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Airborne- Benzene and Its Effect on Blood Indices of 10-12 Years Old Children in Four Regions of Tehran

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ABSTRACT

Background: *There is increasing evidence that environmental pollutants may be contributing to an increase in acute and chronic diseases in a wide range of general population, especially children. Benzene is considered as an important air pollutant of the metropolitan areas.*

Materials and Methods: *The concentration of benzene was measured in four selected regions of Tehran (Bazaar, Dolat-Abad, Chizar, Gheitarieh & Darabad) during a 6-month period. Ambient concentration of benzene in Tehran was in correspondence to overall pollution levels in the air.*

Results: *Mean exposure levels of benzene for male students (10-12 years of age) of the above mentioned areas were 203, 98, 18, and 15 ppb respectively. This measurement for female students (of the same age group and regions) was calculated as 115, 98, 18, and 15 ppb respectively. Differences of peripheral blood cell parameters between male students in different regions were more significant than the differences among female students and this phenomenon could be attributed to lesser exposure of females to air- borne benzene. In general, correlation of peripheral blood contents of HCT, HGB, RBC and Fe of male group was significant in regard to the amount of airborne benzene in different regions of Tehran. Correlation of immune indicators of blood CD_4/ CD_8 of female group was also significant in regard to the amount of airborne benzene.*

Conclusion: *Considering the results of this study and ambient standard of benzene in the European countries at 5 ppb, control of air pollution in Tehran should receive special consideration and the health of school children in polluted areas, must be under continuous surveillances.* ***(Tanaffos 2005; 4(13): 47-55)***

Key Words*: Benzene, Benzene exposure, Air pollution, Environmental exposure, Environmental pollutant, Children's health, CD4/CD8 index, Tehran.*

INTRODUCTION

Benzene is one of the most commonly utilized chemical compounds. It is widely used for the production of petrochemical and hydrocarbon products such as styrene, phenyl compounds, gums, detergents, insecticides, medications, paints and plastic materials etc. (1). Considering the high octane number of benzene, it is one of the important constituents of gasoline. Its concentration in lead freegasoline has been reported up to 5 percent.

According to the report of "Air quality control

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committee" of the United Kingdom in 1997, the level of benzene in urban area was about 2-10 ppb(2). Generally, benzene released from internal combustion engine of motor vehicles was hold responsible for 10% of the Acute Lymphoid Leukemia observed in the United Kingdom (3).

In 1997, a Japanese group performed a research in Tehran studying the level of air pollution. The level of benzene in the air of major streets and squares of Tehran was measured in range of 4-187 ppb (4).

Majority of workers in various chemical industries are exposed to benzene and its products. In the United States of America about 2 million people are exposed to this chemical compound as a result of their occupation. Also it was observed that rate of leukemia in the above mentioned workers with more than 5 yrs. of occupational exposure was 21 times higher than general population. In those who had less than 5 yrs. of occupational exposure, this figure was 6 times (5). Individuals that are somehow in contact with benzene suffer from different blood related malignancies such as chronic myeloid leukemia, acute myeloid leukemia and pre-leukemia. Pre-leukemia reveals itself in the form of pancytopenia, thrombocytopenia, hemolytic anemia and macrocytosis (6). According to a study conducted by WHO in 1996, risk factor of an environmental benzene concentration of 1 $\mu g/m^3$ and life time exposure, was reported as one case of blood malignancy out of million people exposed (10^{-6}). Based on the recommendation of Environmental Organization of the United Kingdom, yearly mean concentration of airborne benzene should be regulated less than 5 ppb. However, environmental standard was advised to be set at 1 ppb level. It is also reminded that there is no specific level of airborne benzene concentration that can be considered as completely safe. However, concentration of 1 ppb in the air is not associated with any significant risks (7).

Benzene enters the human body in various ways. These routes are via respiratory, skin and gastrointestinal systems. Maximum absorption is through the respiratory system. Although the mechanism of genotoxic effects of benzene in the body is unknown, the role of oxidation in causing tissue damage has been shown in different studies and Reactive Oxygen Species such as O_2^-, H_2O_2, OH° formed in biotransformation of benzene (8-9) are responsible for the oxidative tissue damages.

According to the results from previously conducted studies the initial biological manifestation of benzene exposure is alteration in the blood components. These changes are observed in the total number of white blood cells (WBC), WBC differentiation, red blood cells (RBC) and platelets (10). In another study, the biological effect of benzene and aromatic compounds exposure, was reported in term of depression of CD_4/ CD_8 ratio (11).

Considering the progressive increase in the air pollution of Tehran in the past years and also the emergency status that was announced in 1998 as the result of severe air pollution, necessitated the conduction of a research that studied and evaluated the complications that was associated with the environmental exposure to benzene in city of Tehran.

The aim of this study was to determine the environmental concentration of benzene in the city of Tehran and also to evaluate its effect on the hematological indices of 10-12 yrs. old children in 4 areas of Tehran.

MATERIALS AND METHODS

Study population:

Based on the report of air pollution from different areas of Tehran by a Japanese group in 1997, four districts were chosen for this study. In previous study, these districts were considered as clean, slightly polluted, relatively polluted and greatly

polluted (4). In each of these areas two primary schools (girl and boy schools) were randomly picked out. After statistical consultation a total of 216 students were selected. They were divided into 4 groups, having equal number of boys and girls. The parents of the school children were informed about the objectives of the research and a written consent in regard to collecting blood from the subjects was obtained. Prior to monitoring of school children, information regarding socio-economical and health status of subjects was obtained through a questionnaire. Ill-health subjects with chronic blood, respiratory and cardio-vascular disorders receiving therapeutic drugs were excluded from the study. It must be added that all school children in this study were attending governmental primary schools and lived in a close proximity of their schools.

Air sampling of benzene and statistical analysis:

Since, the children lived near their schools, air monitoring was conducted in each schools. The duration of sampling was 6 months. Air samples were obtained from the class rooms (at least 15 times) for 8 hr. Air sampling and analysis of airborne benzene was carried out according to a validated modified method from OSHA (12). In this method environmental air is pumped by a personal sampler pump over a charcoal absorbing tube for duration of 8 hours with flow rate of 20 ml/minute. Activated charcoal tubes were then capped and kept in refrigerator at -20°C until the time of analysis. At the time of analysis of air samples, initially organic material absorbed by the active charcoal bed was extracted by CS_2 solvent and later 2 µl of the sample was injected into the gas chromatography apparatus (with FID detector) and the concentration of benzene in the sample was measured. Definite benzene identification GC chromatograms were cross checked by GC-MS analysis for the 10 percent of samples.

Methods of sampling and blood analysis:

After obtaining the written consent from the parents, 6-8 ml of blood was collected from each student and transferred in lithium heparinized tubes. The tubes were shaken and sent rapidly to the laboratory for analysis. Complete blood count (CBC) was performed according to Davidson et al. method (13). The blood analyses consisted of: hemoglobin (Hb), WBC, RBC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCV), mean corpuscular hemoglobin concentration (MCHC), corpuscular platelet count, differentiation count of WBC, percentage and counts of CD_4 and CD_8 were analyzed by flowcytometric method (14).

Statistical analysis:

Socio-economical status of school children in four regions of Tehran in term of family income and level of education of parents were examined by ANOVA and chi-square respectively. These parameters were matched statistically by exclusion of a few subjects. ANOVA was also used to compare the mean blood indices in the four air monitored regions. A p-value less than 0.05 demonstrated the presence of a significant statistical difference in the blood indices in the four areas of research.

In order to determine the nature of difference in the indices and also to study its relation with level of air pollution, Spearman's coefficient test was used. A significant coefficient pointed towards the relation of increased (decreased) indices with the level of air pollution in the four areas of study.

RESULTS

The concentration of airborne benzene in the classrooms in the regions of Bazaar, Dolat-Abad, Cheezar- Gheitarieh and Darabad were: 203±35 ppb, 115± 16 ppb, 98±23 ppb, 18±4 ppb and 15±3 ppb respectively.

Meanwhile, the blood indices of both male and female subjects (table 1,2) in four regions were calculated and compared. Data were analysed using ANOVA. Significant results were as follows:

1. RBC and Hb values in boys showed significant differences in different areas of study ($p<0.05$)

2. Iron (Fe) values in girls in various areas of study were significant ($p< 0.05$).

Table 1. Blood indices in male subjects (mean ±SD) in the 4 areas of study.

Blood indices	Fe	Fe- TIBC	ferritin	WBC	RBC	Hgb	Hct	MCV	MCH	MCHC	Plt	CD_4/CD_8
Areas												
Baazar n=33	4.72±14.3	389±41.3	44.4±22.9	6.9±1.7	5.1±0.3	14.1±1	41.5±3.7	80.6±4.7	27.5±1	34.2±1.9	269±40	1.2±0.3
Dolatabad n= 17	101±45	624±99	29.6±17	6.6±1.9	5.3±0.4	14.7±1.1	42.5±2.7	79.8±2.8	27.5±1.3	34.4±1.1	305±73.2	1±0.3
Gheitarieh n-21	100±31.4	363±47.8	39.8±17.1	6.4±1.7	5.4±0.4	15±0.9	42.6±2.5	78.5±2.9	27.7±1.1	35.3±0.8	292±59.6	1.2±0.2
Darabad n=30	121±93	418±185.3	41.7±23.4	5.6±1.3	5.5±0.4	14.9±1	42.9±2.4	78.4±6	27.2±2.5	34.7±1	278.6±45.3	1.2±0.6

Table 2. Blood indices in female subjects (mean ±SD) in the 4 areas of study.

Blood indices	Fe	Fe- TIBC	Ferritin	WBC	RBC	Hgb	Hct	MCV	MCH	MCHC	Plt	CD_4/CD_8
Areas												
Baazar N=28	89±37	380.7±55.4	38.8±29.2	6.2±2.07	5.3±0.4	14.6±1.05	32.3±2.9	79.5±6.8	27.3±2.5	34.40±0.98	27±63	1.9±0.39
Dolatabad N= 18	11±41	347±124	26.3±13.1	6.3±1.8	5.4±0.3	15±0.3	43.3±1.9	80.3±3.2	27.9±1.5	34.7±0.9	168±46	0.3±1.3
Gheitarieh n-25	7.2±32.3	392±71.4	36.7±19	6.1±1.2	0.5±0.3	15±1.02	43.1±2.5	78.1±5.2	27.3±2.2	38.9±0.9	267±37.9	1.3±0.30
Darabad n=28	86.4±25.1	354±82.7	38±36.9	5.9±1.5	5.7±1	15.3±5.3	43±8	76.1±11.4	27.2±3.3	43.4±46.9	26±70.9	1.1±0.3

Table 3. Correlation analysis of blood indices in males and females with the degree of benzene air pollution.

Blood indices	Correlation parameters males			Correlation parameters females		
	No. of subjects	Correlation coefficient	p- value	No. of subjects	Correlation coefficient	p- value
Fe	70	0.245*	0.041	99	-0.060	0.558
Fe- TIBC	70	-0.045	0.712	99	-0.123	0.230
Ferritin	71	0.019	0.876	99	0.007	0.945
WBC	71	-0.174	0.148	99	-0.055	0.592
RBC	71	0.344**	0.003	99	0.00	0.998
Hgb	71	0.360**	0.002	99	-0.031	0.757
Hct	71	0.256	0.031	99	-0.128	0.207
MCV	71	-0.048	0.790	99	-0.167	0.099
MCH	71	0.053	0.663	99	0.00	0.999
MCHC	71	0.032	0.789	99	0.124	0.222
Plt	71	0.107	0.374	99	0.01	0.921
CD_4/CD_8	70	-0.033	0.788	99	-0.204*	0.043

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Table 4. ANOVA analysis of the results of the blood indices in both male and female subjects in the 4 areas of study.

Blood indices	Female		Male	
	F	P value	F	P value
Fe	4.752	0.004	2.189	0.098
Fe- TIBC	1.542	0.209	1.003	0.397
Ferritin	0.899	0.445	1.506	0.221
WBC	0.194	0.900	0.896	0.448
RBC	0.647	0.587	4.216	0.009
Hgb	0.352	0.788	3.549	0.019
Hct	0.212	0.888	2.643	0.56
MCV	0.374	0.255	0.840	0.477
MCH	0.325	0.808	0.280	0.836
MCHC	0.815	0.489	0.294	0.38
Plt	0.181	0.909	1.595	0.199
CD_4/CD_8	1.730	0.166	0.325	0.807

* Results mentioned in case of male subjects have been compared with the social and economical parameters.

Categorical analysis was performed to assess the correlation between blood indices of the subjects and the degree of benzene air pollution. Results in the four areas were as follows:

1. There was a significant correlation between (Fe) in boys and degree of benzene air pollution ($p<0.05$).
2. There was a significant correlation between RBC of boys and the degree of benzene air pollution ($p<0.05$).
3. Significant correlation existed between Hb of boys and degree of benzene air pollution ($p<0.05$).
4. Significant correlation existed between hematocrit (HCT) of boys and degree of benzene air pollution ($p<0.05$).
5. Significant correlation existed between depression of CD_4/CD_8 of girls with degree of benzene air pollution ($p<0.05$).

T-test was used to compare the mean of blood indices of both male and female subjects residing in southern (Bazaar and Dolat-Abad) with those living in northern areas (Cheezar-Gheitarieh, and Darabad). The results were as follows (table 4):

1. CD_4/ CD_8 ratio of boys in the two areas of Bazaar and Dolat-Abad showed significant difference ($p<0.05$).
2. There were no significant differences in the blood indices of those living in southern areas and subjects residing in the northern areas.

The blood parameters of students living in southern areas (Bazaar and Dolat-Abad) were compared by t-test (in the form of pooling) with the total blood parameters of school children residing in the northern areas (Cheezar-Gheitarieh, and Darabad). The results showed:

1. (Fe) levels in boys residing in southern areas showed significant differences with those that lived in northern parts ($p<0.05$).
2. (RBC) in boys residing in southern parts showed significant difference compared to those that lived in the northern areas ($p<0.05$).
3. CD_4/CD_8 ratio in girls living in southern areas demonstrated significant difference with that of girl subjects residing in northern areas ($p<0.05$).

DISCUSSION

Based on the results of this study environmental exposure to benzene occurs at concentrations of 15-203 ppb which is a greater value when compared with earlier figure (4-187 ppb) (4). This study which evaluated environmental benzene exposure in the city of Tehran has the following advantages:

1. Sampling was performed in different areas of Tehran with different degrees of air pollution.
2. The evaluation of benzene air pollution was of indoor type i.e. measured inside the buildings. It is notable that the concentration of benzene inside the room is markedly higher than in open-environments. It is note worthy that inhabitants of metropolitan cities spend most of their time (60-70%) in these closed environments. This type of sampling was announced as the best method for evaluating environmental benzene exposure

among the residents of the cities (15).

3. Occupational and industrial exposure to benzene (inside buildings) is very much higher than the environmental type. Occupational exposure to benzene among the Iranian paint spray workers has been reported at 4 ppm levels (16). WHO and OSHA of the United States of America have considered the standard limit for occupational exposure as 100 ppb. This figure has been announced after reviewing the toxicological studies present in this regard. It is noteworthy that this rate is only considered as appropriate standard for workers and in case of vulnerable groups of the society such as children, old age etc, and this level could not be considered as a safe standard (17).

Based on toxicological studies and evaluation of benzene related complications, the standard for environmental benzene was determined by EPA. American researchers have studied the risk of blood malignancy in concentration range of 1-15 ppb of environmental benzene exposure. The risk of blood malignancy in environmental benzene concentrations of 15 ppb and 1 ppb were estimated as 1×10^{-4} and 1×10^{-6} respectively. The basis for setting 1ppb as the environmental standard in America, was the risk of blood malignancy at that level (18).

In comparison with the above standard, it seems that environmental benzene concentration in different areas of Tehran is significantly higher than the environmental benzene concentration of this chemical compound in western countries. The only city that has the same level of benzene air pollution is Mexico City in Mexico (19).

In this study based on the results of the corrected blood indices, significant differences were noted in RBC and Hb parameters of the male school children subjects. Also there was a significant correlation between blood parameters of male students (in regard to HCT, Hb, RBC and Fe) and the degree of benzene air pollution in the four areas of study.

This indicated the possible effect of environmental benzene exposure on the hematopoietic system of the male students. However, this experience did not exist in case of girl subjects, which could be due to a lower environmental exposure to benzene.

There is correlation between the depression of CD_4/CD_8 blood parameters and the degree of benzene air pollution. This fact was proven in earlier studies (11). Based on these reports and results, children living in air polluted areas of Tehran probably have weaker immune system. In this research only RBC of male subjects showed significant correlation with the degree of benzene air pollution. This fact has been reported by other researches for environmental benzene exposure at concentrations of less than 500 ppb (20).

Also in this study the indoor airborne benzene concentration was similar in several regions. For example the concentration of airborne benzene in the girls' schools of Bazaar (high pollution) and Dolatabad areas (moderate pollution) was the same. Also the level of benzene air pollution in both girl and boy schools of Darabad and Gheitarieh-Cheezar was nearly equal, the reasons behind this fact could be gas consuming heaters and sampling from closed environment.

CONCLUSION AND RECOMMENDATIONS

- The level of benzene air pollution in city of Tehran is considerably higher than the environmental standards of industrial countries. Considering the existence of pollutants in the environment, air pollution in Tehran is regarded as a state of environmental and health crisis.
- In this research, a number of effects were seen in the health of school children which could be due to the environmental benzene exposure. Regarding the harmful and injurious

consequences of exposure to other pollutants, threat to the health of school children, even in the closed environment of schools is an important issue that needs special and urgent attention.

- Considering the extent of air pollution (especially that of benzene) in Tehran, a more extensive and comprehensive study is needed to evaluate this issue. Therefore, further elaborated research is needed which encompasses elements such as all ages, different areas of the city, sex, occupation, seasons, different hours of the day and night etc.
- For better documentation of the biological impact of chronic exposure to benzene we recommend chromosomal studies in individuals that are chronically exposed to this chemical compound (21).
- We also advise that in air polluted areas of Tehran, school children undergo periodical health examinations.
- We strongly recommend the conduction and operation of control measures for optimizing the quality of fuel and engine combustion, promoting and encouraging the use of public transportation and last but not the least increasing the knowledge of the public in regard to air pollution and its complications could be very helpful.

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