cord blood, which was in agreement with our results.[7]

Moreover, studies have shown that aromatic air pollution induced non-specific airway reactivity, increased numbers of neutrophils, B-lymphocytes, and

increased mast cell and neutrophil numbers in biopsy specimens [22, 23]. Also, animal and in vitro studies have assessed the effect of air pollution on immune system and allergy. They demonstrated that air pollution shifted primary immune responses towards a Th2 phenotype, characterized by production of antigen-specific IgE, and enhanced allergen-induced immune responses, including increasing IgE production and enhancing cytokines involved in eosinophilic or allergic inflammation, especially IL-4 and IL-5, as well as airway hyperresponsiveness. Air pollution have also been reported to induce B-lymphocyte immunoglobulin isotype switching to IgE.[24]

We concluded that frequent exposure to DAP may lead to increase allergic inflammation through the increase in secretion of IL-4, IL-13 and the percentage of CD4+ CD25+ cells and CD19+. Moreover, our previous study (has not published) showed that the prevalence of allergic diseases was increasing in Bushehr. Finally it is suggested that exposure to ambient DAP could be a cause of increase in the prevalence of allergic diseases in the population living in the southern part of Iran. Further studies is needed to reveal the specific impacts of DAP on the immune system.

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